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TRANSPLANTATION OF AUTOLOGOUS ADULT BRAIN PROGENITOR CELLS IN A NON-HUMAN PRIMATE MODEL OF MOTOR CORTEX LESION

THESIS

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“La vie, dans ce qu'elle a de meilleur, est un processus d'écoulement, de changement où rien n'est fixe. […] On pourrait définir une relation d'aide comme une situation dans laquelle l'on cherche à favoriser une appréciation plus grande des ressources latentes internes de l'individu, ainsi qu'une plus grande possibilité d'expression et un meilleur usage fonctionnel de ces ressources.”

Carl Rogers

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ABBREVIATIONS LIST

ACC	Anterior cingulate cortex
AIP	Anterior intraparietal area
ALS	Amyotrophic lateral sclerosis
Bcl-2	B-cell leukemia/lymphoma-2
BDNF	Brain-derived neurotrophic factor
Bmi-1	B-cell specific murine leukemia virus integration site 1
BrdU	Bromo deoxy uridine
C2	Second cervical
CMA	Cingulate motor area
c-Myc	"Gene similar to myelocytomatosis viral oncogene (v-Myc) by cloning"
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CS	Corticospinal
DAB	Diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DCX	Doublecortin
DG	Dentate gyrus
DIV	Days in vitro
dlPFC	Dorsolateral prefrontal cortex
DTI	Diffusion tensor imaging
ECG	Electrocardiography
EEG	Electroencephalography
EGF	Epidermal growth factor
EMG	Electromyography
ESC	Embryonic stem cells
FA	Fractional anisotropy
FEF	Frontal eye fields
FGF	Fibroblast growth factor
GABA	γ-aminobutyric acid
GalC	Galactocerebroside
G-CSF	Granulocyte colony-stimulating factor
GDNF	Glial cell-derived neurotrophic factor

GFAP	Glial fibrillary acid protein
GM	Grey matter
HD	Huntington's disease
HSC	Hematopoietic stem cell
HUCB	Human umbilical cord blood
ICMS	Intracortical microstimulation
IGF-1	Insulin-like growth factor-1
Klf4	Kruppel-like factor 4
LIF	Leukemia inducible factor
LIN28	"mRNA binding protein associated with differentiation and proliferation of embryonic stem and carcinoma cells"
LIP	Lateral intra-parietal area
LTD	Long term depression
LTP	Long term potentiation
M1	Primary motor cortex
MAP2	Microtubule-associated protein-2
MCA-O	Middle cerebral artery occlusion
MCP1	Monocyte chemoattractant protein 1
MHC	Major histocompatibility complex
MIB1	"Antibody against Ki-67, a protein expressed in proliferating cells"
MIP	Medial intraparietal area
Mk	Monkey
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cell
NANOG	North American Network Operators' Group "Transcription factor involved with self-renewal of undifferentiated embryonic stem cells"
NeuN	Neuronal nuclear
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NPC	Neural progenitor cell
NSC	Neural stem cell

Nurr1	Nuclear receptor related 1
Oct3/4	Octamer3/4
ODR	Occulomotor delayed response
OEC	Olfactory ensheathing cell
OFC	Orbitofrontal cortex
PD	Parkinson's disease
PET	Positron emission tomography
pFBS	Preselected Fetal Bovine Serum
PKH	"Viable membrane fluorescent dyes"
PM	Premotor cortex
PSA-NCAM	Poly-Sialated Neural Cell Adhesion Molecule
Rb	Retinoblastoma
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute culture medium
RT-PCR	Reverse transcriptase polymerase chain reaction
S1	Primary somatosensory cortex
SC	Superior colliculus
SDF-1	Stromal cell-derived factor-1
SGZ	Subgranular zone
Shh	Sonic hedgehog
SMA	Supplementary motor area
SMI-32	"Monoclonal antibody to neurofilament protein"
SOX2	SRY (sex determining region Y)-box 2
SSEP	Somatosensory evoked potential
SVZ	Subventricular zone
TGF-β	Transforming growth factor-β
TH	Tyrosine hydroxylase
TUJ-1	"Class III β-tubulin"
VEGF	Vascular endothelial growth factor
vlPFC	Ventrolateral prefrontal cortex
vmPFC	Ventromedial prefrontal cortex
WM	White matter

RÉSUMÉ

Aider à restaurer les fonctions atteintes suite à une lesion du système nerveux central est un réel défi, dans la mesure ou sa capacité d'auto-réparation est limitée. Bien que la thérapie cellulaire représente une stratégie thérapeutique prometteuse suite à des lésions cérébrales ou des maladies nerveuses, peu d'études ont abordé son bénéfice sur un plan comportemental. De plus, les implantations de cellules embryonnaires et fœtales exogènes sont actuellement limitées, dû aux questions éthiques (source) et de sécurité (problèmes de rejets immunitaires et de formations de tumeurs) qu'elles suscitent.

Nous avons testé ici une stratégie alternative chez des primates non-humains, à savoir l'implantation de cellules corticales progénitrices neurales adultes autologues, avec évaluation de la récupération fonctionnelle. Cette étude a porté sur deux singes recevant une implantation de cellules adultes autologues, comparés à quatre singes contrôles. Dans ce but, une biopsie unilatérale de tissu cortical fut pratiquée chirurgicalement dans le cortex préfrontal dorsolatéral droit de deux sur les six macaques adultes intacts, et les cellules ainsi obtenues furent mises en culture. Tous les singes furent entraînés à effectuer deux tâches de préhension manuelle (tâches du tableau de Brinkman modifié et du tableau de Brinkman tournant) requérant l'opposition du pouce et de l'index (pince de précision). Nous avons démontré ici que la biopsie corticale dans le cortex préfrontal dorsolatéral n'avait qu'un impact transitoire sur le comportement des singes, spécifiquement sur des aspects de stratégie de préhension impliquant un traitement de l'information visuo-spatiale. Une lésion chimique (par injection d'acide iboténique) unilatérale de la représentation de la main dans le cortex moteur primaire fut ensuite pratiquée chez les six singes.

Premièrement, nous avons testé et confirmé l'hypothèse de l'existence d'une correspondance entre les cellules exprimant la doublecortine dans le cerveau primate adulte et les cellules corticales adultes en culture. Chez le primate, des cellules doublecortine positives sont présentes dans l'entier du cortex cérébral adulte, de la glia limitans à la matière blanche, et expriment également des marqueurs gliaux et/ou neuronaux, tels que GFAP ou NeuN. Ces données soutiennent l'idée qu'in vivo, ces cellules doublecortine positives jouent un rôle dans la plasticité corticale et dans les réactions cérébrales suite à une lésion. In vitro, les cellules doublecortine positives forment un écosystème avec des astrocytes et ont le potentiel de réacquérir des caractéristiques de progéniteurs, en lien avec leur potentiel de réparation du cerveau.

Deuxièmement, comme attendu, après la lésion, les singes subirent une perte complète de dextérité manuelle de leur main contralésionnelle. En effet, la dextérité manuelle, une prérogative des primates, est sous le contrôle de la voie corticospinale ; puisque 90-95% des axones de la voie corticospinale décussent, il est admis que ce contrôle est exercé essentiellement sur la main contralatérale. Les quatre singes contrôles récupérèrent progressivement et spontanément une partie de leur dextérité manuelle, atteignant un unique et définitif plateau de récupération après 10 à 100 jours, s'étendant de 39% à 98% du score pré-lésionnel. Les deux singes traités récupérèrent progressivement et atteignirent un premier plateau de récupération spontanée environ 40 et 80 jours post-lésion, représentant 35% et 75% de la performance pré-lesionnelle, respectivement. Contrairement aux singes contrôles, un second plateau de récupération survint 75 jours et 83 jours après la réimplantation cellulaire, correspondant à des rebonds comportementaux reflétant une amélioration de récupération additionnelle de 24% et 25%. Au niveau cellulaire, après avoir été réimplantées dans le cerveau lésé du donneur, les cellules furent capables de migrer vers la région lésée et de se différencier, exprimant MAP2 et SMI-32. Les cellules cérébrales autologues réimplantées ne formèrent aucune tumeur au cours du temps.

Ainsi, l'implantation de cellules progénitrices cérébrales adultes autologues est une approche thérapeutique sûre et permettant une amélioration significative de la récupération fonctionnelle d'environ 25%, agissant probablement par remplacement cellulaire et/ou sécrétion de facteurs stimulant la réorganisation endogène.

SUMMARY

Restoring function following lesion of the central nervous system is a challenging task, as capacity for self-repair is limited. Although cell therapy is a promising therapeutic strategy after brain injury or nervous disease, only few studies have addressed the issue of its behavioral gain. Furthermore, implantations of exogenous embryonic and fetal cells are presently limited by safety and ethical issues. An alternative strategy was tested here in non-human primates, namely the implantation of autologous adult neural progenitor cortical cells with assessment of the functional outcome.

Four monkeys were used as control, compared to two monkeys subjected to autologous adult cell implantation. To this aim, an unilateral biopsy of cortical tissue was performed surgically on the right dorsolateral prefrontal cortex (dlPFC) of two on the six intact adult macaque monkeys, and the obtained cells were put into culture. All monkeys were trained to perform two manual prehension tasks requiring precision grip (modified Brinkman board and rotating Brinkman board tasks). The dlPFC biopsy was shown here to have only a transitory impact on the behaviour of the monkeys, specifically on prehension's strategy aspects implicating a treatment of the visuo-spatial information. The hand representation in the primary motor cortex was then chemically (ibotenic acid) lesioned unilaterally in the six monkeys.

We first tested and confirmed the hypothesis that there is a correspondence between cells expressing doublecortin in the adult primate brain and in the adult cortical cell cultures. Doublecortin-positive cells are present in the whole adult primate cerebral cortex from glia limitans to white matter and also express glial and/or neuronal markers such as GFAP or NeuN. These data support the idea that these doublecortin-positive cells in vivo play a role in cortex plasticity and brain reaction to injury. In vitro, doublecortin-positive cells form an ecosystem with astrocytes and have the potential to reacquire progenitor characteristics, in line with their potential for brain repair.

Second, as expected, after lesion, there was a complete loss of manual dexterity in the contralesional hand. Indeed, manual dexterity, a prerogative of primates, is under the control of the corticospinal (CS) tract; as 90-95% of CS axons decussate, it is assumed that this control is exerted essentially on the contralateral hand. The four control monkeys recovered progressively and spontaneously part of their manual dexterity, reaching a unique and definitive plateau of recovery, ranging from 39% to 98% of the pre-lesion score after 10 to 100 days. The two treated monkeys recovered progressively and reached a first spontaneous

recovery plateau at about 40 and 80 days post-lesion, representing 35% and 75% of the pre-lesion performance, respectively. In contrast to the control monkeys, a second recovery plateau took place 75 days and 83 days after cell implantation, corresponding to behavioral rebounds reflecting an additional enhancement of recovery of 24% and 25%. At the cellular level, after having been reimplanted in the donor injured brain, the cells were able to migrate towards the lesioned area and to differentiate, expressing MAP2 and SMI-32. Importantly, the reimplanted autologous brain cells did not form teratoma or tumor over time.

Overall, autologous adult brain progenitor cell implantation in non-human primate is safe and promotes a significant enhancement of functional recovery of about 25%, acting probably by cell replacement and/or factors' secretion stimulating the endogenous reorganization.

1 INTRODUCTION

1.1 THE HUMAN BRAIN

1.1.1 HISTORICAL OVERVIEW

The encephalon -brain, cerebellum and brain stem- and the spinal cord (Fig.1.1.1), both immersed in the cerebrospinal fluid, constitute the central nervous system, which elicited lots of interrogations in the course of the centuries, both metaphysical, philosophical, anatomical and functional (Parent, 2009; www.techno-science.net; www.neur-one.fr).

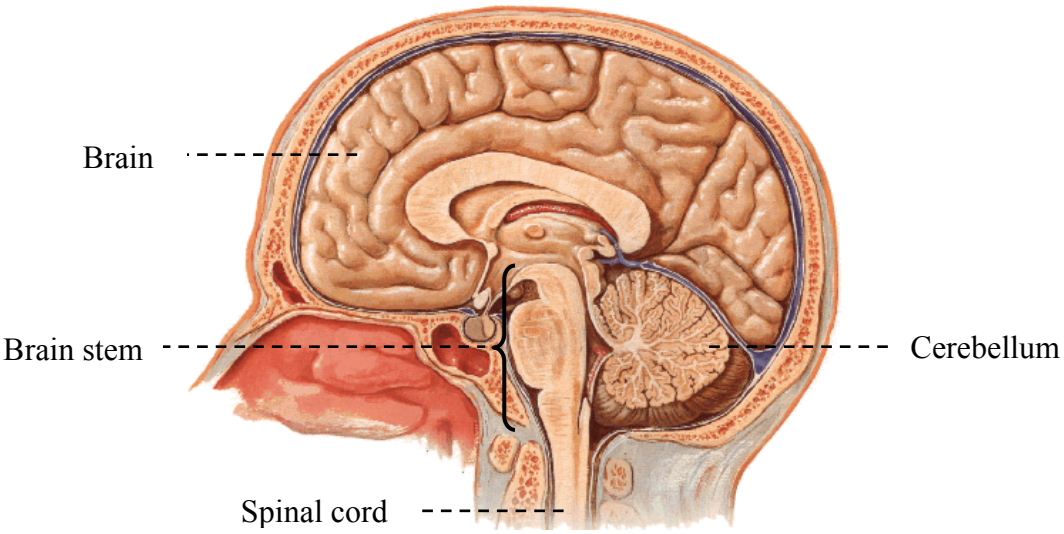


Figure 1.1.1: The central nervous system (CNS) is constituted by the encephalon (brain, cerebellum and brain stem) and the spinal cord (from www.mayoclinic.com).

During Mesolithic, about 12'000 years ago, men began to practice trepanations on the cranium of their congeners with silex tools, with the aim to cure them. In parallel, they took fragments of cranium post-mortem.

In the highlight of the ancient Egypt, doctors did not attribute any particular rule to the brain, neither for the mechanical functioning of the human body nor for the acquisition of the immortality of the soul of the deceased. When embalming, the internal organs of the belly were carefully extracted and conserved in canopic jars to allow a reconstitution of the deceased body in its integrality in the next world, whereas the brain was removed from the cranium and eliminated without ritual or preservation attempt. Nevertheless, the Egyptian papyruses were the first systematic written medical records, and the word "brain" (Fig. 1.1.2B), as well as the earliest description of the meninges and cerebrospinal fluid, first appeared in the Edwin Smith Egyptian surgical papyrus (Fig.1.1.2A) written by an Egyptian

physician named Imhotep in ~1700 BC, inspired by various texts that go back to ~3000 BC. It was found by Edwin Smith in 1862 and translated by Breasted in 1930. This papyrus, that includes 48 detailed descriptions of various cranial and vertebral traumas with various treatment recommendations, is the first to report neurological symptoms shown by patients (Feldman et al., 1991).

A B

Figure 1.1.2: The Edwin Smith Surgical Papyrus (A) and the word "brain" in hieroglyphic (B) (from neurophilosophy.wordpress.com).

In the Greek civilization, until the Hippocratic era and its medical schools, the heart was considered as the center of any thoughts ans feelings, although the localization of the soul, in the sense of vital breath, could not be established there with certainty. Alcmeon (described to be active around 500 BC), on the basis of his dissections, supposed that the optic nerves were « ways of brightness » directed toward the brain, which was the seat of any sensation. Democritus (460-370 BC) considered that the psyche (soul, vital breath, “pneuma”) was constituted by atoms (particles) that were concentrated in the brain, although they could be found in lesser quantity in the whole body. Hippocrate (~460-370 BC) declared that the brain was the seat of the sensations and of intelligence. Plato (~428-347 BC) and Aristotle (~384-322 BC) supported the idea that the source of the perception and the intelligence was the brain and the heart, respectively. For Aristotle, indeed, the brain served in cooling the blood temperature and in regulating the heart temperature. About 350 BC, Herophilus, dissecting human and animal bodies, identified the path of the spinal nerves going from the muscles to the spinal cord, remarking also that each body region was linked to distinct nerves. Nevertheless, he thought that all body affections resulted from “humors” imbalances. Erasistratus (~310-250 BC), also dissecting human bodies, distinguished two main structures

of the encephalon, namely the two hemispheres of the brain and the cerebellum, as well as the convergence of the nerves toward the central nervous system. He also provided evidence for the sensitive and motor roles of the posterior and anterior rachidian nerves, and established a link between the degree of gyrification of the brain of the different animal species and their intelligence degree.

In the ancient Rome, Galen (130-200), who shared Hippocrat's views, developed the notion of psychic pneuma, produced by the brain and storaged in his cavities (ventricles), transported then through the nerves toward all body regions, and constituting the soul organ that links the brain (with the higher cognitive functions), the senses organs and the motor organs.

The Middle Ages was mainly a period during which religion got the upper hand over scientific discoveries, with a disinterest for the antic thoughts and a quasi-prohibition to be interested in the human body, as the intellectual life was mainly confined in the monasteries, where religious exaltation was valorized.

In the Renaissance period, the dissections of human and animal bodies resumed. Leonardo da Vinci (1452-1519) showed the human body from different angles and made wax moldings of the ventricles, thought to work as a pump to produce limb movements, whereas the cerebral cortex was perceived as a protection for the ventricles (Fig. 1.1.3).

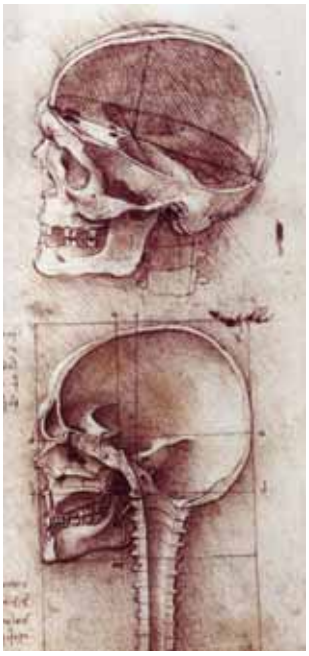
A  B 

Figure 1.1.3: Anatomical drawings of Leonardo da Vinci: A. View of a Skull, 1489; B. Study of Brain Physiology, 1508 (from www.drawingsofleonardo.org).

Vesalius (1514-1564; Fig. 1.1.4) described very precisely the cerebral morphology, but did not contradict the localization of the cerebral functions in the ventricles. Descartes (1596-1650) defended the hydraulic fluid theory of brain function, implying that the cerebrospinal fluid was pumped up through the ventricles to produce limbs movements. He also analyzed the release of movements by sensory signals and realized plans that were close to the actual reflex act, named first by Willis (1621-1675), who showed that the wrinkled cerebral cortex covered subcortical centres, such as the nuclei of the thalamus, and distinguished a grey and a white matter, as well as the corpus callosum that links the two hemispheres, and the sulci and gyri thought to correspond to various functions, opening thus the era of the theory of the cerebral localizations.

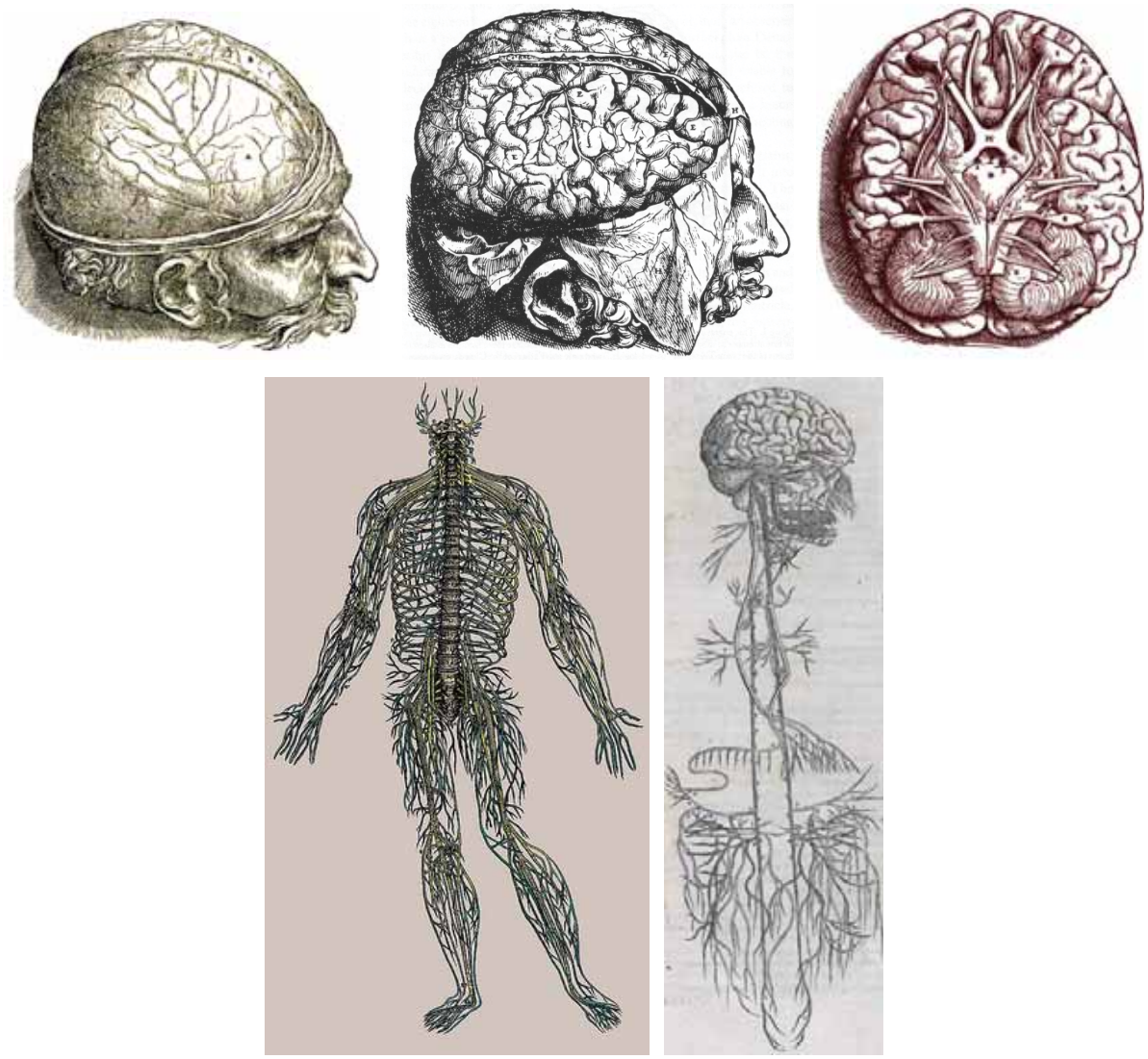


Figure 1.1.4: Anatomical drawings of Vesalius (De humani corporis fabrica, 1543) (from www.kean.edu/~bregal/HIST3321.htm; vesalius.northwestern.edu/).

A turning point in the understanding of the brain structure and function came from the invention of a combined lens system to magnify objects, the first microscope, developed by the father and son Janssen (~1595), and by Galileo (1564-1642). To note that already in the 1st century, Plinius and Seneca described the use of magnifying glasses by old people. During two centuries, the descriptions of nerve fibers and of little glands in the cortex and the white matter succeeded one another in contradictory theories.

In 1839, the concept of « cells » composing all tissues was defined by Schwann and Schleiden, and Meynert (1833-1892; in de Wit, 1993) described a brain organized in different functional areas connected by white matter tracts. The microscopical study of the tissues was improved at the end of the XIXth century with the development of staining techniques, allowing a greater contrast between the observed structures, by Golgi (nitrate silver staining; 1873), Nissl (dahlia violet staining; 1894) and Cajal (reduced nitrate silver staining; 1887). Hence, Camillo Golgi edicted that the brain is composed by an autonomous and homogeneous diffuse nervous network composed of intermingled neurofibrils. This theory was counterfighted by Cajal who wrote the Neuron Theory in 1887 (in Berciano and Lafarga, 2001), stipulating that the nervous cell has independent anatomical, genetic, functional, regenerative and reactive particularities thus establishing the foundations of the modern neurobiology.

The very first to describe the structure of a human brain in regard to its function and pathways were the earliest neuronatomists (Broca, 1863, Cunningham 1892, Wernicke, 1874; in Mendoza and Foundas, 2008) who analyzed the effect of the different localization of the brain lesions over the limbs motility and speech function.

1.1.2 GENERAL DESCRIPTION

The encephalon has three defense lines (Fig. 1.1.5). First the hairs and the skull, which prevent excessive heat and constitute an osseous protection, respectively. Second the meninges, which are three membranes situated between the skull and the brain, protecting the brain and contributing to the blood brain barrier. These three membranes are the Dura mater (a double layered membrane), the Arachnoid (a membrane separated from the dura mater by the subdural space) and the Pia mater (a connective tissue rich in blood vessels). Third the cerebrospinal fluid, located in the ventricles and between the meninges, which serves as shock absorber and as fluid medium to transport substances.

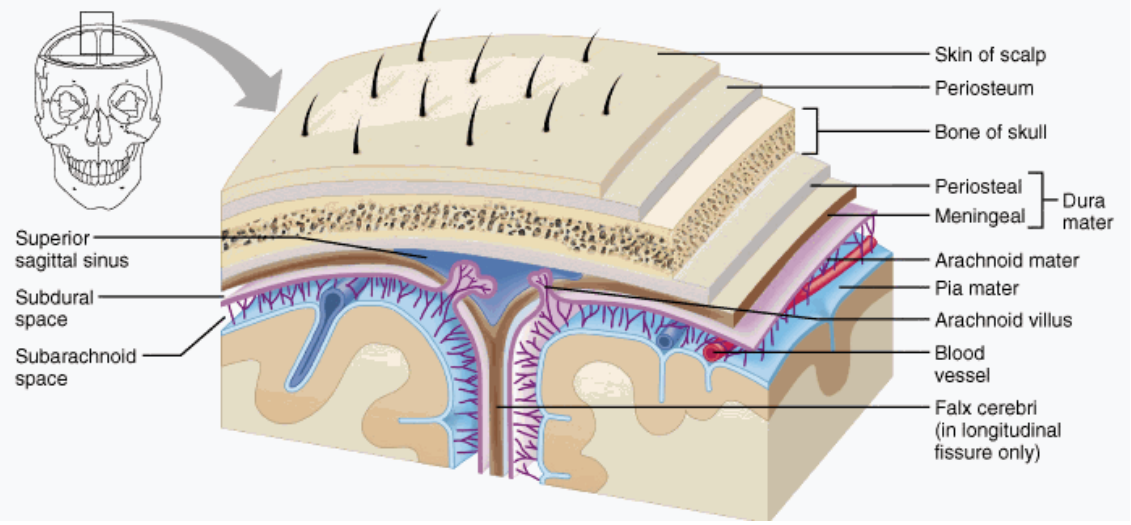


Figure 1.1.5 : Brain defense lines: the hairs and the skull, the meninges and the cerebrospinal fluid (from legacy.owensboro.kctcs.edu/gcaplan/anat/Notes).

Weighting about 1.4kg, the encephalon is formed by the brain, the cerebellum and the brain stem (Fig. 1.1.1).

The brain is constituted by the two cerebral hemispheres, which are subdivided into four lobes, associated to various general functions: frontal (reasoning, planification, locution, movements and emotions), parietal (movements, orientation, recognition and stimuli perception), temporal (perception and recognition of auditory stimuli, memory and locution), and occipital (visual treatment).

The external layer of the brain is named cerebral cortex or grey matter, folded in numerous gyri and sulci, and covering the nuclei situated in the white matter of the hemispheres. The grey matter is composed mainly by neural cell bodies, whereas the white matter is mainly formed by axons.

Various subcortical regions are important (Fig. 1.1.6), among which i) the corpus callosum, which allows the transfer of informations between the two hemispheres, ii) the thalamus, which participates to the transmission of informations between the body and the cerebral cortex and maintains a vast network of connections with the cortex and other parts of the encephalon, such as the basal ganglia, the hypothalamus and the brain stem, iii) the hypothalamus, which manages the hypophysis and the autonomous nervous system, and thus the regulation of the body temperature, the cardiac frequency, the blood pressure, the appetite, the structure of the sleep, the libido and the emotions, iv) the hippocampus which participates to the treatment and the storage of memories, v) the amygdala, which regulates the control and the expression of the mood and the emotions, vi) the basal ganglia, formed by the caudate

nucleus, the putamen and the pallidum, which control the global motor functions such as the posture and the equilibrium, as well as the triggering and the management of voluntary movements; the basal ganglia also play a role in reward learning and in motivation, vii) the ventricles, which are cavities filled with cerebrospinal fluid that is secreted in their wall.

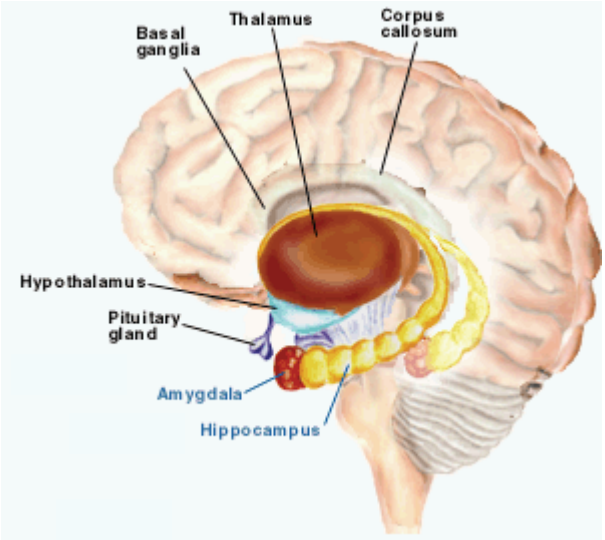


Figure 1.1.6 : Various important subcortical regions : the corpus callosum, the thalamus, the hypothalamus, the hippocampus, the amygdale, the basal ganglia and the ventricles (not represented) (from www.thebrain.mcgill.ca).

The cerebellum is responsible for the psychomotor function, coordinating the influx of sensory inputs coming from the internal ear and the muscles to allow a precise control and coordination of the equilibrium, the posture and the movements.

The brain stem forms the link between the cerebral cortex, the white matter and the spinal cord. In the core of the brain stem is located the reticular formation, which is a group of nuclei that receive information from most sensory systems and from other parts of the encephalon. Some neurons of the reticular formation are relays for the sensory inputs and the motor outputs, whereas some others exert an influence on the conscience state, the respiration, the cardiac frequency, the blood pressure and the attention.

Concerning the cerebral vascular system, the brain is fed by two pairs of arteries : the vertebral arteries (toward the brain stem, the cerebellum, the occipital lobe of the brain and some parts of the thalamus) and the internal carotid arteries (toward the remaining encephalon). The cerebro-vascular system transports the oxygen and the nutriments to the

encephalon. As the encephalon uses about 20 % of the oxygen absorbed by the lungs, the maintain of a constant supply in blood is essential for its normal functioning.

The encephalon and the spinal cord form the central nervous system. The spinal cord (Fig. 1.1.7) measures about 45cm long in men and 43cm long in women. The spinal cord functions primarily in the transmission of neural signals between the brain and the rest of the body, but also contains neural circuits that can independently control numerous reflexes and central pattern generators. It is divided in 31 different segments: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal. At every segment, ventral (motor) and dorsal (sensory) roots form right and left pairs of spinal nerves. The spinal cord is also constituted by grey matter (internal) and white matter (external).

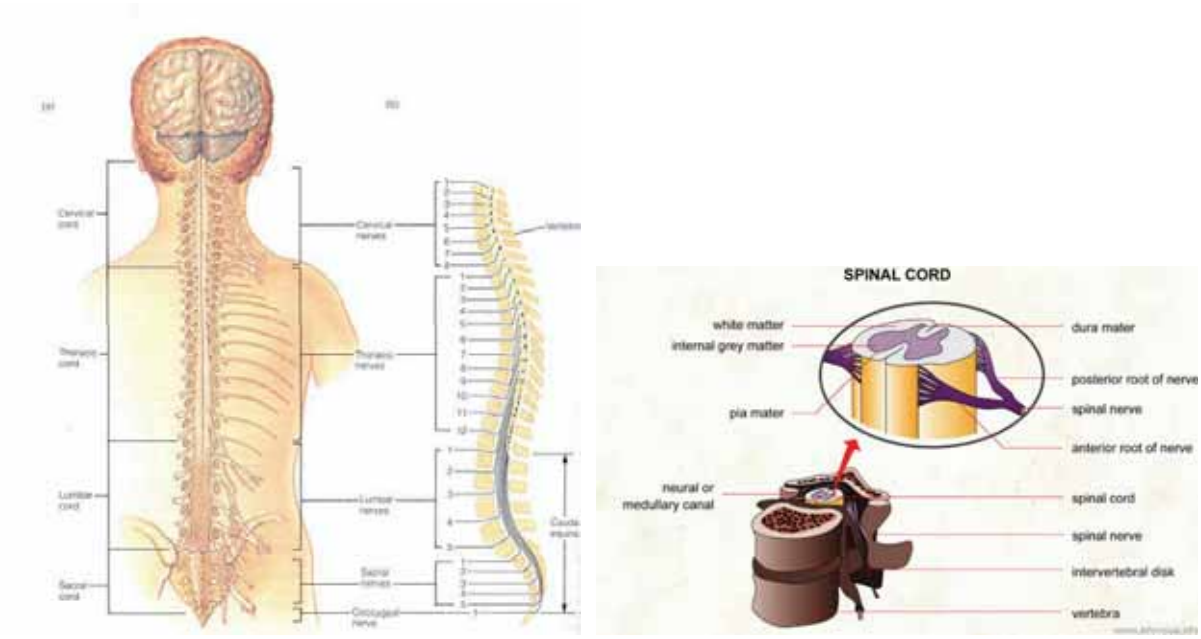


Figure 1.1.7: The spinal cord, divided in 31 different segments: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal. At every segment, ventral (motor) and dorsal (sensory) roots form right and left pairs of spinal nerves. Spinal cord is also constituted by grey matter (internal) and white matter (external) (from jyi.org; www.infovisual.info).

1.1.3 HUMAN BRAIN CELLS

The human cerebral cortex contains over 100 billions neurons and 250 billions glial cells. The neocortex, which contains primary areas receiving projections from the sensory, motor and association areas, is a six-layered structure characterized by regional and laminar specific distributions of a variety of neuronal subtypes and distinct afferent and efferent connections (Fig. 1.1.8) (Brodmann, 1904; Campbell, 1905; Koskinas, 1925; Ramón y Cajal,

1911; von Economo and Vogt and Vogt 1926; in Kesarev and Malofeeva, 1969). The neocortex can be parcellated into a large number of more or less distinct cortical fields according to their microscopic architecture (e.g. Brodmann, 1909; Sanides, 1962). Brodmann (1909) defined 52 functional areas distinguished by their different cellular organization and neuronal populations. Thus, several functional cortical areas such as primary motor cortex (area 4), premotor cortex (area 6), sensory cortex (areas 1, 2 and 3) and visual cortex (area 17) have been identified, each having their morphological particularities. The cortical layers are distinguished on the basis of the number, density, dendritic morphology, size and topographical distribution of particular cells. The six layers are subdivided in a laminated manner as follows (Fig. 1.1.8):

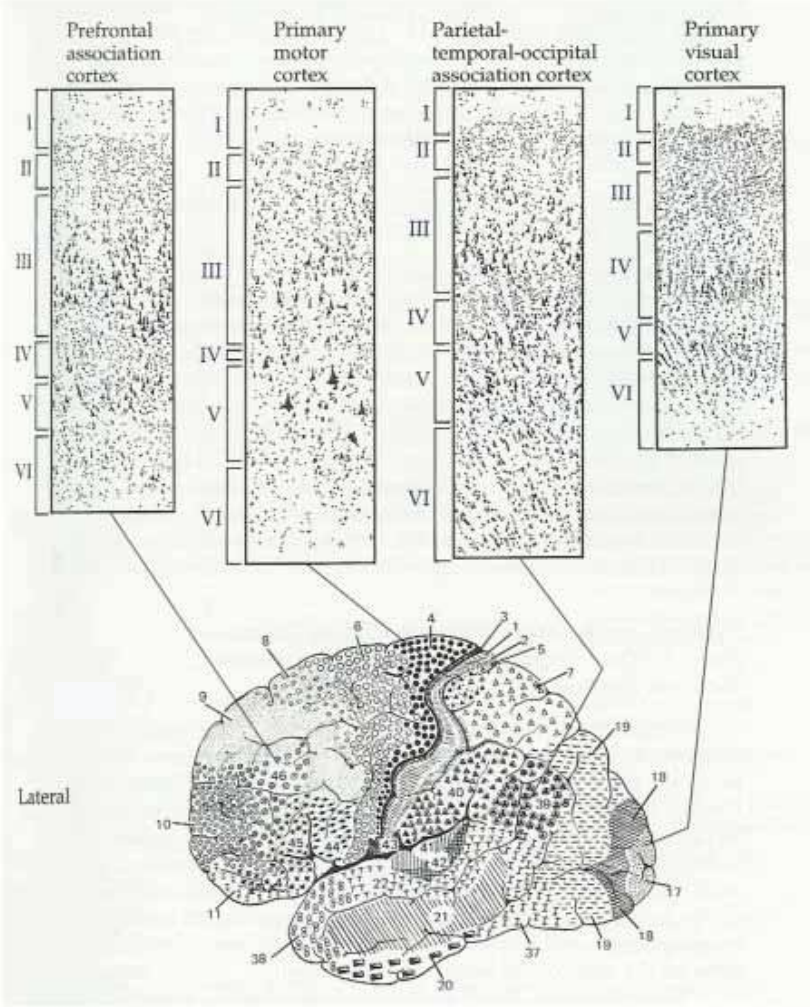


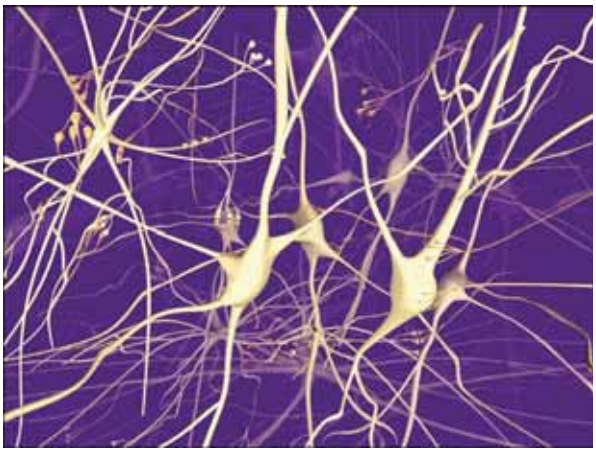
Figure 1.1.8: The cortical layers, which are subdivided in a laminated manner, distinguished on the basis of the number, density, dendritic morphology, size and topographical distribution of particular cells (from faculty.washington.edu).

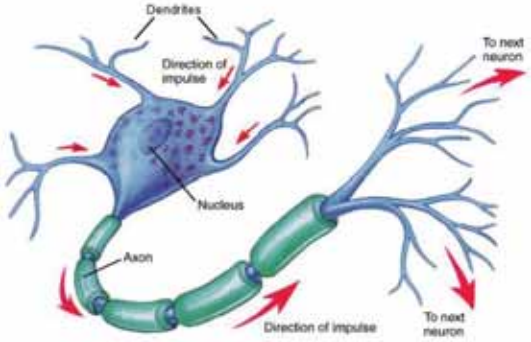
I. Plexiform lamina (Molecular or Zonal layer): it contains axons and dendrites from neurons of the internal layers, as well as Cajal-Retzius cells and stellate cells.
II. External granular lamina: it contains granular neurons, receiving afferences from other cortical areas.
III. External pyramidal lamina: it contains the cell bodies of medium sized pyramidal cells that form connexions with other cortical areas.
IV. Internal granular lamina: it contains mainly stellate cells and occasionally small pyramidal cells, which receive informations from structures external to the cortex, such as the thalamus, as well as from the contralateral cortex.
V. Internal Pyramidal (ganglionic) lamina: it contains the large pyramidal cells that send efferent informations, such as toward the motoneurons. Stellate cells in small numbers may also be present.
VI. Multiform lamina: it contains a considerable range of cell types, which send informations towards the thalamus.

The neurons are formed by a soma and two types of prolongations: the dendrites and the axon. Each individual axon receives informations from up to thousands of other neurons, sending them away up to thousands of other neurons (Fig. 1.1.9). The informations are relayed from one neuron to another by the neurotransmission, which is an indirect processus that takes place in the synaptic junction. Concretely, the information coming from upstream neurons is transmitted through the dendrites (inputs) and then through the axon to be transmitted to one or several downstream neurons (outputs). The contact between two cells is named the synapse (synapse junction). The signal that propagates into the neuron is electrical, whereas at the level of the synapse, the information is delivered through chemical messengers, the neurotransmitters, which are diffused by the neuron, through the synapse to the neighbour neuron. In the post-synaptic neuron, the chemical signal is reconverted in an electrical signal. Before transmitting further the synthesis of the informations he received, a neuron integrates in its soma the collection of the messages received from the dendrites. This integration will lead either to the induction of an action potential (bioelectric signal) to transmit the information further, or to the end of the transmission of information. There are different types of neurons, that can be distinguished by their anatomy, such as the pyramidal cells (cerebral cortex), the stellate cells (cerebral cortex), or the Purkinje cells (cerebellum), or by their function, such as the sensory neurons (directed linked to the organs of the senses and responsible to transmit the sensory information toward the brain; ascending pathway), the

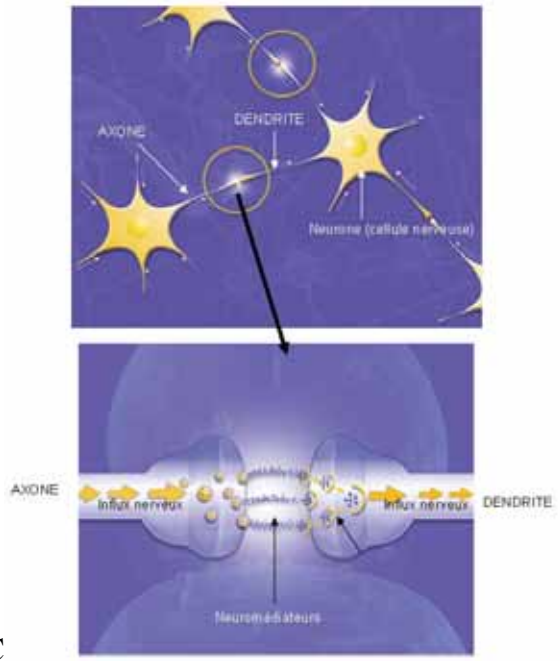
motoneurons (transmit the orders originating for the brain toward the muscles; descending pathway), or the interneurons (local neurons that make the junction between sensory and motor neurons).

A





B



C

Figure 1.1.9 : A. A neuron is integrated in a complex network, receiving and sending information from thousands of other neurons. B. The neurons are formed by a soma and two types of prolongations: the dendrites and the axon, which diffuse the information in an electrical form. C. The information is relayed from one neuron to another by the neurotransmission, which is an indirect processus that takes place in the synaptic junction and includes the release of neurotransmitters (from www.pisani.blog.lemonde.fr/2006/10; www.thebrain.mcgill.ca; www.gsk.fr).

The neuronal architecture is supported by glial cells, which are smaller than the neurons and do not possess neither axons nor dendrites, and which are constituted mostly by astrocytes, oligodendrocytes and microglia (Fig. 1.1.10).

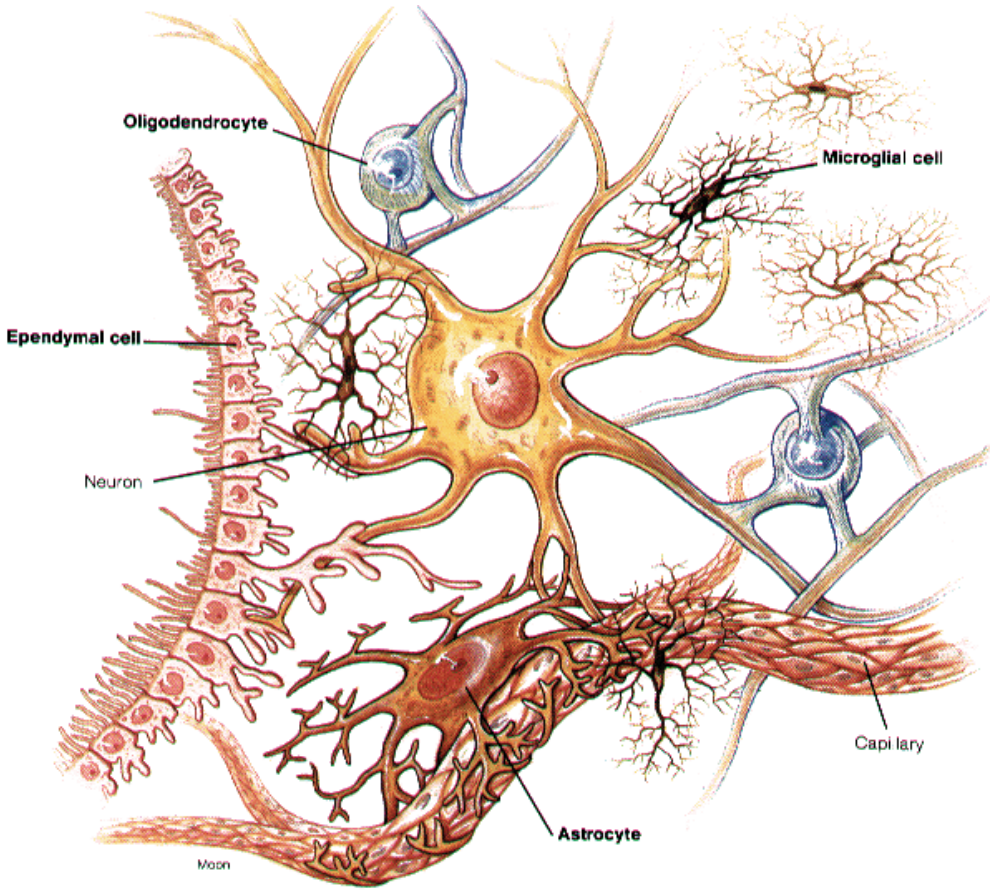


Figure 1.1.10 : Neural ecosystem, formed by neurons, astrocytes, oligodendrocytes and microglial cells, as well as vascular and ependymal cells (from www.med.stanford.edu).

The astrocytes regulate the concentration of diverse substances contained in the extracellular medium and are responsible for support and trophic functions. They are also thought to play an important role to assist the neurons in the information processing. Indeed, the astrocytes, which communicate with the neurons by strategies including the release of chemical substances and the cell-to-cell contact, can release neurotransmitters, such as small peptides, as well as cytokines and neurotrophic molecules. Molecules on the surface of the astrocytes can interact with other proteines on the surface of the neurons to induce changes in the neuronal function. Furthermore, astrocytes produce neurotransmitter receptors and can respond to various neurotransmitters, in addition to release neurotransmitters through a process that does not implicate synaptic vesicles (Attwell et al., 1993).

The oligodendrocytes, as Schwann cells at the peripherical nerve level, form the myelin sheath around the axons of the central nervous system. This biological isolation serves to optimize the conduction velocity of the information.

The microglia is a cell type that “cleans” the extracellular medium from the waste and ensures support and immunological functions.

Finally, the endothelial cells line the blood vessels.

When two neurons are interconnected, the type of information of the pre-synaptic neuron can be excitatory or inhibitory, leading the post-synaptic neuron to be activated or inhibited from discharging, respectively. The ability of the neurons to perform this complex task of transmission is due to the unequal distribution of electrically charged particles on either side of its semi-permeable cell membrane.

A neuron forms synaptic connections with axon terminals from many different nerve cells. Several excitatory as well as inhibitory ionic disturbances can therefore occur simultaneously, or nearly so, in neighboring portions of the post-synaptic cell membrane. Both temporal and spatial summations of excitatory and inhibitory post-synaptic potentials occur. The outcome of these interactions determines the size of the membrane potential at any point in time.

Humans consist of 2/3 salt water, the principal salts being the positively charged cations of sodium (Na+), potassium (K+), calcium (Ca2+) and magnesium (Mg+), and the negatively charged anions of chloride (Cl-) and phosphate (P-) as well as various organic acids (A-). All of these chemicals (and more) are contained in both the extracellular fluid surrounding each neuron, and intercellular fluids (cytosol) of neurons, and both contain high concentrations of compounds, called electrolytes, that conduct an electric current and are decomposed by it into atoms called ions capable of carrying positive or negative electrical charges. Their concentrations are not the same: the fluid inside neurons contains more negative anions and/or fewer positive cations than the surrounding extracellular fluid from which it is separated by the semipermeable cell membrane (the permeability of this membrane to specific ions is determined by the presence of specific ionic channels). The unequal distribution of positive and negative charges between the inside and outside of a neuron results in an electrical potential of -70 mV. This resting membrane potential of the cell comes about because of interplay of several forces including i) diffusion, ii) electrostatic pressure, and iii) active sodium and calcium pumps.

Action potentials (Fig. 1.1.11) represent the transitory changes of the resting potential of the membrane. In most axons, the depolarization activates the action potential and causes a transitory change in the membrane that overbalances briefly the permeability to allow the transfer of sodium ions instead of the potassium ones. The opening of channels sensitive to variations of voltage in the membrane allows to the sodium ions to decrease the concentration gradient to penetrate in the cell. This produces the ascending phase of the action potential, the membrane potential becoming briefly positive. The descending phase of the action potential is caused by the consecutive closing of the sodium channels and the opening of potassium channels, restoring the negative rest potential of the membrane. The action potential is followed by a transitory hyperpolarization, during which the evacuation of potassium ions is more important than at rest.

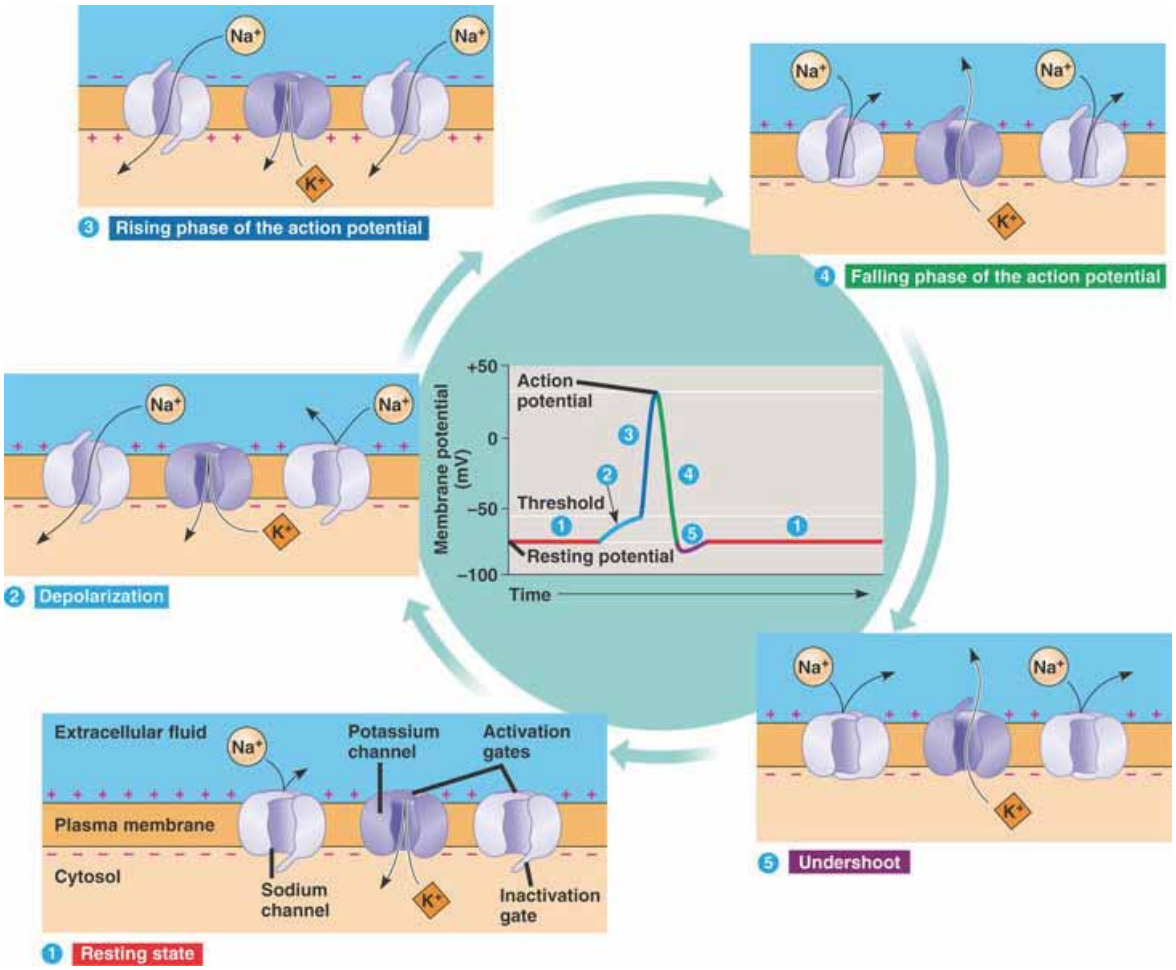


Figure 1.1.11: Action potential process, allowing the transmission of the electrical information (from www.kvhs.nbed.nb.ca).

Once the action potential reaches the termination of the axon, the change of membrane potential induces the activation of calcium channels, which increases the concentration of

calcium ions in the presynaptic neuron. This increase of intracellular calcium causes the fusion of the synaptic vesicles with the presynaptic membrane, and neurotransmitters are released in the synaptic junction (Fig. 1.1.12). The neurotransmitters are produced and stored in a neuron, liberated following a depolarization of this neuron, and diffused in the synaptic junction to bind to the specific receptors on the membrane of the postsynaptic neuron. The binding of a neurotransmitter to its receptor on the postsynaptic membrane can activate some channels in the postsynaptic neuron, leading to a change of the resting potential of the membrane, generating excitatory or inhibitory postsynaptic potentials that modify the excitability of the postsynaptic neuron. If the threshold is reached, the post-synaptic neuron will generate action potentials. After having generated the postsynaptic potential, the neurotransmitter must be quickly evacuated from the synaptic junction, so that the postsynaptic cell can begin another cycle of signals' production.

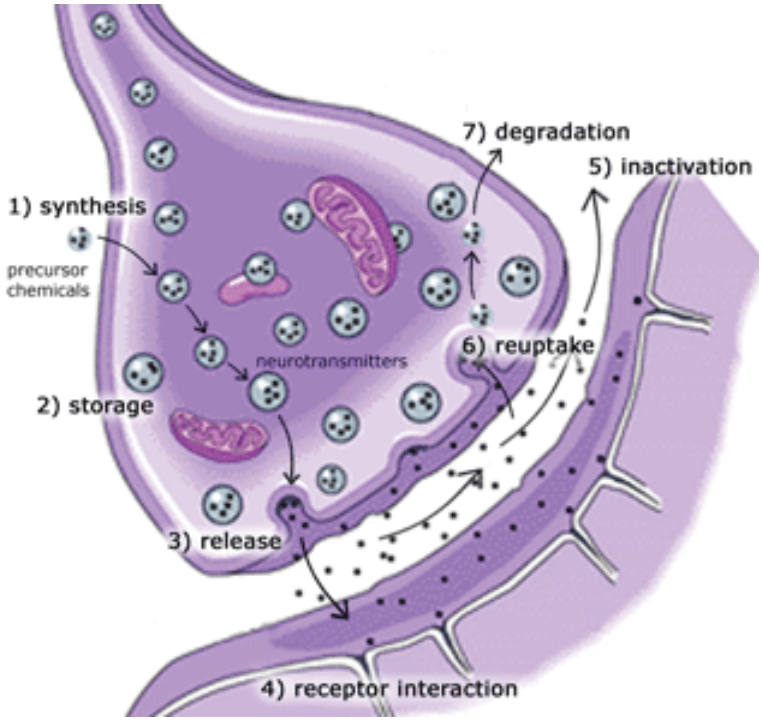


Figure 1.1.12: The neurotransmission, namely the chemical relay of information from one neuron to another, takes place in the synaptic junction and includes the release of neurotransmitters, binding to the specific receptors on the membrane of the postsynaptic neuron (from www.glittra.com).

The neurotransmitters can be classified in two main categories (Table 1.1.1) : the « classical » neurotransmitters with small molecules, and the neuropeptides that are relatively more voluminous.

Neurotransmitters with small molecules			
Type	**Neurotransmitter**	**Postsynaptic effect**	**Neuropeptides**
	Acetylcholin	Excitatory/Inhibitory	Corticoliberin
Amino acids	Gamma-aminobutyric acid (GABA)	Inhibitory	Corticotrophin (ACTH)
	Glycin	Inhibitory	Bêta-endorphin
	Glutamate	Excitatory	P substance
	Aspartate	Excitatory	Neurotensin
Biogenic amines	Dopamine	Excitatory/Inhibitory	Somatostatin
	Noradrenaline	Excitatory/Inhibitory	Bradykinin
	Serotonin	Excitatory/Inhibitory	Vasopressin
	Histamine	Excitatory/Inhibitory	Angiotensin II

Table 1.1.1: The two main categories of neurotransmitters, which can have excitatory or inhibitory postsynaptic effects. To note that acetylcholine, dopamine, noradrenaline, serotonin and histamine can have either excitatory or inhibitory actions depending on receptors.

1.2 MOTOR SYSTEM

1.2.1 MOTOR CORTEX

In 1870, Fritsch and Hitzig stimulated electrically the cerebral cortex of an anaesthetized dog (Taylor and Gross, 2003) and described a map of the muscles in the frontal lobe. Later, Ferrier (1873) obtained a similar motor map in the monkey brain. Brodmann (1903, 1909), based on the histological appearance of the cortical layers, originally described area 4 as an agranular zone (indeed, the motor cortex is deprived of layer IV), delineated by the presence of Betz cells, a subpopulation of giant infragranular pyramidal neurons in cortical layer V (Betz, 1874).

The pyramidal neurons govern the skelettical muscles to realize voluntary movements. Their axons project towards the motoneurons of the spinal cord, constituting the cortico-spinal or pyramidal tract. During the 1930s, Penfield highlighted various cortical areas implicated in the human motricity (Penfield and Boldrey, 1937). As he practicated surgeries with the aim to attenuate patients epileptic crises by surgically removing the responsible brain area, he stimulated electrically cortical regions and observed the responses, delimiting the vital regions in order to avoid injuring them during the intervention. He thus showed that stimulations of the precentral gyrus, corresponding to the primary motor cortex (M1;

Brodmann's area 4), elicited contralateral muscles contractions. In area 4, mainly responsible for the execution of movements, the cortical columns are arranged in a functional somatotopic way, illustrated first by the motor homunculus of Penfield, representing the body muscle territories on the cortical surface.

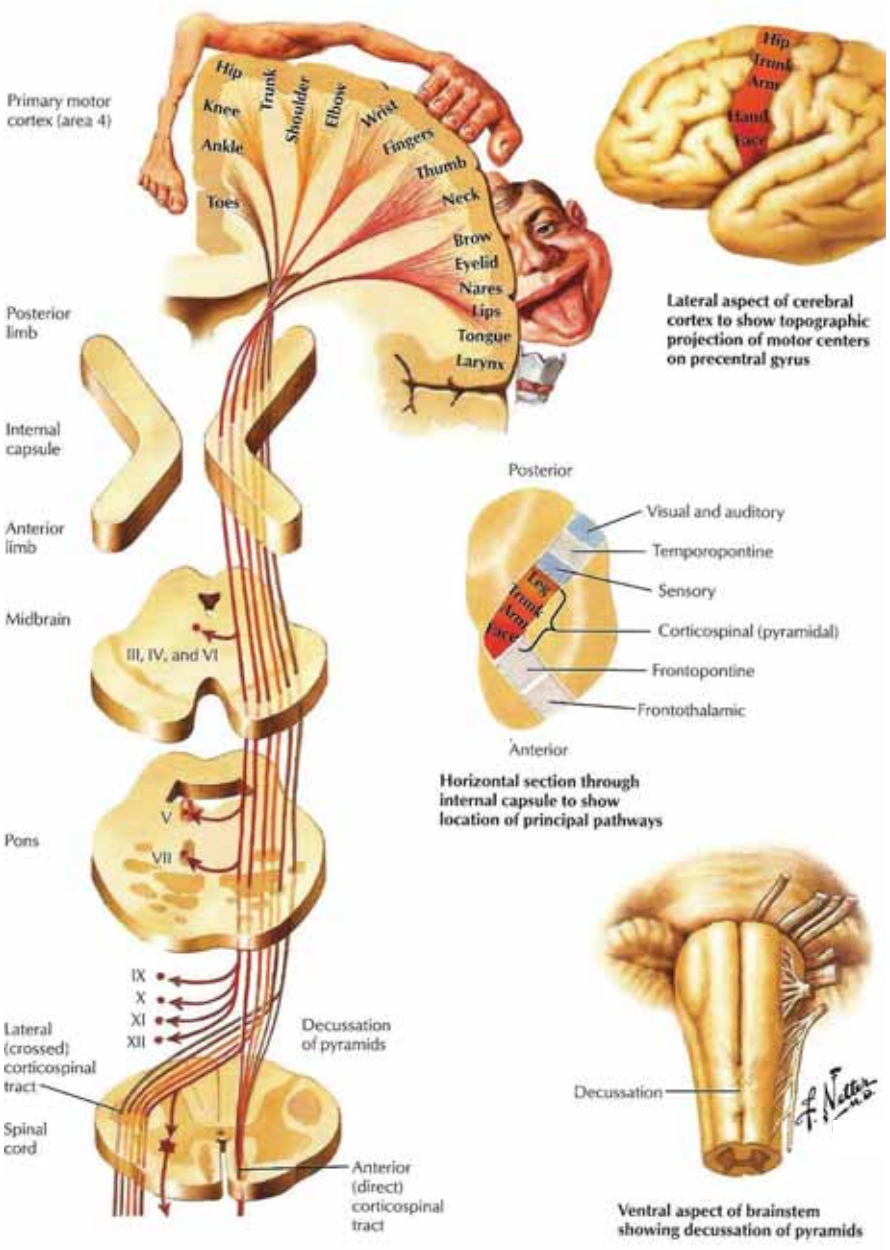


Figure 1.2.1 : Pyramidal system. In area 4, responsible for the execution of movements, the cortical columns are arranged in a functional somatotopic way, illustrated first by the motor homunculus of Penfield. The fibers originating from neurons of layer V pass by the capsula interna, the midbrain and the pons, and about 80% of the corticospinal fibers cross the medial line at the level of the pyramidal decussation, corresponding to the lateral corticospinal tract, which controls the distal muscles, whereas 20% of these fibers project bilaterally, 10% decussating just before contacting their targets and 10% staying ipsilateral, constituting the ventral corticospinal tract that controls the axial muscles (from "Netter's Neurology", Royden Jones, 2005).

From lateral to medial are elicited movements of the pharynx and the larynx, the tongue and the face, the hand and the fingers, the upper limb, the trunk, the leg (Fig. 1.2.1). The hand and the face regions are over represented as compared to other body parts, reflecting the higher precision of movements executed by face and hand muscles.

Other cortical areas are implicated in motricity (Fig. 1.2.2). Rouiller and Olivier (2004) suggested that at least one of the following criteria should be filled to define an area as motor : i) to contain corticospinal neurons, ii) to project directly on the primary motor cortex, iii) to activate muscles in response to intracortical microstimulations, iv) to be connected to the motor part of the thalamus, v) to have a neuronal activity strongly linked to the execution of a motor task.

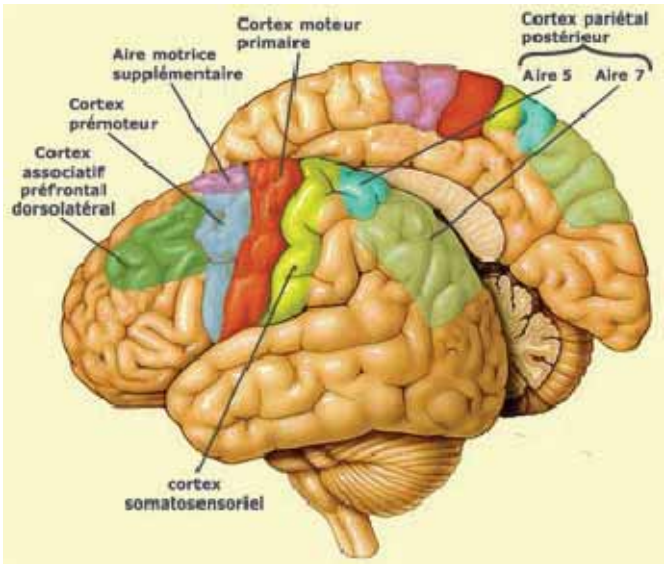


Figure 1.2.2 : Cortical areas implicated in the control of movements in primates (cingulate motor cortex not represented here) (from www.futura-sciences.com).

Area 6, situated rostrally to area 4, is subdivided into two regions located in the lateral and medial part of area 6, respectively, namely the premotor cortex (PM), which integrates sensory informations to plan and organize movements (Dum and Strick, 2005; Luppino et al., 1991; Matelli et al., 1985; Ming-The et al., 1994; Rizzolatti et al., 1988; Tachibana et al., 2004; Todd et al., 1996; Wise, 1985) and the supplementary motor area (SMA), which plays a role in the planification and the coordination of multiple limbs movements as well as of complex movements (Dum and Strick, 2005; He et al., 1995; Matsuzaka et al., 1992; Mushiake et al., 1990). The cingulate motor cortex (CMA; He et al.,1995), situated in the cingulate gyrus, on the medial side of the hemispheres, is implicated in higher-order cognitive

control of movements, such as processing the reward information for motor selection (Shima and Tanji, 1998).

All these regions can be subdivided in several parts (Rouiller and Olivier, 2004). In addition, motor functions have been described for the posterior parietal cortex (areas 5 and 7), which guides voluntary movements in space, and the dorsolateral prefrontal cortex (areas 8,9 and 46), which contributes to the selection of voluntary movements to make according to higher-order instructions, rules, and self-generated thoughts.

A somatotopy is also present in the SMA, PM and CMA. According to the description of Penfield, each body part is represented once on the motor cortex. However, the actual intracortical microstimulation techniques allowing to stimulate the cortex more selectively, showed that the Penfield homunculus was not totally correct. Indeed, the somatotopic map in the primary motor cortex is overlapping and intermingled, which suggests that it is organized to promote coordination among the muscles (Donoghue et al., 1992). Schieber (Schieber, 2001; Schieber and Hibbard, 1993) demonstrated that the movement of each finger activated neurons distributed in the whole M1 hand area and that one single M1 neuron was related to different movements (Fig. 1.2.3).

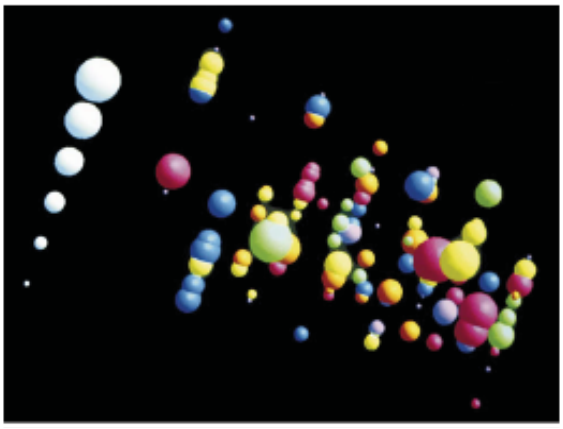


Figure 1.2.3: Distributed activation in M1 during finger movements. Colored spheres each represent a single neuron recorded in the left hemisphere M1 as a monkey performed individuated right hand movements. Each neuron was consistently related to at least 1 movement, although most neurons were related to multiple different finger and/or wrist movements. The sphere representing each neuron is centered at the location of the recorded neuron in the anterior bank of the central sulcus, with the hemispheric surface *above*, white matter *below*, lateral to the viewer's *right* and medial to the *left*. Each sphere is sized according to its greatest change in discharge rate during any of the movements; the white spheres at *left* constitute a scale from 0 to 200 spikes/s, with centers 1 mm apart. Each sphere representing a neuron is colored according to the movement for which that neuron's greatest discharge occurred: thumb, red; index finger, orange; middle, yellow; ring, green; little, blue; wrist, violet. Neurons best related to movements of each digit or the wrist were intermingled throughout the same cortical territory (from Schieber and Hibbard 1993).

Some years later, the same author (Schieber, 1999, 2001, 2002) developed the idea that two somatotopic gradients existed for the fingers that superimpose like a mosaic, leading

to various different combinations of fingers muscles activation, to allow a wide repertoire of muscle synergies (Fig. 1.2.4).

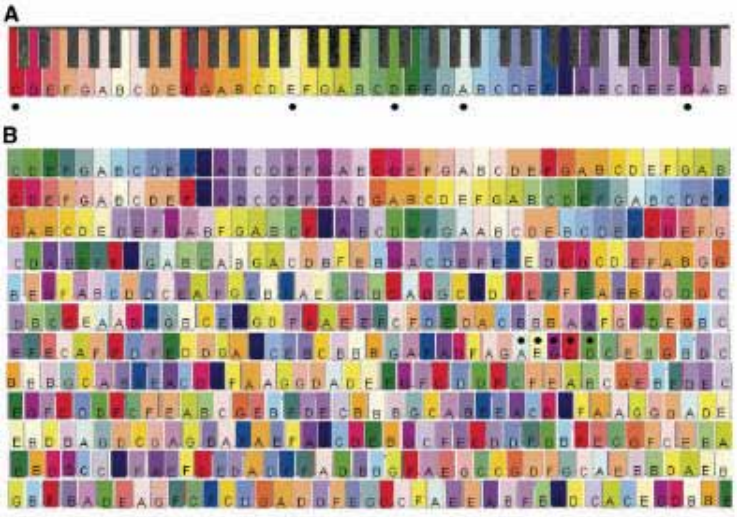


Figure 1.2.4: Cortical pianos. A: The standard piano keyboard constitutes a well-ordered map of musical notes (the somatotopic representation in M1), which limits the combinations of notes (the muscle contractions and movements). B: A nonstandard keyboard can be created by re-representing each note at multiple locations and in a wide variety of orders. In this way, M1 provides the capacity to generate a huge repertoire of movements, as well as the potential to generate previously unperformed movements, by accessing virtually any different combination of muscle contractions and body part movements with equal facility (from Schieber, 2001).

Park et al. (2001) showed that in macaca mulatta, the cortical map of the M1 hand region was organized in a concentric manner, with a central region corresponding to the distal muscles (wrist, fingers, hand), surrounded by a region in the form of a horseshoe, coinciding with the proximal muscles (shoulder, elbow). These two regions are separated by a co-facilitation zone, activating proximal as well as distal muscles. Similar results had already been obtained in long-tailed macaques by Sessle and Wiesendanger (1982), who showed multiple representations of a movement in the M1 hand area. A great proportion of neurons in the primary motor cortex are tuned to movements parameters of high order and of movements sequences and directions, which suggests a more abstract coding than a simple body map (Caminiti et al., 1990; Crowe et al., 2004; Georgopoulos et al., 1986, 1989; Kakei et al., 1999; Lu and Ashe, 2005; Reina et al., 200; Scott and Kalaska, 1995, 1997; Sergio and Kalaska, 2003). Neuronal correlates have been found for diverse variables including the speed, the force, the joint angle and the muscular activity (Cheney et al., 1985; Evarts, 1968; Georgopoulos et al., 1992; Holdefer and Miller, 200; Kakei et al., 1999; Li, Padoa-Schioppa et al., 2001; Reina et al., 2001).

The organisation of M1 is thus much more complex than initially described by Penfield (Penfield and Rasmussen, 1950; Penfield and Jasper, 1954). Indeed, it seems that the

neurons are tuned to a combination of movement parameters. This complexity allows probably the plasticity of the motor cortex (Nudo et al., 2001).

1.2.2 CORTICOSPINAL TRACT AND OTHER DESCENDING PATHWAYS

The neurons of layer V that make synapses with motoneurons via interneurons of the spinal cord, which themselves project on the muscular fibers, are named corticospinal neurons (Rouiller et Olivier, 2004), and this pathway is named corticospinal (Kandel et al., 2000). Retrograde tracing studies in the spinal cord of macaque monkeys revealed that corticospinal neurons are not only present in M1 but also in other motor cortical areas of the primate frontal lobe (Dum and Strick, 1991, 1996; Galea and Smith, 1994; Picard and Strick, 1996), namely in PM, SMA and CMA (Fig. 1.2.5). According to the classical theory, these fibers pass by the capsula interna, the midbrain and the pons, and about 80% of the corticospinal fibers cross the medial line at the level of the pyramidal decussation, corresponding to the lateral corticospinal tract, which controls the distal muscles, whereas 20% of these fibers project bilaterally, 10% decussating just before contacting their targets and 10% staying ipsilateral, constituting the ventral corticospinal tract that controls the axial muscles (Fig. 1.2.1). This means that the left hemisphere, respectively the right one, controls the right, respectively left, half of the body. The axons of the corticospinal neurons form the pyramidal tract, before making synapses with the interneurons, which will themselves make synapses with motoneurons. To note here that there are also corticospinal neurons in the primary somatosensory cortex (S1) and in the posterior parietal cortex, which terminate in the spinal cord dorsal horn (Fetz, 1968) and in the dorsal column nuclei (Bentivoglio and Rustioni, 1986), contrarily to the corticospinal neurons of the frontal lobe, which target the ventral horn and the intermediate zone of the spinal cord.

The singularity of the primates resides in their capacity to execute precise and rapid fingers movements, such as the opposition between the thumb and index fingers (Kandel et al., 2000), which is possible via the cortico-motoneuronal pathway (Lemon, 1993). This pathway forms a monosynaptic direct link between the corticospinal neurons and the motoneurons of the ventral horn of the cervical spinal cord controlling the distal muscles of the hand (Rouiller et al., 1998).

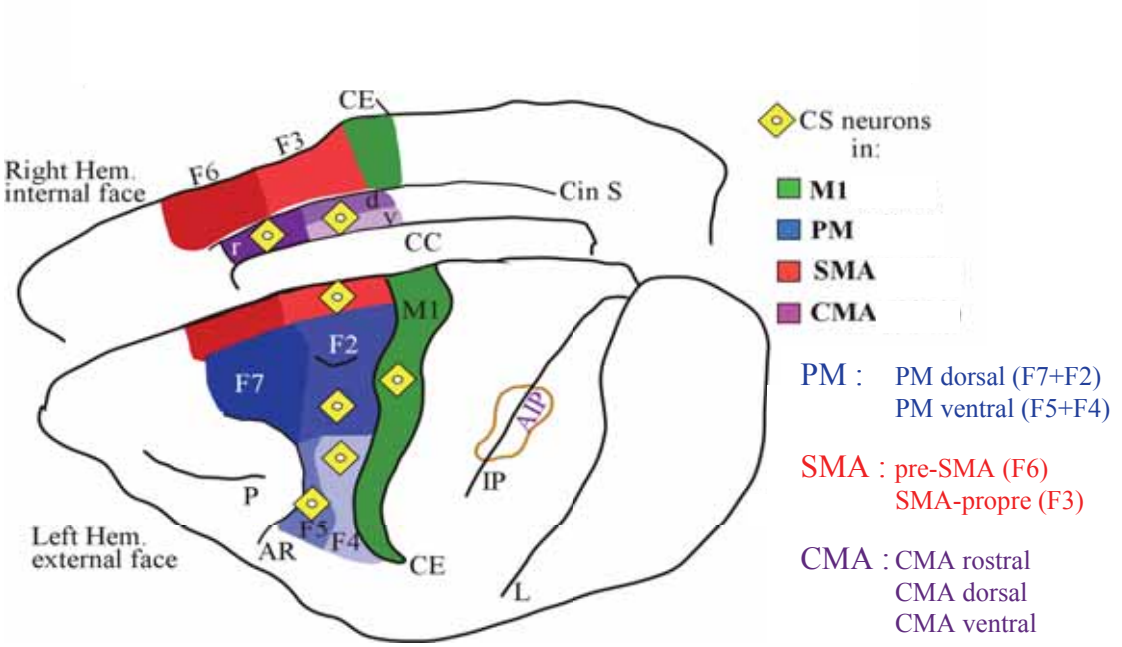


Figure 1.2.5: Origins of corticospinal projections in the frontal lobe of primates. M1: Primary motor cortex; PM: Premotor cortex; SMA: Supplementary motor area; CMA: Cingulate motor area; AIP: anterior intraparietal area; AR: arcuate sulcus; CC: corpus callosum; CE: central sulcus; CinS: cingulate sulcus; CS: corticospinal neurons; IP: intraparietal sulcus; L: lateral sulcus; P : sulcus principalis; F1 – F6: classification of motor cortical areas as proposed by Matelli et al. (1985, 1991); Area 4,6,7,23,24 classification of motor cortical areas as proposed by Brodmann (1909) (from www.unifr.ch/neuro/rouiller/teaching/post-graduate/slideshow/diapocadre1.html).

The second descending pathway of the lateral system (Fig. 1.2.6A) is the rubrospinal tract, which originates from neurons of the red nucleus, situated in the midbrain. This nucleus receives informations from the frontal lobe. In the course of the evolution of primates, a decrease of this indirect pathway occurred for the benefit of a more and more important management of the motor control via the corticospinal tract.

The other main efferent system is the ventromedial system (Fig. 1.2.6B), constituted by four tracts (among which the above mentioned ventral corticospinal tract) that originate in various regions of the brain stem, and which contribute essentially to the postural control and to reflex movements. The vestibulospinal tract originates from vestibular nuclei that receive informations from the internal ear (vestibular organ). It contributes to maintain the head in a correct position. The tectospinal tract originates from the superior colliculus, which receives visual informations from the retina, as well as somatosensory and auditory informations; this tract contributes thus to the orientation of the sight. Finally, the reticulospinal tract originates from the reticular formation, which receives informations from most sensorial systems and from other parts of the encephalon. This tract acts in coordinating automatic movements of locomotion and posture.

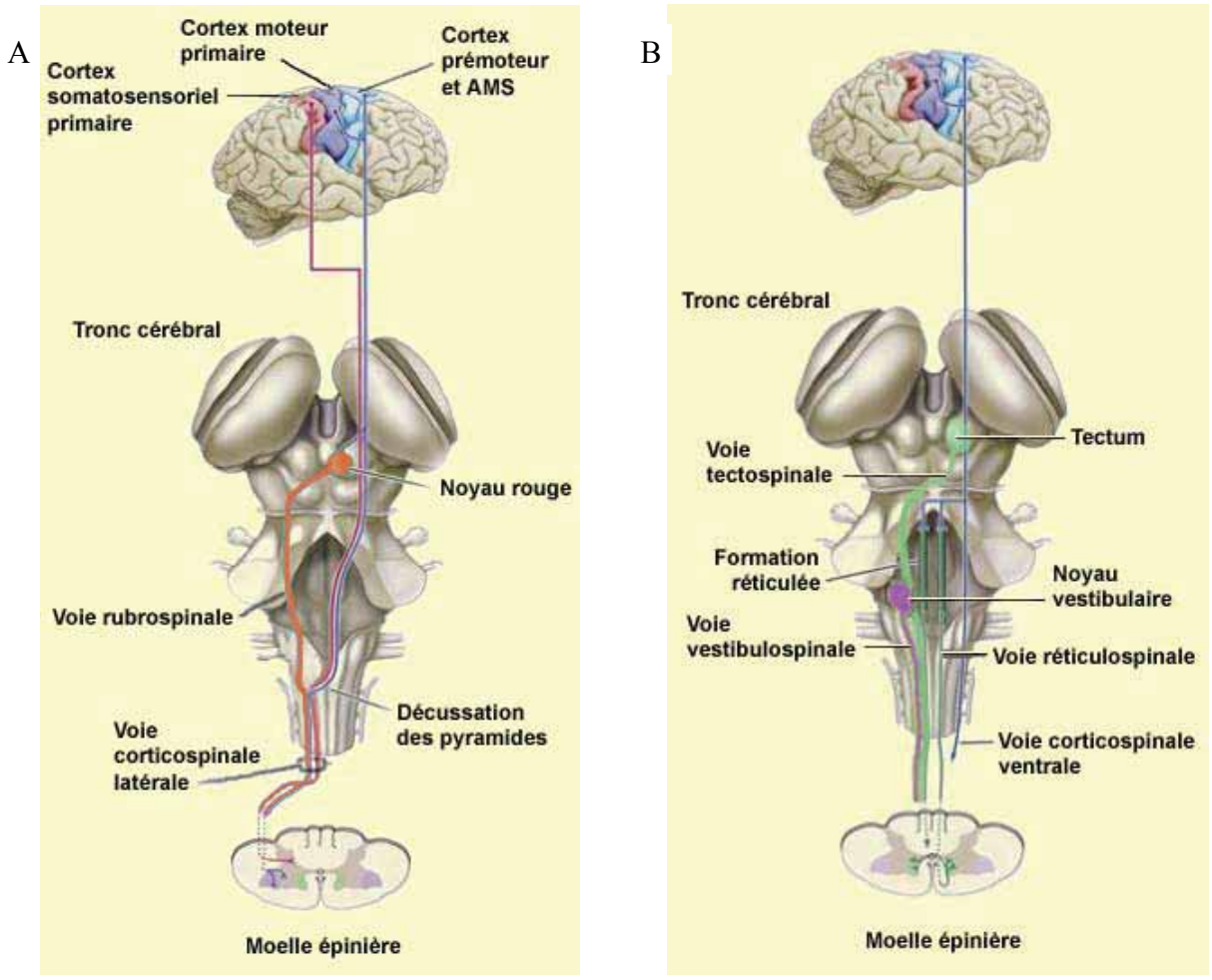


Figure 1.2.6: Lateral (A) and ventromedial (B) systems (from www.thebrain.mcgill.ca).

All these indirect pathways, particularly the cortico-rubrospinal pathway and the cortico-reticulospinal pathway, are also known to play a role in taking over some of the functions of damaged corticospinal projections after a lesion.

1.2.3 OTHER MOTOR STRUCTURES: CEREBELLUM AND BASAL GANGLIA

Other structures playing an important role in the motor execution and programming are the cerebellum and the basal ganglia.

The cerebellum (Fig. 1.2.7), which receives inputs from the cortex, the spinal cord and the vestibular organ, plays a role of control, coordination and adjustment of the movements. It is divided in the following three parts.

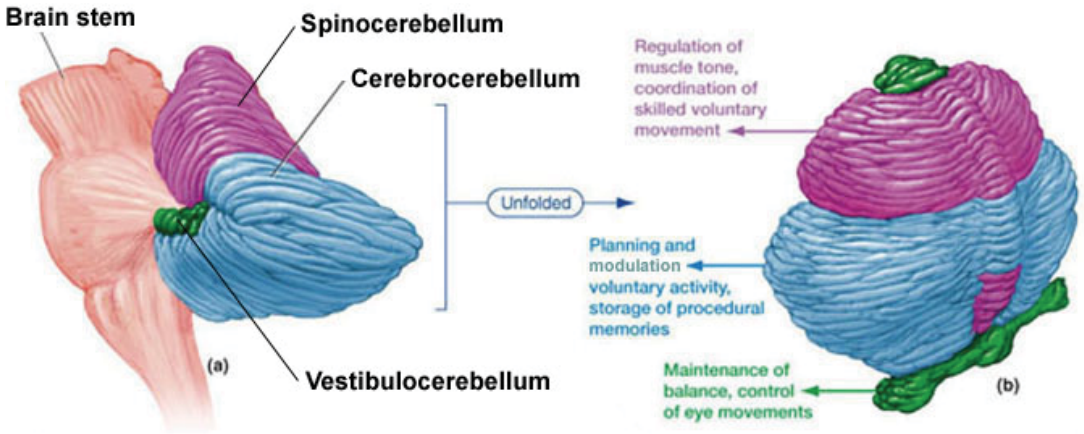


Figure 1.2.7: Cerebellum, subdivided in three parts playing specicif roles in motor control (from www.colorado.edu).

First, the archeocerebellum or vestibulocerebellum, which receives vestibular sensory information related to the equilibrium; the information is brought to Purkinje cells (GABAergic principal neurons of the cerebellum) through the climbing fibers. The cerebellar information goes from Purkinje cells to the spinal motoneurons through the vestibulospinal pathway. Second, the paleocerebellum or spinocerebellum, which controls the execution of movements and the postural tonus, receiving proprioceptive informations from the spinal cord. In return, the Purkinje cells send informations to motoneurons through two possible ways : the olivospinal (inferior olive) and the rubrospinal (red nucleus) pathways. Third, the neocerebellum or cerebrocerebellum, which is implicated in the planification and the control of voluntary movements. It receives motor information from the cortex through the pons, and in turn Purkinje cells send information that is received by the thalamus, which projects to the cortex and the striatum, itself projecting toward the red nucleus that sends the information to motoneurons.

The basal ganglia (Fig. 1.2.8) is a group of nuclei lying deep in the subcortical white matter of the hemispheres including i) the caudate nucleus and the putamen, which form the striatum, ii) the globus pallidus, iii) the substantia nigra, and iv) the subthalamic nucleus.

The motor components of the basal ganglia make a subcortical loop that links most areas of the cortex with pools of upper motor neurons in the primary motor and premotor cortex and in the brainstem. The neurons in this loop respond in anticipation of and during movements, and their effects on upper motor neurons are required for the normal initiation of voluntary movements.

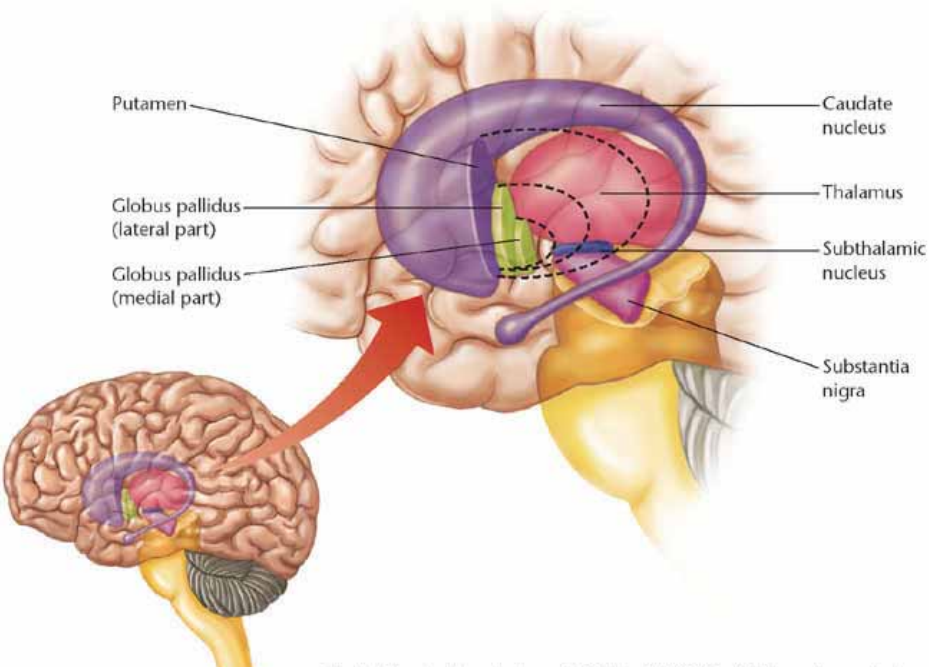


Figure 1.2.8: Nuclei of the basal ganglia (from www.colorado.edu).

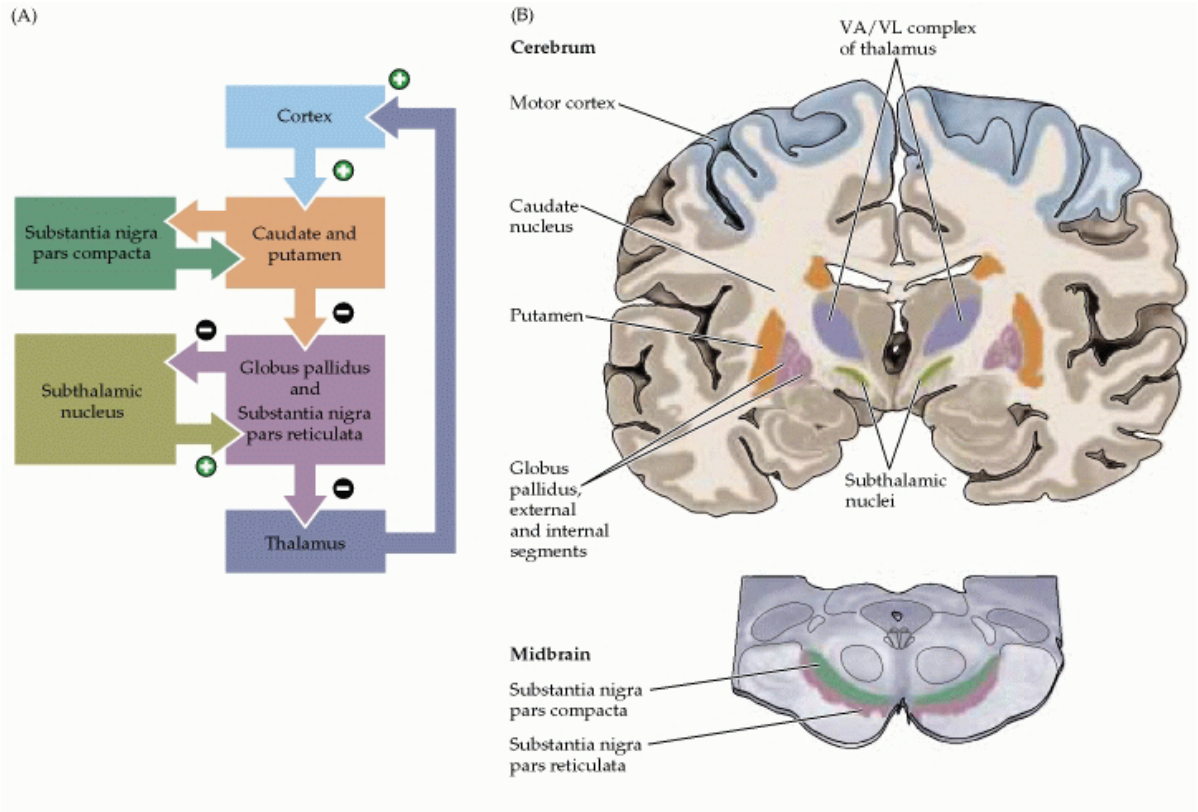


Figure 1.2.9: Motor components of the human basal ganglia. A. Basic circuits of the basal ganglia pathway: (+) and (-) denote excitatory and inhibitory connections. B. Idealized coronal section through the brain showing the anatomical locations of structures involved in the basal ganglia loops. Most of these structures are in the telencephalon, although the substantia nigra is in the midbrain and the thalamic and subthalamic nuclei are in the diencephalon. The ventral anterior and ventral lateral thalamic nuclei (VA/VL complex) are the targets of the basal ganglia, relaying the modulatory effects of the basal ganglia to upper motor neurons in the cortex (from www.ncbi.nlm.nih.gov).

The information input pathways take place in the striatum and the output pathways originate in the globus pallidus and pars reticulata of the substantia nigra (Fig. 1.2.9).

The caudate nucleus receives afferents from associative and multimodal cortical areas, whereas the putamen receives afferents from primary sensory areas. The striatum receives also dopaminergic afferents from the substantia nigra (pars compacta), which in turn receives projections from the striatum.

Two exit pathways can be distinguished: a direct one, which projects from the striatum to the internal globus pallidus, and an indirect one, which establishes a first relay on the external globus pallidus that projects in the subthalamic nucleus, which itself sends the information towards the internal globus pallidus. The indirect pathway apparently serves to modulate the disinhibitory actions of the direct pathway.

1.2.4 PREHENSION MOVEMENT

In the context of this work, the fine manual prehension of monkeys, using precision grip (opposition between the thumb and index fingers; Napier, 1956), will be assessed. Such movements were already described by Brinkman (1984; Fig. 1.2.10).

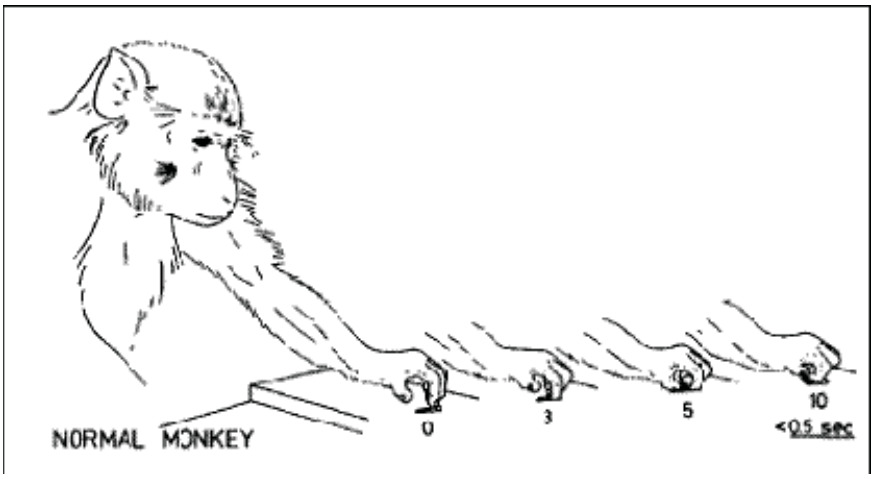


Figure 1.2.10 : Brinkman test. Sequences illustrating the succesion of movements realized by an intact macaque monkey to retrieve a food pellet using precision grip. Numbers below each sequence indicate the number of images since the beginning of the recording, with a resolution of 24 frames per second (from Brinkman, 1984).

The parameters of the movement, determined according to the visual information and the recognition of the objects, include spatial aspects, such as the direction in which the arm must be projected, the distance to cover or the trajectory to follow, as well as the force and the speed of the gesture.

At the beginning of the years 1980, Ungerleider and Mishkin showed that the cerebral circuits of the vision are organized in two distinct pathways originating from the primary visual area (Fig. 1.2.11), treating in parallel specific attributes of the visual information: i) the ventral pathway, which passes through the temporal lobe and of which neurons code for parameters that are intrinsic to the object, such as its size, its color, its form, ii) the dorsal pathway, which progresses through the parietal lobe and of which neurons code for the position of the objects and their move in the space.

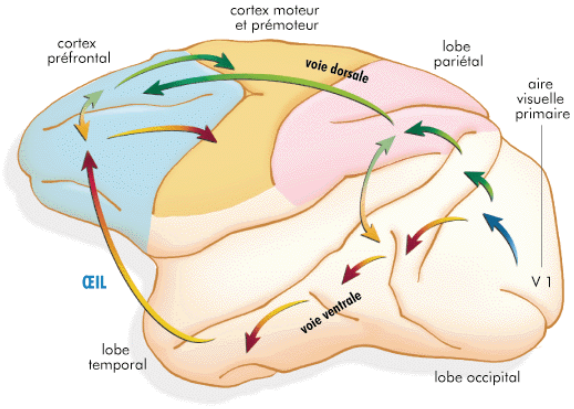


Figure 1.2.11 : Two distinct « classical » pathways (ventral and dorsal) leading from vision to action (from Boussaoud, 2003).

To guide the prehension and the manipulation of the objects, the brain has to detect, identify and localize the target. Some neurons of the parietal cortex seem to integrate informations to localize the targets in relation to the head, others take into account proprioceptive signals related to the head position in relation to the body axis, whereas neurons of the medial temporal area, situated at the junction between the parietal and temporal lobes, code for the direction and the speed of movement when the object is moving (Colby et al., 1993).

This classical schema according to which the visual information reaches the premotor cortex by indirect pathways implicating the prefrontal cortex (Boussaoud et al., 1996) has evolved. Indeed, the parietal areas are now considered as the main source of visual informations sent to the premotor cortex. Therefore, the above mentioned dorsal pathway itself is divided in two distinct components (Fig. 1.2.12; Tanne-Gariepy et al., 2002) : i) a medial pathway, which is specialized in the location of the targets and treats the spatial coordinates of the object, and which projects to PMd, and ii) a lateral pathway, which is

implicated in the treatment of the forms of the objects in order to manipulate them, and which projects to PMv. The neurons of the AIP region (anterior intraparietal) are responsive to shape, size, and orientation of the objects to be grasped, as well as for the context information related to hand grasping movements and manipulation of the hands themselves, both to viewed and remembered stimuli (Baumann et al., 2009; Jeannerod et al., 1995; Murata at al., 1996, 2000). It seems thus that these two pathways control the transport of the hand towards the object and the grasp of the object, respectively.

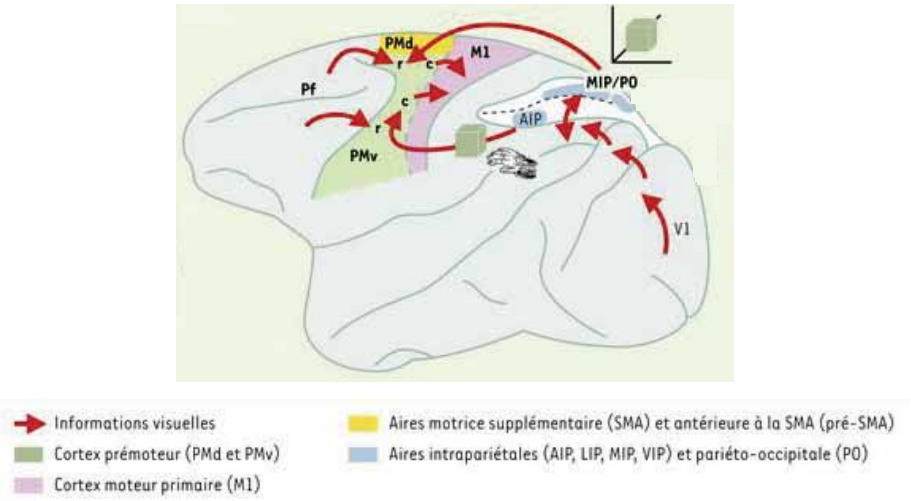


Figure 1.2.12 : The parieto-premotor pathways are organized in two components : one is specialized in the spatial coding (schematized by the green cube in a tridimensional space), and the other coding for the tridimensional characteristics of the object and for its manipulation (represented by a cube and a hand) (from Boussaoud, 2003**)**. Pf: prefrontal cortex; PMd et PMv : dorsal and ventral premotor cortex, respectively; r and c : rostral and caudal subdivisions, respectively ; SMA: supplementary motor area ; M1: primary motor cortex; AIP, LIP, MIP and VIP: anterior, lateral, medial and ventral intraparietal areas, respectively; PO: parieto-occipital area, (from Boussaoud, 2003).

Thus, the activity of the neurons in the parietal areas elicit that of premotor neurons that activate the primary motor cortex, from which the motor orders are sent to the spinal cord and then to the appropriate muscles.

To note that a direct access exists from the premotor cortex, which can send directly its commands in the spinal cord. Furthermore, the sensorimotor repertory involves the connections network implicating the basal ganglia.

1.3 BRIEF COMPARISON BETWEEN HUMAN AND MONKEY BRAINS

The human brain is 4.8 times the size of a hypothetical monkey of the same body weight (MacLeod et al., 2003). Indeed, the proportions of the human brain are not those that would be predicted by a plot of the changes in proportions in other primates as brain size

increases (Rilling, 2006). For example, the neocortex is 35% larger than predicted for a primate with a brain as large (Rilling and Insel, 1999). The prefrontal cortex forms 28.5% of the neocortex in the human brain but only 11.3% in the macaque brain (Elston, 2007). When looking at the degree of gyrification (Zilles et al., 1989), for which mammals that have smooth-surfaced or nonconvoluted brains are called "lissencephalics" and those that have folded or convoluted brains "gyrencephalics" (Hofman MA, 1985, 1989), monkeys show a clearly simpler sulcal pattern than humans (Fig. 1.3.1).

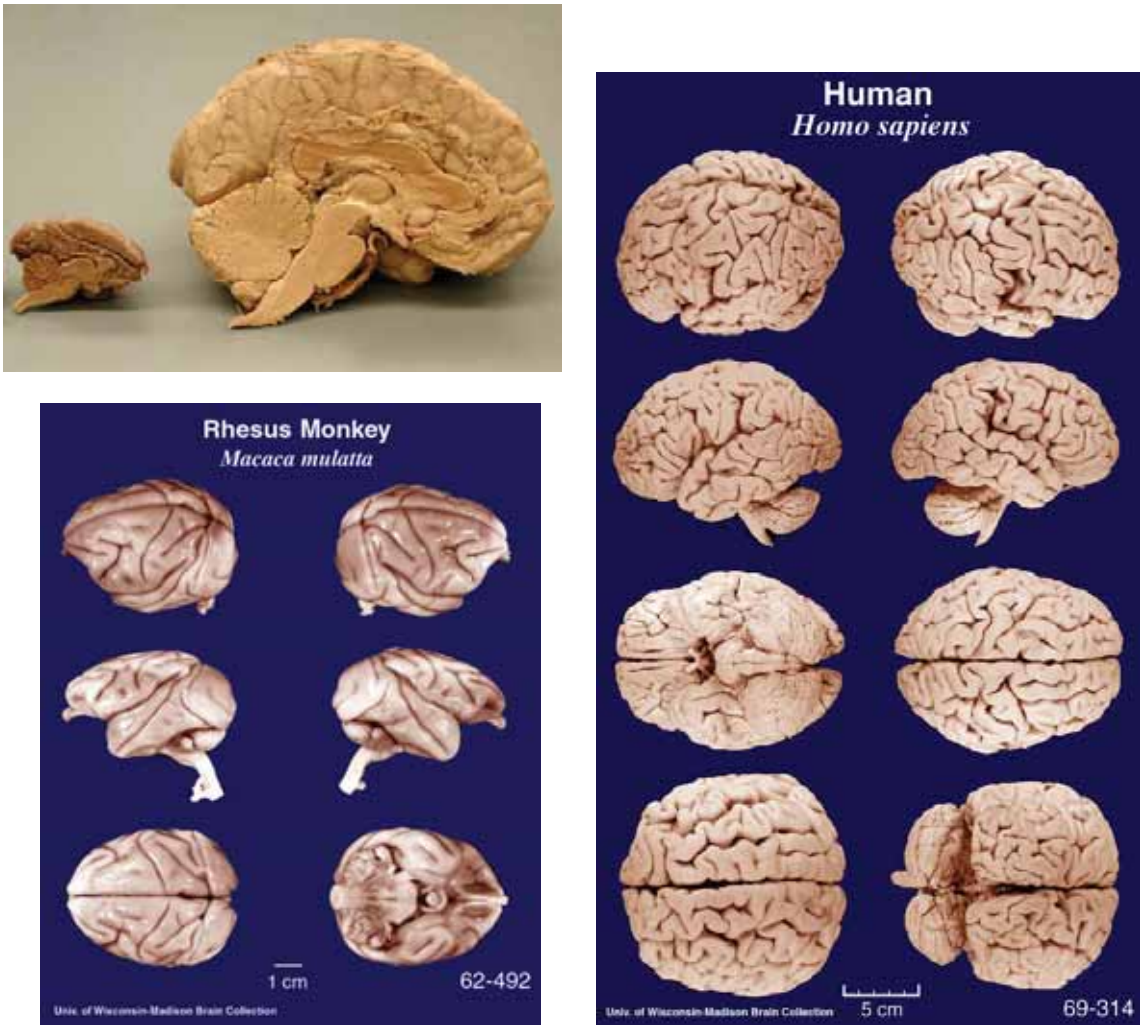


Figure 1.3.1 : Comparison between macaque and human brains. The human brain is proportionally greater, the degree of gyrification is more important in human, but despite some structural differences, the main strctures of the human brain and their complexity are present in macaques (from www.brainmuseum.org).

There are then consequential changes in the microstructure. For example, the maximum spine density of layer III pyramidal neurones in the prefrontal cortex is 70% greater in the human than in the macaque brain (Elston, 2007). To note that the value for the human brain is what would be predicted for a primate with a granular frontal cortex that was as large.

But not all the differences in microstructure are the result of differences in size. For example, Buxhoeveden et al. (2001) showed in the human brain an asymmetry in the size of the mini-columns in the posterior auditory association cortex within Wernicke's area between the left and right hemisphere that is absent in the monkey brain. There are also more magnopyramidal cells in the left rather than right superior temporal cortex in the human brain (Hutsler, 2003), and in the left rather than the right Broca's area (Hayes and Lewis, 1993). Other differences that have been discussed in relation to the human ability to reflect on one's own thoughts and those of others are the absence of monkey's homologue of the paracingulate area 32, which is activated when participants reflect on mental states (Amodio and Frith, 2006), and the absence of 'spindle cells' or 'von Economo neurones', found in human in the anterior cingulate cortex and anterior insula (Allman et al., 2002).

Nevertheless, the great similarity between monkey and human central nervous systems, of which complexity is not found in rodents, is the reason why monkey models are often used in research, particularly in neurosciences. For example, the neocortex, with its white matter, forms just 28% of the brain in the rat, compared with 72% in the macaque monkey and 90% in the human (Passingham, 2009). There are also many human abilities that are found in monkeys, allowing to give them the same tasks as to humans, such as visual conditioning tasks (Nixon et al., 2004; Toni et al., 2002), spatial working memory tasks (Funahashi et al., 1989; Leung et al., 2002), oddity tasks (Buckley et al., 2001; Lee, Buckley et al., 2006) or visual matching and non-matching rules (Bunge et al., 2003; Wallis et al., 2001). Furthermore, monkeys show the same cortical areas and a somatotopy comparable to humans (Fig., 1.3.2; Schieber, 2001).

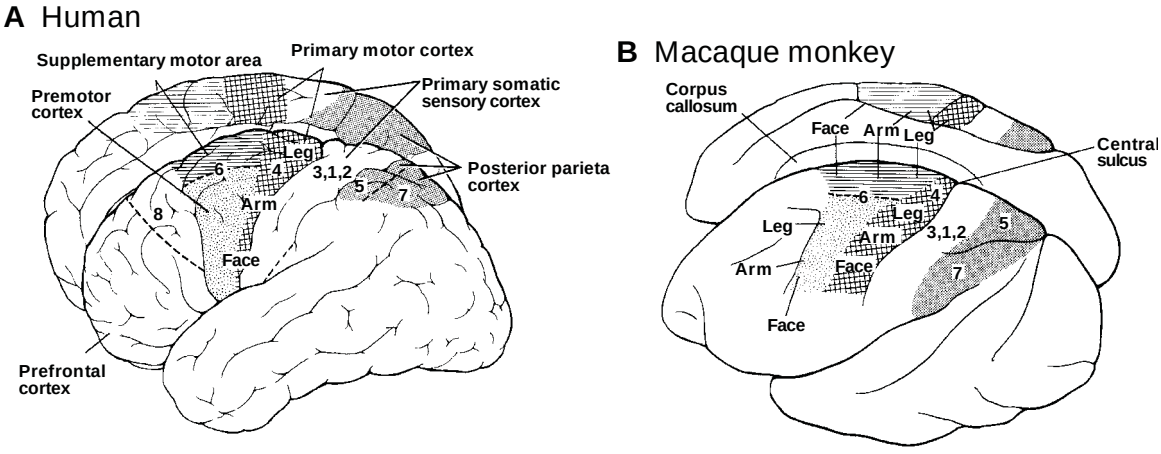


Figure 1.3.2 : Comparable organization and somatotopy of the cortical areas implicated in motricity between human (A) and macaque (B) (from Kandel et al., 2000).

These important similarities allow thus to develop models that permit to study various processes, in their normal and pathological forms, as well as to set new efficacious therapies (Nudo et al., 2001). In our context, the interest is directed towards the motor prehension, the cerebral plasticity, the reorganization and the recovery following a motor cortex lesion, as well as the impact of an autologous cell therapy on this recovery. Primates are the only animals that possess the corticomotoneuronal pathway that establishes a direct link between the motor cortex and the motoneurons, and which allows to achieve fine fingers movements, such as studied in the present context, where monkeys perform a task requiring precision grip (Napier, 1956). Indeed, there is a relationship between the development of the corticospinal tract and the emergence of fine motor control abilities (Lemon, 2008; Fig. 1.3.3).

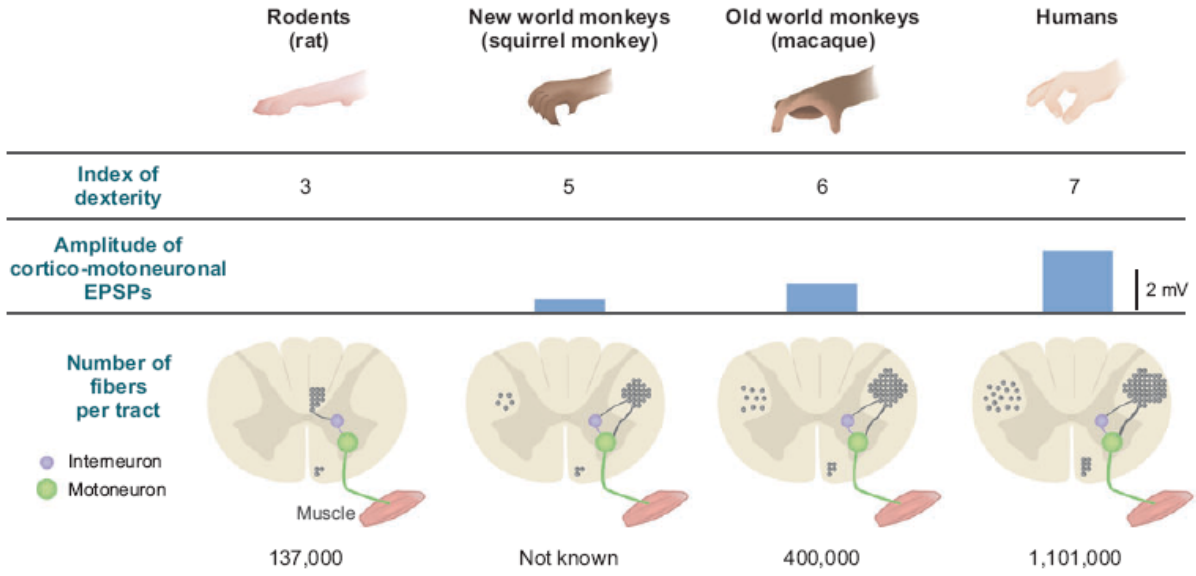


Figure 1.3.3: Relationship between the development of the corticospinal tract and the emergence of fine motor control abilities (from Courtine et al. 2007, in Lemon, 2008).

In rodents, there are no direct connections between corticospinal neurons and the cervical motoneurons which innervate forelimb muscles; brainstem pathways and spinal interneurons relay cortical input to motor neurons. Furthermore, most of the corticospinal tract fibers in rodents travel in the dorsal columns. In non-human primates and humans, direct corticospinal connections with motoneurons have evolved, together with an increase in the size and number of the corticospinal fibers. This is reflected by an increase in the size of the excitatory postsynaptic potential (EPSP) elicited by cortical neurons in hand motoneurons. Furthermore, the primate corticospinal tract is located mostly in the lateral columns, and a significant proportion of CS fibers (~10%) descend ipsilaterally. Development of the corticospinal tract correlates with the improvement in the index of dexterity, particularly in

the ability to perform finger-thumb precision grip (Courtine et al. 2007; Heffner and Masterton, 1983; Lemon, 2008).

Differences in the organization of CS projections across species may explain species dependent effects of CNS disorders (Lemon, 2008). Therefore, one should exercise caution in selecting which animal model should be adopted and in interpreting the results obtained (Courtine et al. 2007; Lemon and Griffiths, 2005; Lemon, 2008). Along this line, work on monkeys is essential for understanding the various mechanisms of the brain before testing whether the results can be generalized to the human brain (Passingham, 2009).

1.4 STROKE AND TRAUMATIC BRAIN INJURY

1.4.1 STROKE

Stroke is the most frequent cause of acquired handicaps at the adult age and affects between 12'000 and 14'000 persons in Switzerland, where it is the third mortality cause (after cardio-vascular diseases and cancers). With the lenghtening of life expectancy, one can expect an enhancement of these numbers. Between 80% and 85% of people survive from stroke, but will suffer from sequelas that could reach various degrees. Schematically, one third of them will stay dependent and require constant care, one third will recover a relative autonomy, whereas the last third recovers in a large scale its previous functional capacity (www.fragile.ch). Strokes can be ischemic infarcts (80%) or hemorragies (20%) (Fig. 1.4.1).

In ischemic stroke, the blood supply to a part of the brain is decreased, leading to dysfunction of the brain tissue in that area, due to four possible reasons: thrombosis, embolism, systemic hypoperfusion and venous thrombosis (Donnan et al., 2008; Shuaib et al., 1991; Stam, 2005). The brain tissue ceases to function if deprived of oxygen for more than 60 to 90 seconds, and after a few hours, it will suffer irreversible injury possibly leading to the death of tissue. Due to the collateral circulation, there is a spectrum of severity. Thus, part of the tissue may immediately die, while other parts may only be injured and could potentially recover (ischemic penumbra). A major cause of neuronal injury is the release of the excitatory neurotransmitter glutamate due to a dysfunction of ion pumps. This release of glutamate produces an influx of calcium which i) activates enzymes that digest the cell's proteins, lipids and nuclear material and ii) leads to the failure of mitochondria, which can lead further towards energy depletion and may trigger cell death due to apoptosis. Ischemia also induces a production of oxygen free radicals and other reactive oxygen species. These react with and damage a number of cellular and extracellular elements. Damage to the blood vessel lining or

endothelium is particularly important. Free radicals also directly initiate elements of the apoptosis cascade. Ischemia and infarction can also result in a loss of structural integrity of brain tissue and blood vessels, partly through the release of matrix metalloproteases, which are zinc- and calcium-dependent enzymes that break down collagen, hyaluronic acid, and other elements of connective tissue. Other proteases also contribute to this process. The loss of vascular structural integrity results in a breakdown of the protective blood brain barrier that contributes to cerebral edema, which can cause secondary progression of the brain injury (www.ninds.nih.gov/disorders/stroke/detail_stroke.htm).

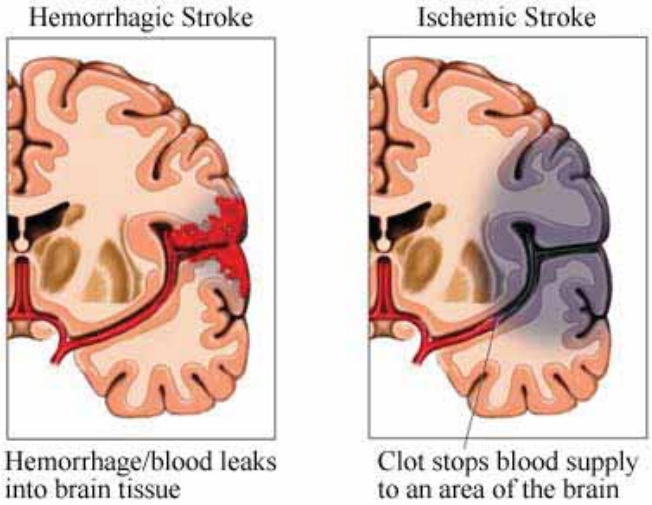


Figure 1.4.1: Hemorrhagic (accumulation of blood anywhere within the skull vault) and ischemic (decrease of blood supply to a part of the brain) stroke (from www.injuryboard.com).

In hemorrhagic stroke, there is an accumulation of blood anywhere within the skull vault, causing a tissue injury by compression. In addition, the pressure may lead to a loss of blood supply to the affected tissue with resulting infarction, and the blood released by the brain hemorrhage appears to have direct toxic effects on the brain tissue and vasculature (www.ninds.nih.gov/disorders/stroke/detail_stroke.htm).

1.4.2 TRAUMATIC BRAIN INJURY

Traumatic brain injury (Fig. 1.4.2) can be of various types, differentiated by the gravity degree and the origin of the lesions. Three gravity types are distinguished, namely light, mild and severe, which are determined according to the Glasgow scale measuring the conscience state of the person, and according to the coma duration consecutively to the accident. Between 3’000 et 5’000 persons per year are victim of cranio-cerebral trauma in Switzerland (www.fragile.ch). At least 60% are due to circulation accidents, the other being

consequent to sport, hobby and professional accidents. Most victims are men (80%), particularly young men (more than 50% of them are less than 30 years old). One third of the victims suffer from severe brain trauma, of which the possible consequences are paralyses, motricity disorders (spasticity, apraxia…), sensory disorders (disorders of the touch, insensibilité to the cold and the warm…), language disorders (aphasia), elocution and deglutition disorders (dysarthrie), equilibrium disorders and epilepsia.

Brain trauma can be caused by a direct impact or by acceleration alone. In addition to the damage caused at the moment of injury, brain trauma causes secondary injury, a variety of events that take place in the minutes and days following the injury. These processes, which include alterations in cerebral blood flow and pressure within the skull, contribute substantially to the damage from the initial injury.

Figure 1.4.2: Brain trauma (from www.ehow.com).

Primary brain injury results immediately from the initial trauma (Collins and Dean, 2002) and includes contusion, damage to blood vessels, and axonal shearing, in which the axons of neurons are stretched and torn (Rehman et al., 2008). The blood brain barrier and meninges may be damaged in the primary injury, and neurons may die (Blissitt, 2006). Cells are killed in a nonspecific manner in primary injury (Hannay et al., 2004).

Brain damages (see Fig. 1.4.3 for the cellular level deficits) are rather caused by secondary injury, a complex set of cellular processes and biochemical cascades that occur in the minutes to days following the trauma (Xiong et al., 2000). These secondary processes can dramatically worsen the damage caused by primary injury (Park et al., 2008) and account for the greatest number of traumatic brain injury deaths occurring in hospitals (Ghajar, 2000). Secondary injury events include damage to the blood-brain barrier, release of factors that cause inflammation, free radicals overload, excessive release of the neurotransmitter

glutamate (excitotoxicity), influx of calcium and sodium ions into neurons, and dysfunction of mitochondria. Injured axons in the brain's white matter may separate from their cell bodies as a result of secondary injury (Park et al., 2008), potentially killing those neurons. Other factors in secondary injury are changes in the blood flow of the brain, ischemia, cerebral hypoxia, cerebral edema, and raised intracranial pressure that can cause cell death (Scalea et al., 2007), as well as meningitis and brain abcess (Maas et al., 2008).

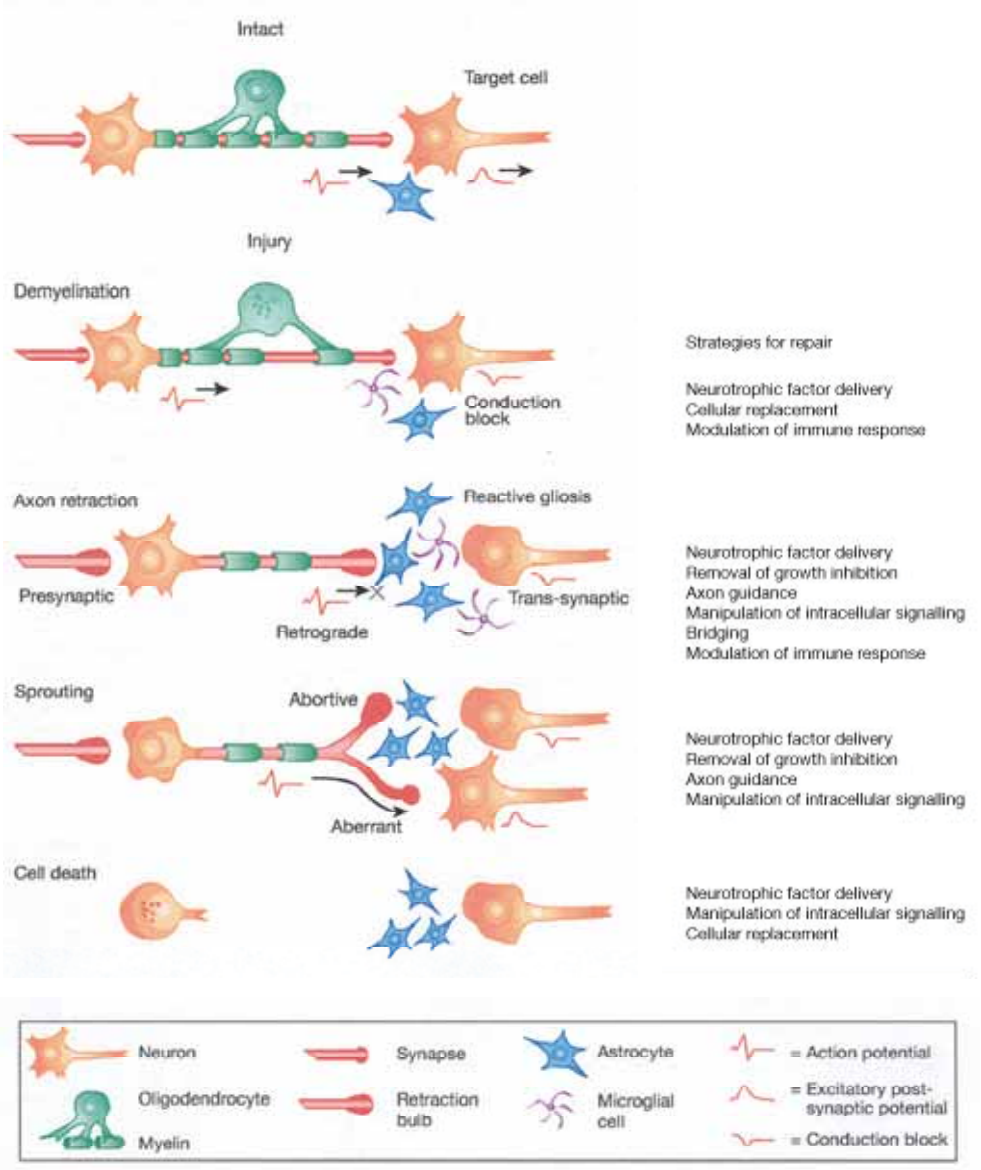


Figure 1.4.3: Various cellular structural and functional deficits following brain damages. Demyelination: A demyelinated axon may maintain both its afferent and efferent connections but, due to a loss of myelination, poor or failed conduction results. Axonal retraction: Injury to an axon itself or to the original cellular target of the axon can result in degeneration. Presynaptic, retrograde and transsynaptic degeneration can occur. Synaptic conduction across a pathway is lost and a reactive cellular response, including astrocytes and microglia, forms. Sprouting: Axonal sprouting has been described for surviving neurons. It is typically abortive when a sprouting axon encounters an inhibitory matrix or scar, loss of neurotrophin support, or the presence of continuing inflammation or toxicity. Aberrant sprouting can occur when an axon reconnects to an inappropriate target. Synaptic conduction is restored but this pathway does not result in functional restoration. Cell death: When a neuron is completely deprived of its source of growth factors and exposed to high levels of toxic molecules or inflammatory attack, it can undergo cell death (from Horner and Gage, 2000).

Figure 1.4.3 illustrates the various cellular structural and functional deficits that can occur following brain damages, namely i) demyelination, ii) axonal retraction, iii) presynaptic, retrograde and transsynaptic degeneration, iv) abortive or aberrant sprouting, and v) cell death; these phenomena are generally accompanied by a reactive cellular response, implicating astrocytes and microglia.

Since the cascade of injury is mostly complete within 24-48 hours, neuroprotective treatment, to be as most effective as possible, should be started within 3-6 hours after the injury. In practice, this is difficult. Later treatment is restorative, aiming to promote repair processes such as angiogenesis, neurogenesis, and synaptogenesis. Neurorestorative treatment can begin later than neuroprotective treatment and therefore many more stroke patients could potentially benefit from it (Table 1.4.1).

Neuroprotection	Goal	↓ Cell dysfunction/death caused by secondary cascades	
	Examples	Antiapoptotic molecules, free radical scavengers, antiexcitotoxic molecules, trophic factors	
Neuroregeneration	Goal	↑ Plasticity (reorganization of neuronal circuits)	↑ Axonal growth to reconnect brain with target cells
	Examples	Trophic support, enhance neurogenesis	Trophic support, ↓ inhibitory components of glial scar, physical guidance channels with chemical cues

Table 1.4.1: Therapeutic strategies for regeneration and repair in the injured CNS: neuroprotection (earlier) and neuroregeneration (later) (from Molly et al., 2008).

1.4.3 MOTOR REHABILITATION

The sequelas of a stroke or a traumatic brain injury will of course depend on the cerebral regions affected and their degree and duration are difficult to predict. The rehabilitation is performed on different levels, including neuropsychology, ergotherapy, physiotherapy and logopedy. The motor rehabilitation itself contains two main types of approaches, namely the standard motor rehabilitation and the intensive motor rehabilitation (Schaechter, 2004).

The standard motor rehabilitation involves an eclectic mix of approaches, including neurofacilitation techniques (combining several approaches aimed at retraining motor control

by promoting normal or inhibiting abnormal movement), task-specific training (aimed at improving skill in performing selected movements or functional tasks) and task-oriented training (aimed at retraining functional tasks by taking into account the interplay of many systems, including the musculoskeletal, perceptual, cognitive and neural systems). The intensity of standard motor rehabilitation is typically about 30–60 min per day early after stroke, and tends to decrease later after stroke. The period of time during which stroke patients receive motor rehabilitation depends on their degree of impairment and functional deficits, but it usually does not continue for more than 6 months. Under these typical conditions of post-stroke rehabilitative care, recovery of motor function has been observed to be most rapid during the first month post-stroke, to slow down during subsequent months, and to reach a plateau at about 6 months post-stroke (Hendricks et al., 2002). Initial stroke severity substantially shifts this time course, such that stroke patients with milder motor deficits usually exhibit a truncated time course of motor recovery. After completing standard rehabilitation, approximately 50–60% of stroke patients still experience some degree of motor impairment (Hendricks et al.,2002), and approximately 50% are at least partially dependent in activities-of-daily-living (Gresham et al., 1995).

For its part, the intensive motor rehabilitation has been shown to favourably impacts motor recovery in hemiparetic stroke patients. Meta-analyses and systematic reviews have concluded that greater intensity of therapy, provided by increasing the amount of therapy during the post-stroke period, modestly improves functional outcomes in stroke patients (Kwakkel et al., 1997; Steultjens et al., 2003; Teasell et al., 2003). Other authors having reviewed the post-stroke rehabilitation literature suggested that the efficacy of post-stroke motor therapy is related to the degree to which the neuromuscularsystem is challenged by repetitive volitional movement (Woldag and Hummelsheim, 2002; Richards and Pohl, 1999; Woldag and Barreca et al., 2003). Several rehabilitation approaches considered have shown efficacy in improving motor function after stroke. For example, lower limb strength and function have been shown to be increased by progressive aerobic conditioning (Smith et al., 1999; Teixeira-Salmela et al., 1999), resistance training (Teixeira-Salmela et al., 1999; Weiss et al.,2000), and circuit training (Dean et al., 2000). Upper limb motor control and function have been shown to be improved by constraint-induced movement therapy (Kunkel et al., 1999; Kwakkel, 2006; Miltner et al., 1999; Taub et al.,1993; Wolf et al., 2006; to note here that application of CIMT very early after stroke at high intensity can be deleterious, as shown by Dromerick et al., 2009), bilateral training (Altschuler et al., 1999; Hesse et al., 2003; Mudie and Matyas, 2000; Whitall et al., 2000), robot-assisted training (Aisen et al., 1997;

Fasoli et al., 2003; Lum et al., 2002; Volpe et al., 2000), and virtual reality training (Fischer et al., 2007; Holden et al.,2001; Johnson-Frey, 2004; Merians et al., 2002, 2006; Mulder, 2007; Page et al., 2007; Sharma et al., 2006).

Furthermore, it seems that certain intensive therapies are more beneficial than others to particular patient subgroups. For example, constraint-induced movement therapy, a training that aims to overcome learned-non use of the stroke-affected limb (Taub, 1993), was shown to produce greater gains in dexterity and functional use of the affected upper limb of chronic stroke patients with sensory disorder or hemineglect, respectively, than a neurodevelopmental therapy-based training of equal amount of time (van der Lee et al., 1999). In severely impaired stroke patients, greater improvements in motor function of the affected upper limb were induced by bilateral training, in which the unaffected limb simultaneously practiced selected functional tasks, as compared to unilateral practice of the same functional tasks (Mudie and Matyas, 2000). In contrast, in mildly impaired stroke patients, bilateral training of selected motor tasks tended to be less efficacious in improving aiming movements of the affected upper limb than unilateral training of the same motor tasks (Platz et al., 2001).

Additionnally to these rehabilitative approaches, transcranial magnetic stimulation (TMS) has also proven some efficacy in helping recovering from stroke. Indeed, electromagnetic stimulation can modulate a number of functions and behaviours and in this sense repetitive TMS can have inhibitory or excitatory effects on cortical activity (Valero-Cabre et al., 2007). As such, goals can include increasing activity in ipsilesional cortical regions that are underactive (Khedr et al., 2005; Kim YH et al., 2006), or in contralesional cortical regions that are overactive and a source of potentially harmful inhibition (Fregni et al., 2006). Transcranial direct current stimulation has also shown promise in initial studies and epidural motor cortex stimulation can also improve motor function after stroke (Webster et al., 2006). In these approaches, brain mapping studies might be useful to direct the site of stimulation (Cramer et al., 2005).

Nevertheless, despite the efficiency of various rehabilitative approaches, a large amount of patients having suffered from stroke or brain trauma show remaining deficits that affect their every day life. It is thus of great importance to understand the brain plasticity specific processes mediating motor recovery occurring spontaneously as well as promoted by rehabilitation, in order to develop rehabilitative and therapeutic methods to improve the recovery of these patients (Nudo, 2001).

1.5 PLASTICITY OF THE CENTRAL NERVOUS SYSTEM

A major recent finding of the neurosciences is the plasticity of the adult mammalian brains, namely their ability to reorganize according to the practice of activity or not, the learning or a lesion of the nervous system (see for review Nudo et al., 2001). Although some authors evoked this idea early (ex. Kalaska and Pomeranz, 1979; Wall and Egger, 1971), the first clear demonstration of the reorganization of the adult primate brains following a peripherical nervous lesion was shown by Merzenich et al. (1983a,b), who found that following a deprivation of normal inputs in the hand representation area in the primary somatosensory cortex (area 3b) by cutting off one peripherical nerve in the hand, the neurons of the deafferented region began to respond to stimulations of body parts normally innervating adjacent territories. Since then, an amount of studies have expanded this finding to the visual, auditory and motor systems, to subcortical regions including nuclei in the thalamus, and to other mammalian species (Donoghue et al., 1990; Jain et al., 1997, 1998; Nudo et al., 1990; Nudo and Milliken, 1996; Pons et al., 1988; Sanes et al., 1988, 1992), as well as after amputation of a limb (Chen et al., 1998; Florence and Kaas, 1995).

Observations were also conducted in humans by surface recordings and imaging techniques (Flor et al., 1995). To practice movements has been found to result in an increase of the performance and the reorganization of the motor cortex (Karni et al., 1995; Pascual-Leone et al., 1994) and motor learning was demonstrated to be dependent on plasticity (Donoghue et al., 1996).

The plasticity can play a beneficial role in the functional recovery following a lesion of the central nervous system (Bütefisch et al., 1995; Nudo, Milliken et al., 1996). In the affected hemisphere, a possible mechansism implicated in the recovery is the reorganization of the lesioned area and the management of the lesioned area's function by the undamaged adjacent tissue (Rouiller et al. 2004). Indeed, Rouiller et al. (Liu and Rouiller, 1999; Rouiller et al., 1998) and Nudo et al. (2001) showed that after a small unilateral lesion of M1, the partial recovery of the contralesional hand's dexterity that took place was related to the intrinsic reorganization around the lesion of the primary motor cortical map, whereas a larger unilateral M1 lesion would lead to the management of the lesioned function by other non primary motor cortex regions, such as PM (Rouiller et al., 1998 ; Rouiller and Olivier, 2004). Dancause et al. (2005) described a specific form of neuroanatomical reorganization distant from infarct, whereby primates with primary motor cortex injury produced a novel projection from ventral premotor cortex to somatosensory cortex.

Functional imaging studies have also shown a functional reorganization of the cortical representations. For example, a systematic posterior shift of the activation was observed after recovery from stroke (Carey et al., 2002; Pineiro et al., 2001). Furthermore, it has been demonstrated that following a stroke in the motor cortex, an activation linked to the sensorimotor activity took place in the somatosensory cortex (Cramer et al., 1997).

When the lesion affects a wide motor cortex region, the contralateral motor cortex seems to play a role in the recovery (Nudo et al., 2001; Rouiller and Olivier, 2004). Neuroimaging studies in stroke patients showed an increased recruitment of the unaffected hemisphere during paretic hand movements (Cao et al., 1998; Chollet et al., 1991; Cramer et al., 1997; Weiller et al., 1992). Furthermore, the use of the TMS technique induced an increase of the excitatory activity in the unaffected motor cortex of stroke patients with good recovery, a phenomenon that was not observed in those having a poor recovery (Bütefisch et al., 2003). The type of relation between motor function enhancement and contralateral cerebral activation related to the movement in the fMRI stays blur, as far as some studies showed an association between the enhancement of the motor function and an increase in the contralateral motor area activation (Johansen-Berg et al., 2002) by moving the affected limb, whereas others found a negative correlation between the amount of contralateral motor cerebral activation and the outcome (Calautti et al., 2007; Ward et al., 2003) or the enhancement of the function following a therapy (Wittenberg et al., 2003; Calautti and Baron, 2003).

The rehabilitation following a stroke seems to play an important role in the plasticity. In adult monkeys, the effect of the affected limb training on the reorganization following small lesions in the hand area of M1 was studied by Nudo and Milliken (1996). In monkeys who did not receive any post-infarct behavioural training, the representation of the unaffected hand decreased in size, whereas in monkeys receiving such training, there was a retention of the representation of the unaffected hand. In some cases, the hand territory was enlarged on the elbow and shoulder representation (Nudo, Wise et al., 1996). In humans, the motor training of the proximal arm when the forearm was deafferented resulted in a remarkable increase in the cortical representation of the muscle implicated in the use of the proximal arm (Ziemann et al., 2001). In stroke patients, the deafferentation of the proximal arm potentiated the enhancement induced by the training of the manual function and the increases in the cortical representation of the hand muscles (Muellbacher et al., 2002). Another example is the constraint-induced movement therapy, which encourages the use of the affected limb by constraining the unaffected limb (Taub et al., 1993) and which was shown to enhance the

motor function of patients having had a stroke (Wittenberg et al., 2003) and to induce a shift of the motor cortical map (Liepert et al., 1998; Traversa et al., 1997; Wittenberg et al., 2003).

The plasticity of the adult brain as potential therapeutic tools opened thus new perspectives, although the mechanisms that underly it, that seem to be multiple, are not totally understood. Nevertheless, the motor plasticity mechanisms are linked to the concept that the motor cortex contains multiple representations that overlap (Donoghue et al., 1992; Sanes et al., 1995; Schieber and Hibbard, 1993), functionally connected through a wide horizontal network (Huntley and Jones, 1991). The main mechanisms suggested to mediate the reorganization in the cerebral cortex implicate the unmasking of existing, but latent horizontal connexions (Bütefish, 2004; Sanes and Donoghue, 2000) and the modulation of the synaptic efficacy such as the long term potentiation (LTP[1]) (Hess and Donoghue, 1994; Hess et al., 1994; Hess et al., 1996) or the long term depression (LTD[2]) (Hess and Donoghue, 1996). These mechanisms, which include also the synaptogenesis and the synaptic withdrawal, as well as the dendritic arborisations growth or axonal sprouting (Fig.1.5.1; Darian-Smith and Gilbert, 1994; Jain et al., 2000), could reveal preexisting inputs that are normally not expressed (Jain, 2002). Such a modification of the synaptic efficacy was demonstrated in the horizontal connexions in the motor cortex of rats trained to perform a skilled motor task (Rioult-Pedotti et al., 1998). Other mechansisms comprise also an increased activation and migration of endogenous neural stem cells (see the following chapter about endogenous neurogenesis), and changes in glia, inflammation, and angiogenesis (Carmichael, 2006; Komitova et al., 2006; Nudo, 2007). Genetic factors probably also have important influences on post-stroke repair. For example, brain-derived neurotrophic factor (BDNF) is among the most abundant growth factors in mammalian brain, being necessary for many neuronal functions. Kleim et al. (2006) found that individuals with the BDNF val66met polymorphism (a single nucleotide polymorphism producing a valine to methionine amino acid substitution

[1] Long-term potentiation (LTP) is a long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously and changes thus the strength of the synapses. LTP improves the postsynaptic cell's sensitivity to neurotransmitter in large part by increasing the activity of existing receptors and by increasing the number of receptors on the postsynaptic cell surface (Winder and Sweatt, 2001).

[2] Long-term depression (LTD) is an activity-dependent reduction in the efficacy of neuronal synapses lasting hours or longer. LTD is thought to result mainly from changes in postsynaptic receptor density, although changes in presynaptic release may also play a role. LTD is one of several processes that serves to selectively weaken specific sets of synapses in order to make constructive use of synaptic strengthening caused by LTP. This is necessary because, if allowed to continue increasing in strength, synapses would ultimately reach a ceiling level of efficiency, which would inhibit the encoding of new information (Winder and Sweatt, 2001).

at codon 66) in one or both alleles exhibited significantly impaired short-term experience-dependent motor cortex plasticity. Given the importance of cortical plasticity to behavioral recovery after stroke, this finding suggests that this polymorphism might have an important influence on behavioral outcome after stroke. Siironen et al. (2007) found that the presence of this polymorphism was associated with a poorer outcome after subarachnoid hemorrhage, supporting this hypothesis.

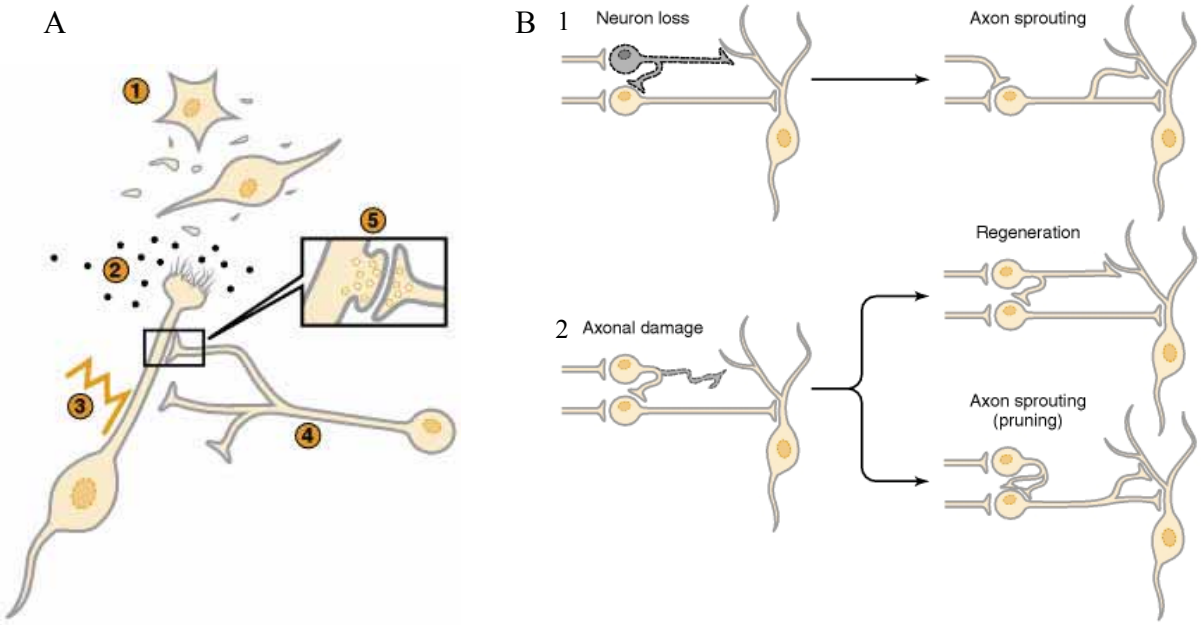


Figure 1.5.1: A. Stages in the mechanisms of axon sprouting and reactive synaptogenesis. The CNS has to face the complex problem of clearing the damage as the circuitry is being rebuilt and remodeled. 1 : Microglia and astrocytes clear the products of degeneration. 2 : Neurite sprouting factors are needed. 3 : Cell adhesion molecules and extracellular matrix support growth. 4 : Mechanisms must operate to specify the target. 5 : The appropriate machinery to create new functional synapses needs to be mobilized. Many of these mechanisms are similar to those essential for normal development. B. After injury, the CNS can mobilize a number of responses. 1: If neurons degenerate, the natural response is that the axons of healthy neurons sprout and form new connections to replace those of the lost neurons, a process referred to as axon sprouting. This process includes the branching and growth of axons and formation of new synapses in reaction to an abnormal stimulus such as an injury, termed reactive synaptogenesis. 2: When an axon is severed, the ideal response would be for the axon to regenerate, but in the adult CNS this does not occur successfully in the absence of interventions. Indeed, in the adult CNS, neurite growth inhibitors contribute to the absence of regenerative fiber growth (Schnell and Schwab, 1990).The damaged cell mobilizes a growth response, such as increased collateralization on available target cells, termed pruning. Thus, in either case, the circuitry can be rebuilt, particularly when homologous cells are available. In other cases, the incorrect inputs can be formed and only regeneration will restore function (from Carl, 1999).

The neurotransmitter systems implicated in the mediation of many of these plasticity mechanisms include the GABAergic inhibitory system (Brasil-Neto et al., 1993; Chen et al., 2002; Donoghue et al., 1990; Garraghty et al., 1991; Hess et al., 1996; Jacobs and Donoghue, 1991; Levy et al., 2002; Nudo et al., 2001; Sanes et al., 1988; Schnitzler and Benecke, 1994; von Giesen et al., 1994; Witte, 1998; Ziemann et al., 2001), as well as the glutamatergic

excitatory system with the activation of the N-methyl-D-aspartate receptors (NMDA) (Hess et al., 1994, 1996; Nudo et al., 2001; Rema et al., 1998; Witte, 1998).

However, direct causal relations between the changes in the topographic maps and some of these mechanisms, which are partly activity-dependent (Jones and Schallert, 1994; Jones et al., 1996; Kandel et al., 2000 ; Nudo et al., 2001; Rouiller and Olivier, 2004; Stroemer et al., 1995), are lacking. A better understanding of these processes might assist in defining therapeutic targets for improving poststroke brain repair in humans via pharmacologic (Kolb et al., 2007; Li et al., 2007; Ramic et al., 2006; Schabit et al., 2007; Schneider et al., 2007; Tsai et al., 2006), cell-based (Horita et al., 2006; Zhang and Pardridge, 2006), immune-based (Papadopoulos et al., 2006; Ziv et al., 2007), gene transfer (Shimamura et al., 2006), and physical (Ramanathan et al., 2006) therapeutic approaches.

To note here that stroke patients often show compensatory movements instead of a real recovery (Nudo et al, 2001). Furthermore, the cortical plasticity depends on factors such as the extent to which eloquent neocortical areas are injured (Heiss and Thiel, 2006; Talelli et al., 2006), the location of the injury (Hamzei et al., 2006), the size of the lesion and the age of the patient (Rouiller and Olivier, 2004), as well as the type of rehabilitative training (Cramer and Riley, 2008; Rouiller and Olivier, 2004) or the degree of environmental enrichment (Hicks et al., 2007).

1.6 NEUROGENESIS AND NEURORESTORATIVE CELL THERAPIES

1.6.1 ENDOGENOUS NEUROGENESIS

The dogma that cells of the central nervous system (CNS) were unable of regeneration was challenged in the early 1960s when neurogenesis was first described in the adult mammalian brain by Altman (Altman and Das, 1965; Altman, 1969). These results were replicated using tritiated thymidine labelling followed by electron microscopy by Kaplan (Kaplan and Hinds, 1977; Kaplan, 1981), although these studies were largely ignored. The pioneering work of the Nottebohm laboratory (Goldman and Nottebohm, 1983; Nottebohm et al., 1990; Nottebohm, 2004), showing the functional relevance of adult neurogenesis in songbirds during the spring mating season, was followed by Reynolds and Weiss (1992) reporting the isolation of precursor cells from the adult mouse brain and their differentiation into neurons in vitro.

Many studies (e.g. Arias-Carrión et al., 2004; Eriksson et al., 1998; Gould, Reeves, Fallah et al., 1999; Gould, Reeves, Graziano et al., 1999; Johansson et al., 1999; Snyder and Vescovi,

2000; Zhao et al., 2003) further confirmed the presence of neurogenesis in the adult brain of rodents, primates, and humans.

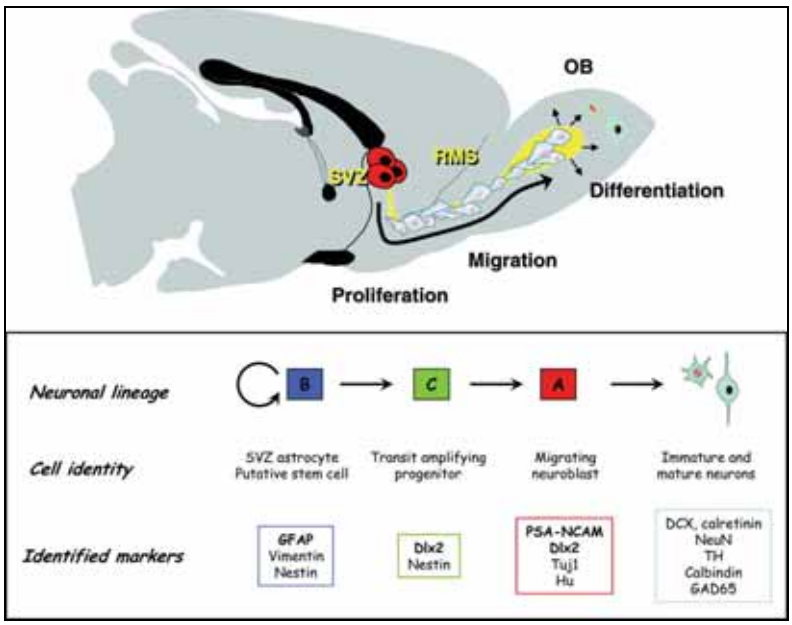


Figure 1.6.1 : Neurogenesis in the subventricular zone (SVZ)/olfactory bulb (OB) system. *Top panel*: a sagittal view of a rodent brain showing the sites of neurogenesis in the SVZ/OB system. Cells proliferate mainly in the SVZ, migrate along the rostral migratory stream (RMS) to reach the OB, where they migrate radially and undergo terminal differentiation. *Bottom panel*: a sequence of cell types involved in neuronal lineage and specific markers allowing cell identification are presented. Markers appearing in bold are specific to each stage. This region in the rodent is composed of three main cell types: B cells, C cells and A cells (from Abrous et al., 2005).

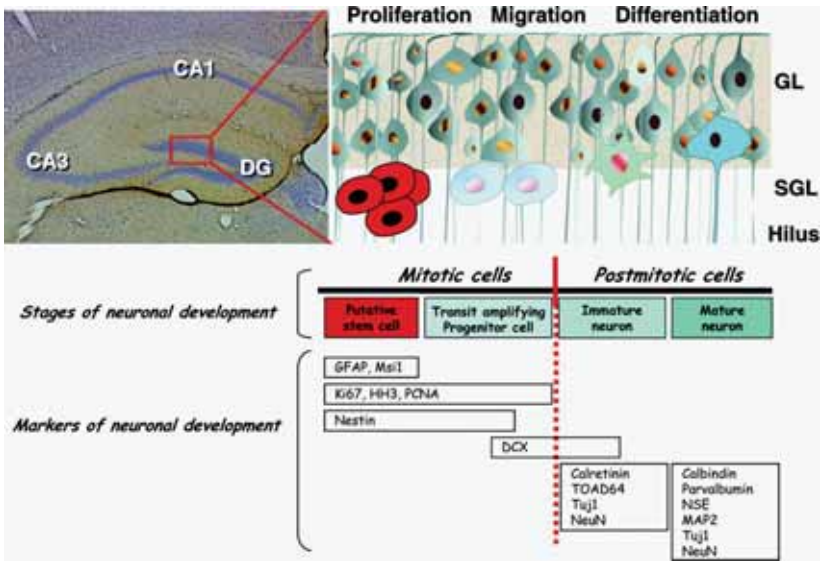


Figure 1.6.2 : Neurogenesis in the hippocampal system. *Top panel*: a frontal view of a rodent brain showing the sites of neurogenesis in the dentate gyrus (DG) of the hippocampal formation (HF). Cells proliferate in the subgranular layer (SGL) located at the interface between the hilus and the granular layer (GL), where they migrate and differentiate into mature neurons. *Bottom panel*: a sequence of cell types involved in neuronal development, along with specific markers allowing cell identification (from Abrous et al., 2005).

Indeed, in the adult mammalian brain, neurogenesis, which can lead to the generation of the three major cell lineages (neurons, astrocytes and oligodendrocytes) persists in at least two germinal zones: i) the subventricular zone (SVZ), which is a region that surrounds the lateral ventricles of the forebrain; ii) the subgranular zone (SGZ), which is the germinal zone of the adult hippocampal formation and is located within the dentate gyrus (DG) (Fig.1.6.1 and Fig. 1.6.2), reported in mice, rats, as well as non-human and human primates (Abrous et al., 2005; Curtis et al., 2007; Eriksson et al., 1998; Gage et al., 1998; Jin et al., 2001; Josefson, 1999; Kim et al., 2004; Lledo et al., 2006; McDermott and Lantos, 1991; Ming and Song, 2005; Ostenfeld and Svendsen, 2003; Quiñones-Hinojosa et al., 2006; Sanai et al., 2004; Yoshimura et al, 2001). In the rodent brain, the SVZ generates thousands of olfactory bulb neurons daily (Curtis et al., 2007; Lois and Alvarez-Buylla, 1993; Luskin, 1993).

Furthermore, in addition to these well-established neurogenic regions, the striatum, the spinal cord, the corpus callosum, the hypothalamus and the neocortex were shown to be the site of neurogenesis (Altman and Das, 1965; Altman, 1969; Alvarez-Buylla et al., 2001; Arsenijevic et al., 2001; Assanah et al., 2006; Darsalia et al., 2007; Gage, 2000; Jiang et al., 2001; Lie et al., 2002; Lois and Alvarez-Buylla, 1993; Palmer et al., 1995, 1999; Reynolds and Weiss, 1992; Roy et al., 1999; Shihabuddin et al. 1997; Shin et al., 2008; Tropepe et al., 2000; van der Kooy and Weiss, 2000; Weiss et al., 1996; Yamamoto et al., 2001; Walton et al., 2006; Windrem et al., 2004).

The neuroblasts originating from endogenous neurogenesis migrate, sometimes along very long distances, to their final destination where they differentiate and functionally integrate into pre-existing networks (Alvarez-Buylla and Garcia-Verdugo, 2002; Gage, 2002). Neurons destined for the olfactory bulbs migrate out of the subventricular zone of the lateral ventricle to the olfactory bulbs where they differentiate into granule layer and peri-glomerular interneurons (Lledo et al. 2004). Within the hippocampus, newborn neurons are produced near the granule cell layer, ultimately differentiate and integrate into this densely packed population of neurons (Kempermann and Gage 2000).

Several studies have shown that focal or global cerebral ischemia stimulates neurogenesis in adult rodents and monkeys (Arvidsson et al. 2002; Briones et al., 2005; Choi et al., 2003; Jin et al., 2001; Kawai et al., 2004; Kee et al., 2001; Nakatomi et al., 2002; Parent et al., 2002; Salazar-Colocho et al., 2008; Takasawa et al., 2002; Taupin, 2006a; Thored et al., 2006; Tonchev et al., 2003, 2005; Wang et al., 2008; Widestrand et al., 2007; Yagita et al., 2001; Yamashita et al., 2006; Zhang, Zhang et al., 2001). Furthermore, migration and projections of these new stem/progenitor cells toward distant injured brain regions have been

shown after stroke or ischemia (Arvidsson et al., 2002; Chen et al., 2004; Hill et al., 2004; Jin et al., 2006; Kee et al., 2001; Magavi et al., 2000; Martino and Pluchino, 2006; Nakatomi et al., 2002; Rice et al., 2003; Thored et al., 2006; Zhang, Zhang et al., 2004; Zhang et al., 2005).

Furthermore, an optimal extent of neurogenesis seems required, as too much neurogenesis may cause various dysfunctions, such as status epilepticus and allodynia, whereas too few neurogenesis, which can be caused by aging, stress and drugs, can lead to neurodegenerescence (Abrous et al., 2005; Karten et al., 2005).

Nevertheless, although the endogenous cells from neurogenesis may contribute to recovery of the damaged tissue, the impact of this process is limited. This limitation has been attributed to a low rate of neurogenesis, to the death of most of migrated cells (about 80%). Furthermore, only 0.2% of the cells damaged by stroke are replaced via neurogenesis. Finally, the presence of active inhibitors of axonal regeneration associated with myelin, such as Nogo A and glial scarring (Nadareishvili and Hallenbeck, 2003; Schwab, 2004; Yiu and He, 2006) limit recovery post-injury.

1.6.2 STIMULATION OF THE ENDOGENOUS NEUROGENESIS

One general regenerative strategy is to stimulate the endogenous neurogenesis, to prevent death of the migrating cells and to increase differentiation of the migrated neural precursors (Fig. 1.6.3; Jin, LaFevre-Bernt et al., 2005; Jin et al. 2006). Various parameters can contribute to this, such as: i) physical exercise and environmental enrichment (Brown et al., 2003; Kempermann et al., 2000; Komitova, Mattsson et al., 2005; Komitova, Zhao et al., 2005; Lee et al., 2008; Matsumori et al., 2006; Nygren et al., 2006; van Praag et al., 1999), as well as antidepressant (Duman et al., 2001), ii) growth factors, such as GDNF, BDNF, IGF-1, FGF-2, EGF, VEGF, G-CSF, MCP1 and erythropoietin (Altman, 1962; Baldauf et al., 2005; Brines et al., 2000; Cairns et al., 2003; Chmielnicki et al., 2004; Craig et al., 1996; Dempsey and Kalluri, 2007; Doggrell, 2004; Gluckman et al., 1992; Greenberg and Jin, 2006; Ehrenreich et al., 2002; Esneault et al., 2008; Gustafsson et al., 2003; Horita et al., 2006; Jin et al., 2002, 2004; Kalluri and Dempsey, 2008; Kuhn et al., 1997; Lin et al., 1997, 2005; Nakatomi et al., 2002; Ninomiya et al., 2006; Schabitz et al., 2007; Schanzer et al., 2004; Schneider et al., 2006; Sehara et al., 2007; Shingo et al., 2001; Shyu et al., 2004; Sun et al., 2003; Takami et al., 1992; Tsai et al., 2006; Tureyen et al., 2005; Wang et al., 2004, 2007;

Watanabe et al., 2004; Wiltrout et al., 2007; Yan et al., 2006, 2007; Yao et al., 1999; Zhu et al., 2005, 2008), iii) anti-inflammatory drugs, such as antibodies against the pro-inflammatory cytokine interleukin-6 (Monje et al., 2003), indomethacin, which is an immunomodulator, and minocycline, which is a tetracycline derivative known to inhibit microglial activation (Hoehn et al., 2005; Kluska et al., 2005; Liu et al., 2007), iv) Galectin-1, which is a β-galactosidase-binding lectin regulating neural progenitor cells (NPC) proliferation (Ishibashi et al., 2007), v) bone morphogenic protein-7, which is a trophic protein that belongs to the TGF-β (transforming growth factors) superfamily (Chang et al., 2003; Chou et al., 2006), vi) estradiol, which increases the number of newborn neurons (Suzuki et al., 2007), vii) tissue kallikrein, a serine proteinase stimulating the production of kinin, which is a potent vasodilatator (Ling et al., 2008; Xia et al., 2006), viii) Bcl-2, which is a regulator of apoptosis (Sasaki et al., 2006), ix) substance-P, which participates in the immune/hematopoietic functions (Park et al., 2007).

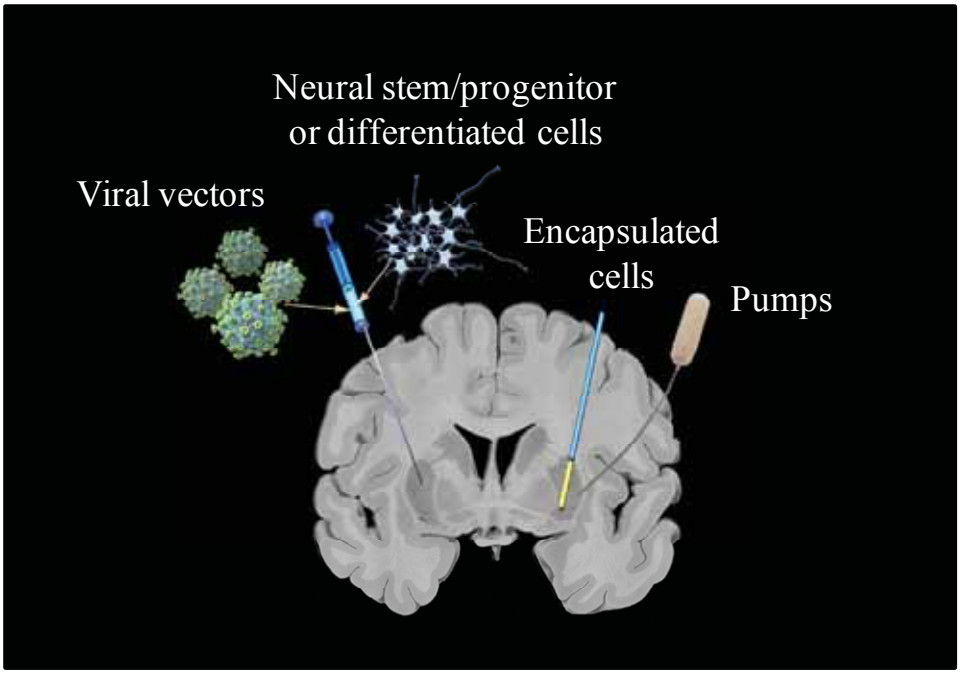


Figure 1.6.3: Various strategies to stimulate the endogenous neurogenesis (from Brunet, Stem Cells Symposium, on April 26-27, 2005, Geneva).

To note also that neurotrophins have been more or less successfully administered in various neurodegenerative diseases, such as Parkinson's disease (Gill et al., 2003; Kirik et al., 2004; Kordower, Palfi et al., 1999; Nutt et al., 2003; Patel et al., 2005; Slevin et al., 2005;), Huntington's disease (Bloch et al., 2004; de Almeida et al., 2001 ; Kordower, Isacson et al., 1999; Mittoux et al., 2000) and amyotrophic lateral sclerosis (Aebischer et al. 1996; ALS

CNTF Group, 1996; Hagg & Varon 1993; Henderson et al. 1994; Kaspar et al., 2003, 2005; Klein et al., 2005; Miller et al. 1996a,b.; Nagano et al., 2005; Penn et al. 1997), by using bolus injection of purified protein, chronic pump infusion and direct delivery of transgenes encoding trophic factors via replication-deficient viral particles, as well as by cellular biopumb mediated delivery of neuroprotective agents, such as GDNF, hCNTF and IGF-1.

1.6.3 TRANSPLANTATION OF EXOGENOUS STEM/PROGENITOR CELLS

Beside these strategies to stimulate the endogenous neurogenesis and reorganization, another strategy is the transplantation of exogenous stem or progenitor cells, in which cells are delivered intracerebrally or are infused by an intravenous or intra-arterial route. While initially, it was thought that cell therapy might work by a 'cell replacement' mechanism, a large body of evidence is emerging that cell therapy works also by providing trophic or 'chaperone' support to the injured tissue and brain (Ormerod et al., 2008). In this sense, this approach contributes also to support the endogenous neurogenesis.

The most ancient report on cerebral tissue transplantation raises to 1890, when Thompson described attempts to transplant adult cats or dogs cortical pieces in the cortex of adult dogs. This report indicated that a certain type of tissue remained present in the reimplantation site after 6 weeks. Although other studies on various transplantations took place (Forssman, 1900; Saltykow, 1905) most actual neuroscientists consider the work of Dunn (1917), which began in 1903, as being the first pertinent experiments on the transplantation of cerebral tissue. In her experiments, Dunn transplanted cerebral cortex of immature (neonatal) rats in other neonatal animals, and in some cases, a small number of surviving neurons were found.

Since then, numerous studies proposed a number of stem and progenitor cell types as therapy for neurological disease ranging from neural stem cells to bone marrow derived stem cells to embryonic stem cells (see for review Taupin, 2006b).

Stem cells (Fig. 1.6.4) are proliferative cells that have the capacity to self-renew (by generating identical daughter cells) as well as giving rise to progeny that become progressively more lineage-restricted, leading eventually to a terminally differentiated cell. The pluripotent stem cells, such as the embryonic stem cells (ESC) have the ability to differentiate into all types of cells that develop from the three germ layers: mesoderm, endoderm, and ectoderm. Stem cells may also be multipotent, namely able to give rise to a

restricted range of differentiated progeny, or restricted to producing a single differentiated phenotype. These cells are more heterogenous (Morrison et al., 1999).

Progenitor cells, issued from stem cells divisions, have acquired a certain level of specialization and are physiologically functional. Although they have a more limited capacity for self-renewal (Clevers, 2005; Potten and Loeffler, 1990), they can be used therapeutically.

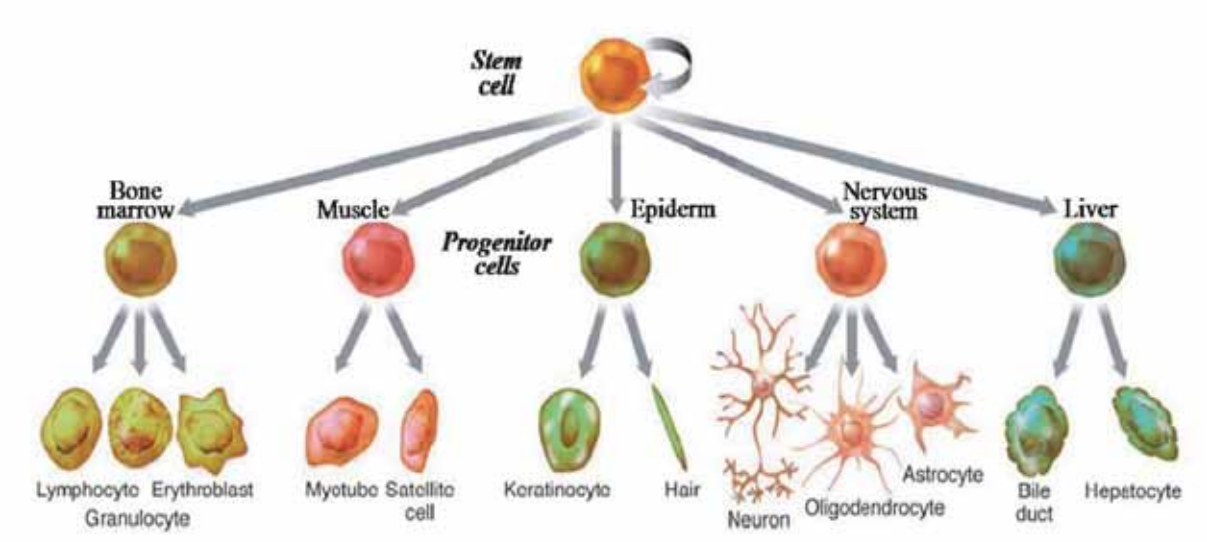


Figure 1.6.4: Pluripotent stem cells have a self-renew capacity and generate the lineages derived from the three germ layers of the body, endoderm, mesoderm, and neurectoderm. Multipotent stem cells have a self-renew capacity and generate lineage-specific cell types restricted to the tissues from which they are derived. Progenitor cells, having a limited capacity to self-renew, give rise to specific cell types. Recent evidences suggest that adult stem cells may have a broader potential; they may generate cell types of tissues other than the ones they have derived (adapted from Taupin, 2006b).

In the context of brain trauma or stroke, there are several manners by which the transplants could be used to repair the brain (Fig. 1.6.5; Freed, 2000): i) by releasing neurotransmitters at synaptic level for cells that form new synapses when transplanted, ii) by releasing neurotransmitters in the extracellular space, for cells that do not form synapses when transplanted, iii) by releasing growth factors, such as NGF, BDNF, GDNF (Rosenberg et al., 1988), iv) by releasing an antibody, a toxin or an enzyme (Schnell and Schwab, 1990), v) by depositing a substrate, such as laminin and fibronectin that can promote the neuritis extension, vi) by interconnecting bridge to receive inputs and make outputs (Wictorin et al., 1989), vii) by providing a physical or metabolic support for the surrounding cells (Pearlman et al., 1991, 1993), viii) by inhibiting an abnormal growth (Harwood, 1992; Postone, 1987; Sherman, 1989; Stevens, 1990; van der Zee et al., 1995), ix) by degrading the effects of a toxin (Mattson and Rychlik, 1990; Przedborski et al., 1991), x) by remyelination (Archer et al., 1994; Groves et al. 1993; Tontsch et al., 1994; Utzschneider et al., 1994). Possibly, the overall

success of functional outcome is mediated by a combination of these factors (see Padma Srivastava, 2009 for review).

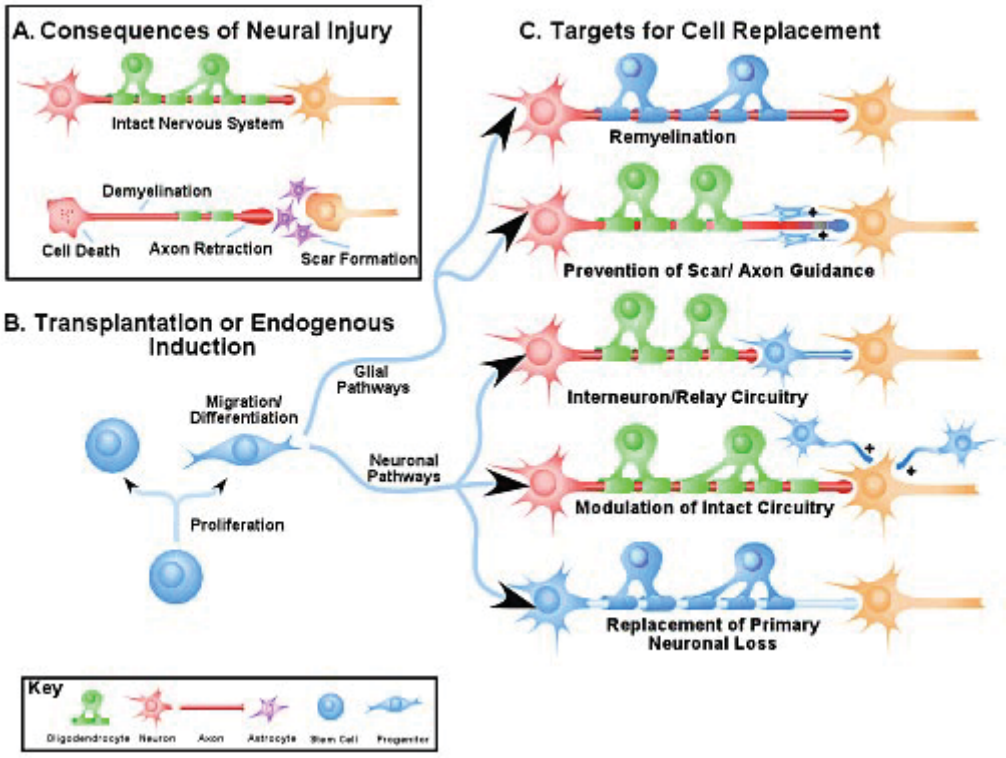


Figure 1.6.5: After neuronal injury (A), cells from endogenous neurogenesis or transplanted cells may proliferate, migrate and differentiate in glial or neuronal cell types (B), in order to participate to cell replacement by various ways (C) (from Freed, 2000).

Furthermore, the various grafts and their immunological implications are the following (Freed, 2000): i) autografts, which are performed in one unique individual and do not induce any immune reaction, ii) syngrafts or isografts, which are performed between two animals that are genetically identical (monozygotes twins) and do not induce any immune reaction too, iii) allografts, which are performed between two individuals of the same species that are genetically different and induce immunological reactions, needing thus immunosuppresors, iv) xenografts, which are performed between animals of two different species and virtually always rejected, recquiring thus immunosuppressors too.

The various cell types that could be cultured and transplanted in a therapeutical context for brain trauma or stroke are the following:

First, the embryonic and fetal stem cells (here pooled as ESC) have the potential to differentiate into any fetal or adult cell in the body, and possess a theoretically unlimited

potential to proliferate (Hoffman and Carpenter 2005; Josefson, 1999; Kim et al., 2004; Ostenfeld and Svendsen, 2003; Rakic, 2004). Human embryonic stem cells (hESCs) are derived from the blastocyst inner cell mass of excess embryos (Papaioannou et al., 1975) generated by in vitro fertilization (Fig. 1.6.6).

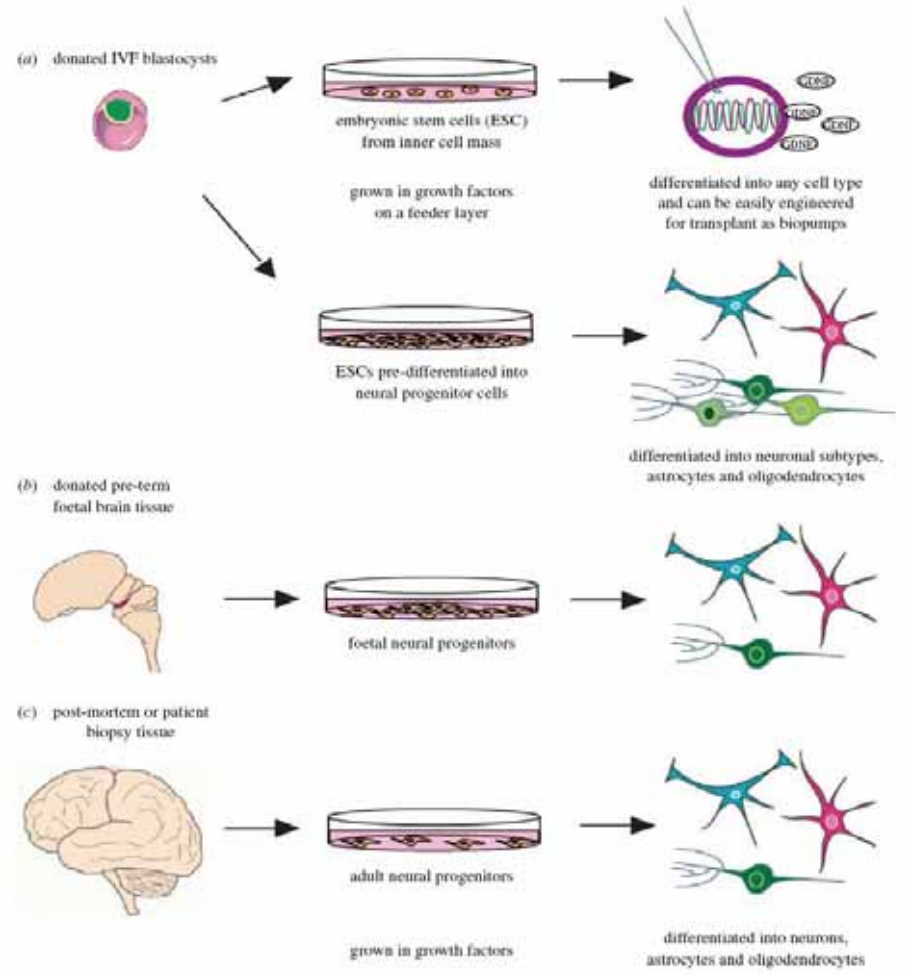


Figure 1.6.6: Human stem cells grown from different tissue sources have properties that each may be advantageous for different therapeutic strategies. (a) Human embryonic stem cells (hESCs) derived from excess blastocysts (in vitro fertilization). In their primitive form, hESCs can be easily engineered to secrete neurotransmitters or growth factors to stop disease progression or to augment intact circuitry, respectively. When hESCs are differentiated into neural progenitors, they can then make neurons, astrocytes and oligodendroctyes, the precursors of which could potentially be used for regeneration. (b) Foetal neural progenitors harvested from the brain tissue of discarded foetuses. They readily generate neurons and astrocytes in vitro and generate neurons mber implantation
post-mortem tissue or from patient biopsies to generate immunologically matched transplants. These cells generate neurons, astrocytes and oligodendrocyte precursors in culture and neurons when transplanted into neurogenic regions of the adult brain (from Ormerod et al., 2008).

The derivation and long-term maintenance of ESC in vitro has been extensively standardized and ESC are currently thought to be one of the ideal stem cells for transplantation (Evans and Kaufman, 1981; Martin, 1981; Thomson et al., 1998; Thomson

and Odorico, 2000). Some studies showed that transplantation in a rat ischemic brain either of genetically altered ESC that over-express the anti-apoptotic gene Bcl-2, or of mouse ESC derived neural progenitor cells (NPC) with an hypoxic pre-treatment, resulted in an increased survival, neuronal differentiation and functional outcome (Wei et al., 2005; Theus et al., 2008). Various studies have used ESC in the context of neurodegenerative diseases, such as Parkinson's disease (PD) and of Huntignton's disease (HD). In both rat and non-human primate models of PD and HD, foetal tissue-derived neuroblasts transplanted into the striatum survive and reverse both motor and cognitive deficits (Freeman, 1997; Kordower et al. 1997; McBride et al., 2004; Peschanski et al. 2004). Promising preclinical model results have led to several clinical trials for PD and a few for HD (Bjorklund et al., 2003; Clarkson, 2001; Dunnett and Bjorklund, 1997; Freeman, 1997; Hauser et al., 1999; Kordower et al., 1995, 1997; Le Belle and Svendsen, 2002; Lindvall et al., 1989; Lindvall and Bjorklund, 2004; Peschanski et al. 2004; Winkler et al. 2005). However, grafts of human fetal tissue did not lead to permanent positive effects (Bachoud-Levi et al., 2000 for HD; Clarkson, 2001; Freed et al., 2001; Olanow et al., 2003 for PD), and even deleterious effects were reported (Freed et al., 2001; Olanow et al., 2003; Whalley, 2009). Furthermore, the therapeutic use of ESCs was hindered by the intrinsic capability of ESC to generate teratomas and to give rise to extraneural cell types when grafted to the brain (Bjorklund et al., 2002; Bongso et al., 2008; Erdo et al., 2003).

Hence, to avoid the risk of teratoma formation, the use of ESC differentiated into neuronal progenitors (see below the fifth cell type: neural stem/progenitor cells) for transplantation (Takagi et al., 2005), as wells as the depletion or ablation of the undifferentiated cells (Arase et al. 1999; Singh et al. 2005), were tested. These ESC differentiated into neuronal progenitors can generate neurons, oligodendrocytes and glia, both in culture and when transplanted into the adult rodent CNS (Cummings et al. 2005;Fricker et al. 1999 ; Keirstead et al. 2005; Reubinoff et al. 2001; Svendsen et al. 1996, 1998; Tabar et al. 2005; Vescovi et al. 1999; Zhang, Wernig et al. 2001). Such cells improved the transplant safety in animal models and demonstrated physiological characteristics of neurons with voltage-gated sodium currents that enable action potentials (Bühnemann et al., 2006).

Furthermore, protocols have been developed to obtain *in vitro* high numbers of cell type-specific neural precursors (eg. motor neurons, dopaminergic neurons, oligodendroglial cells) from ESC prior grafting (Barberi et al., 2003; Brustle et al., 1999; Kawasaki et al., 2002; Wernig et al., 2002; Wichterle et al., 2002;). Indeed, exposing stem cells to a few key patterning stimuli in a stepwise fashion can trigger neuron subtype-specific programmes. For

example, dopamine neuron development has been well characterized and, in vitro, a developmental dopamine neuron-like pattern of gene expression can be recapitulated to induce the generation of midbrain dopamine neurons from human embryonic stem cells (hESC). Neutralized hESC differentiate into dopaminergic neurons following exposure to FGF-8 and Shh combined with Nurr1 overexpression (Ben-Hur et al. 2004; Chung et al., 2002; Kim et al., 2002; Sonntag et al. 2005; Takagi et al., 2005). In a mouse model of Parkinson's disease, nuclear transfer-derived ESC that were predifferentiated into dopaminergic neurons corrected motor deficit (Barberi et al., 2003). Similarly, differentiation of spinal motoneurons has been achieved by exposing ESCs to a specific sequence of environmental stimuli, known to regulate motoneuron generation *in situ*, such as retinoic acid and Shh (Wichterle et al., 2002).

It seems therefore that strategies such as cell sorting for expression of markers of neural progenitor differentiation to eliminate undifferentiated ESCs (Chung et al., 2006) represents the actual safest mode to use ESC. Nevertheless, in vitro patterning does not prevent the generation of unintended neuronal subtypes; a significant portion of cells that survive transplant do not express the desired markers (Mendez et al. 2005). Furthermore, the major ethical and legal issues (Brevig et al., 1999; Issacson and Deacon, 1997) concerning hECS remain.

Second, the immortalized cell lines are derived from cell tumors or by infecting neuroepithelial precursor cells from CNS regions before their terminal mitosis, with a retrovirus encoding an immortalizing oncogene (Magnuson et al., 1995; Staines et al., 1994). The most promising cells of this kind in stroke therapy are the immortalized human neural stem cell line NT2N, which was derived from human testicular germ cell tumor. The efficacy of NT2N cells was investigated in animals (Borlongan et al., 1998; Saporta et al., 1999), with significant improved functional recovery, as well as in humans, where the transplantation was shown to be feasible, safe and tolerable (Hara et al., 2008).

Third, the mesenchymal, stromal and hematopoietic stem cells can be derived from bone marrow, umbilical cord blood, placenta, fat, spleen or thymus. These non-neural adult stem cell represent alternatives that have been proposed for treating CNS diseases. These are multipotent cells that can be induced to differentiate into osteoblasts, chondrocytes, myocytes and adipocytes (Krampera et al., 2007; Wu et al., 2006). Kucia et al. (2005) suggested that the bone marrow contains a heterogenous population of stem cells that might have neuronal, glial,

endothelial, or cardiac fate, and that during injury or stress, these cells may be mobilized into the blood and then migrate and home to an SDF-1 gradient in an injured organ.

Under specific conditions of cell culture, mesenchymal stem cells (MSC) can adopt a neuronal phenotype (Azizi, et al., 1998; Bossolasco et al., 2005; Cho et al., KJ., 2005; Guo et al., 2005; Woodbury et al., 2000, 2002), providing a source of transplantable autologous neurons or glia for CNS therapy (Crain et al. 2005 ; Joannides et al. 2004; Kokai et al. 2005 ; Ortiz-Gonzalez et al. 2004; Toma et al. 2005). In stroke rodents studies, MSC were shown to migrate to the damaged area and to differentiate into neurons (Shen et al., 2007; Wu et al., 2008), to stimulate endogenous neural progenitor proliferation, neurogenesis, synaptogenesis and angiogenesis (Chen, Li et al., 2003; Chen, Zhang et al., 2003; Pavlichenko et al., 2008), to prevent neuronal apoptosis (Okazaki et al., 2008), and to decrease mortality, infarct volume and neurological deficits (Andrews et al., 2008; Chen et al., 2001; Kim et al., 2008; Lee et al., 2003; Li et al., 2001a; Okazaki et al., 2008; Pavlichenko et al., 2008; Shen et al., 2006; Sokolova et al., 2006; Wu et al., 2008). Bang et al. (Bang et al., 2005) reported a phase I trial of autologous MSC in ischemic stroke, where the treatment was proved to be feasible and safe, with a trend towards functional improvement. The mechanism of action of these cells is probably not direct cell replacement, but rather acting as trophic factory, elaborating a host of trophic and growth factors (Caplan and Dennis, 2006; Chopp and Li, 2002; Liu et al., 2006; Nomura et al., 2005). This was also true for the multipotent adult progenitor cells subset of MSC; indeed, Zhao et al. (Zhao et al., 2002) showed that transplantation of such cells in a rodent stroke model resulted in improved sensorimotor function, and that although some of the transplanted cells expressed neuronal markers, their small number suggested that improvement was from a trophic effect. In the context of Parkinson's disease, MSC could be differentiated in culture into cells with many characteristics of dopaminergic neurons (Guo et al., 2005) expressing genes characteristics of them (Kramer et al., 2006). MSC transplanted intrastrially in a mouse MPTP model survived, expressed tyrosine hydroxylase activity, and promoted functional improvement (Li et al., 2001b).

Hematopoietic stem cells (HSC) were also shown to differentiate into neurons, astroglia and vascular endothelial cells when implanted into rats brains following ischemia (Mezey et al., 2000; Shyu et al., 2006), and to reduce infarct volume, inflammation and peripheral immune activation, leading thus to neuroprotection (Schwarting et al., 2008).

Transplantation of human umbilical cord blood (HUCB) stem cells were also reported to be effective in animal models of cerebral ischemia (Borlongan et al., 2004; Chen et al., 2001; Taguchi et al., 2004; Vendrame et al., 2004). However, not all groups have found an

effectiveness of HUCB in improving the outcome and few delivered cord blood cells were seen in the brains of rodents with stroke (Makinen et al., 2006). Umbilical cord blood derived CD34 cells improved functional outcome and increased angiogenesis and associated neurogenesis in mice (Borlongan et al., 2004; Taguchi et al., 2004). A substantial disadvantage of HUCB stem cell transplantation is its allogenic nature, requiring permanent immunosuppressive treatment to avoid graft rejection (Haas et al., 2005).
To note that MSC in general may elicit an immune response in MHC mismatched recipients (Eliopoulos et al., 2005). Nevertheless, if MSC can be used in allogeneic transplantation, this would represent an interest for cell therapy, as it may permit isolation from healthy donors (vs autologous).

Fourth, olfactory ensheathing cells (OEC) are cells that ensheath the axons of the olfactory receptor neurons, which are continuously replaced throughout life in mammals (Doucette, 1984, 1991, 1995). After transplantation into the CNS, these OEC survive, and in models of dorsal spinal transsection, they bridge the transsected zone, myelinate axons, and improve locomotor activity and sensorimotor reflexes (Doucette, 1995; Ramón-Cueto et al., 2000; Sasaki et al., 2004). Xenotransplanted OEC also have been shown to effectively remyelinate regions of injured spinal cord in a non-human primate model (Radtke et al., 2004). Furthermore, two phase I trials of autologous OEC in spinal cord injury patients have demonstrated safety and feasibility (Feron et al., 2005; Lima et al., 2006).

Fifth, neural stem/progenitor cells (NPC) are mutipotent cells that occur in fetal or adult brain and are partially specialized. They divide and give rise to differentiated neural lineages (neuronal, astroglial, glial and oligodendroglial) (Josefson, 1999; Kim et al., 2004; Ostenfeld and Svendsen, 2003; Rakic, 2004). Adult neural stem/progenitor cells persist throughout life and mediate limited repair and restoration through adulthood (see "Endogenous neurogenesis"). Adult neural stem cells can be harvested from brain tissue, post-mortem or through biopsy and expanded in culture. Indeed, at the end of the 1990s, it was demonstrated that proliferating cells isolated from the forebrain (Cattaneo and McKay, 1990; Reynolds et al., 1992; Temple, 1989) could give rise *in vitro* to large spheres of cells termed "neurospheres". These neurospheres possessed neurons and glia, but largely consisted of cells expressing the intermediate filament previously associated with neuroepithelial cells, nestin. An entire neurosphere could be generated from a single cell and this neurosphere could be subsequently dissociated to produce a new neurosphere that also contained neurons and

glia. Thus, these neurosphere-producing cells had the properties of a stem cell: they were self-renewing and multipotent. These findings were then reproduced using adult human CNS tissue (Brunet et al., 2002). These cells exhibit genetic stability and differentiate into limited numbers of neurons, astrocytes and oligodendrocytes, both in vitro and upon transplant into the rodent CNS (Arsenijevic et al. 2001; Nunes et al. 2003; Palmer et al. 2001 ; Roy et al. 2000). For their part, hESC can be cultured in such a way that they yield a large number of neural stem or progenitor cells (Kitajima et al., 2005; Li et al., 2005; O'Shea, 2001, 2002; Singh et al., 2005; Tropepe et al., 2001). These protocols have not been fully optimized or tested in a large number of cell lines, but could theoretically yield an extremely large number of cells.

In culture, precursor cells removed from the brain are often cultured in growth factor-supplemented serum-free medium (such as EGF or FGF-2) (Johe et al., 1996) and with hormonal supplements (such as N2 or B27, Reynolds et al., 1992). In some cases of human NSCs cultures, LIF has also been added to stimulate proliferation (Bartlett et al., 1998; Carpenter et al., 1999). It is also known that the differentiation program can be influenced by exposure to various factors, such as growth factors or cytokines (Galli et al., 2000; Johe et al., 1996; Williams et al., 1997). A number of investigators have attempted to purify neural stem cells using cell sorting under a variety of conditions (Capela and Temple, 2002; Keyoung et al., 2001; Kim and Morshead, 2003; Nakano et al., 2005; Rietze et al., 2001; Uchida et al., 2000). Furthermore, numerous specific genes/pathways have been identified as important regulators of neural stem cell proliferation, many of which are important for several other cell types, including other stem cells. Some of these are: Bmi-1 (Molofsky et al., 2005), P21 (Kippin TE et al., 2005), nucleostemin (Tsai and McKay, 2002), maternal embryonic leucine zipper kinase (Nakano et al., 2005), P53 (Meletis et al., 2006), Rb (Karsten et al., 2003), and AKT (role in blocking apoptosis and thereby promoting cell survival) (Sinor and Lillien, 2004) among others.

However, one cannot assume that studies of gene expression or cell fate on cultured cells necessarily implies similar mechanisms in vivo. For example, although spinal cord neural stem cells can be cultured for extensive periods and retain expression of key spinal cord genes, they lose characteristic gene expression of the dorsal or ventral cord from which they are originally derived (Gabay et al., 2003). It has also been demonstrated that, at least under some conditions, cultured neural stem cells will transform into tumor-like cells (Morshead et al., 2002).

Concerning the biochemical properties of adult neuronal stem cells, certain phenotypic markers can discriminate between different cell types or different maturational states of the same cell. These markers include neuronal nuclear antigen (NeuN) (Mullen et al., 1992) and microtubule-associated protein-2 (MAP2) (Binder et al., 1984), which identify mature neurons; glial fibrillary acid protein (GFAP) for astrocytes (Debus et al., 1983); and galactocerebroside (GalC) for oligodendrocytes (Ranscht et al., 1982). The doublecortin (DCX) gene encodes isoforms of a highly hydrophilic protein that is highly expressed in migrating and differentiating neurons in fetal and adult brain (des Portes et al., 1998; Francis et al., 1999). A recent study showed that DCX positive cells retain their multipotentiality (Walker et al., 2007). In addition, PSA-NCAM may be involved in cell migration (Alonso et al., 1999; Bonfanti and Theodosis, 1994; Rousselot et al., 1995). Neural precursor cells can also be identified using markers including nestin, a protein expressed by NSC (Kaneko et al., 2000), β-tubulinIII (TUJ-1), an earlier marker for the neuronal lineage in vivo (Fanarraga et al., 1999), and Hu, a neuron-specific RNA-binding protein that begins to be expressed in neuronal nuclei and somata soon after differentiation (Marusich et al., 1994).

Previous studies investigating the regenerative capacity of rodent or human embryonic or fetal derived neural stem/ progenitor cells reported differentiation of grafted neural stem cells into neurons and astroglia as well as functional recovery (Aihara et al., 1994; Aoki et al., 1993; Darsalia et al., 2007; Farber et al. 1988; Ishibashi et al., 2004; Jin, Sun et al., 2005; Kelly et al., 2004; Mattsson et al., 1997). Oligodendrocyte progenitor cells harvested from adult white matter have even remyelinated the newborn shiverer mouse brain upon transplant (Windrem et al., 2004). Zhang et al. (Zhang et al., 2003) investigated the effects of adult neural progenitor cell transplantation in the context of stroke in adult rats. These progenitor cells, transplanted 48 h following MCA-O, survived and migrated toward the ischemic area as assessed by histological and radiological analysis (MRI). Some functional recovery was reported, but the underlying mechanisms need to be elucidated. NPCs derived from monkey ESC and transplanted into ischemic rodent striatum were observed to migrate into the area surrounding the ischemic lesion and differentiate into various types of neurons and glia (Hayashi et al., 2006). Furthermore, Brunet et al. (2002, 2005) showed that non-human primate adult NPC could be reimplanted in the donor, where they migrated toward the ibotenic acid lesioned cortical region and differentiated. In the context of PD, the same authors (Brunet et al., 2009) showed that these primate adult NPC reimplanted in the caudate nucleus of PD donor survived, migrated bilaterally in the whole striatum, and seemed to have a neuroprotective effect over time. To note here that induced pluripotent stem cells derived

from skin fibroblasts (Dimos et al., 2008; Ebert et al., 2009; Lowry et al., 2008; Okita, 2008) are a source of cells that provide the advantage to be also suitable for autologous transplantation, but which present the disadvantage to be genetically modified by gene transfection with four genes, such as Oct3/4, SOX2, c-Myc, Klf4, NANOG and LIN28, implicating manipulations to stop their proliferation and to maintain their differentiation after implantation. Furthermore, c-Myc and LIN28 are associated to tumor formation (Viswanathan et al., 2009; Okita, 2008).

In PD, hNSC have been grafted into rat brains where they survive, differentiate into neurones and glial cells, express tyrosine hydroxylase, and ameliorate neurological deficits (Svendsen et al., 1997; Svendsen et al., 1996). In the case of HD, the studies of Kim (2004) and Lee et al. (2005), investigating the effects of hNSC transplantation in the striatum of HD rats, showed that transplanted hNSCs can integrate well in the striatum and support the survival of host striatal neurons against neuronal injury indicating that early intervention using brain transplantation with human NSC overexpressing neurotrophic factors might be effective in blocking the progression of clinical pathology in HD.

One of the important characteristics of neural stem/progenitor cells is their ability to migrate towards distant areas of injury (Imitola et al., 2004; Kabos et al., 2003). It has indeed been shown that transplanted stem/precursor cells are able to follow, via the blood stream or cerebrospinal fluid circulation, gradients of pro-inflammatory cytokines and chemokines that are released at the site of brain lesions, such as SDF-1. While promoting interaction between transplanted NPCs and activated endothelial/ependymal cells around inflammated CNS tissues, this chemoattractive gradient leads to selective and specific homing of transplanted cells in inflammated damaged CNS areas (Aboody et al., 2000; Ben-Hur et al., 2003; Imitola et al., 2004; Pluchino et al., 2003, 2005).

It has been shown that one major therapeutic effect of neural stem/progenitor cells is their ability to rescue injured and dysfunctional endogenous neurones and serve as a chaperone cells, by exerting direct neuroprotection through neutralization of free radicals, inflammatory cytokines, excitotoxins, lipases peroxidises and other toxic metabolites that are released following an ischemic event (Ourednik et al., 2002, 2004). In addition to these effects, it has also been shown that adult NPC may exert an immunomodulatory action, while in an undifferentiated state, causing a profound downregulation of inflammatory Tcells and macrophages within inflamed brain areas (Chen et al., 2006; Einstein et al., 2007; Park et al., 2002; Zhang, Li et al., 2004).

Overall, stem/progenitor cells are obvious candidates for neuroprotection, local augmentation or full restoration, since they can be expanded and differentiated in culture and successfully transplanted. Cell therapy could replace lacking neurons to allow the reestablishment of a functional neuronal circuitry. Additionnally, glial cells such astroglia and oligodendroglia have to be replaced to maintain neuronal circuitry and to establish proper nerve conduction. Differentiated neurons may reveal histochemical characteristics of host cells, producing neurotransmitters and forming dendritic branches that grow out into the cell environment. Electrophysiological studies of these neurons may show characteristics of resting potentials, membrane currents and action potentials very similar to mature neurons (Bühnemann et al., 2006). However, only a small percentage of transplanted cells undergo terminal differentiation in the host tissue (Martino and Pluchino, 2006) and the number of differentiated transplanted cells remains very small compared with the large number of cells that are lost following a stroke. Nevertheless, transplanted cells could remedy the absences of chemical signals, of factors, of metabolites, of enzymes, of neurotransmitters, or other defective or lacking components in a damaged brain and in the penumbra surrounding the infarct area, helping to promote survival, migration and differentiation of endogenous precursor cells (Lee et al., 2007). That cell-based therapies may enhance the reorganization of white matter tracts surrounding an ischemic infarct has recently been shown by magnetic resonance imaging using fractional anisotropy (FA) and diffusion tensor imaging (DTI) sequences (Jiang et al., 2006). Cell therapies could also deliver the genetic material needed in the treatment of hereditary illnesses (Zigova et al., 2002).

1.7 AIM OF THE PRESENT STUDY

Numerous difficulties emerged from stem cell therapies using embryonic and fetal cells, although when engineering them, either by inducing proliferation and/or differentiation, or by genetically modifying them. Indeed, additionally to the biological (immune rejection (Bradley et al., 2002) and poor supply), ethical and legal questions limiting a widespread use of these cells at present, various studies reported formations of teratomas, signs of pathology acquired by the transplanted cells, as well as allodynia in a case of intraspinal graft (Bjorklund et al., 2002; Bongso et al., 2008; Erdo et al., 2003; Hofstetter et al., 2005; Whalley, 2009). In this problematical context, the autologous cell transplantation mode seems to represent a promising approach in the use of adult stem/progenitor cells to brain repair. The feasibility and safety of such an approach was demonstrated by Brunet et al. (2005). Particularly, the

objective here is to preleve cells in a healthy non-eloquent region of the brain (prefrontal cortex), which showed neural progenitor characteristics in vitro (see the results chapter "Adult brain progenitor cells"), to put them in culture without any kind of purification and proliferation or differentiation induction, nor genetic engineering, and to reimplant them in a focal ibotenic acid injured motor cortex area in macaque monkeys. This strategy is expected to help recovering from brain injury, by cell replacement and/or by secreting factors that could promote the endogenous neurogenesis and general reorganization, without encountering any of the above mentioned potential troubles.

Furthermore, as already underlined, the use of primates in the present context is particularly pertinent and needed, as the organization of the CNS is very different between rodents and humans, and there are much more anatomical, physiological and functional resemblances between non-human primates and humans. For example, rodents would not be able to perform the kind of tasks requiring precision grip used in the present investigation to assess fine manual prehension, whereas the motor skills of non-human primates closely resemble those of humans (fig. 1.3.3). Moreover, effectiveness and eventual secondary effects of the treatment are more likely to be observed before transferring the therapeutical method to the clinic (Capitanio and Emborg, 2008).

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2 GENERAL MATERIAL AND METHODS

2.1 SUBJECTS : LONG-TAILED MACAQUE

Long-tailed macaque, crabe-eating macaque, Javanese macaque or Buffon macaque (Macaca Fascicularis; Fig. 2.1.1) is a species that belongs to the Primates order and to the Cercopithecidae family (Fig. 2.1.2). They are generally grey to brown coloured. Males measure from 40 to 65 cm and weight between 6.5 and 12 kg. Females measure 40 to 50 cm and weight between 5 and 9kg.

Young macaques grow slowly. They attain their sexual maturity at the age of four years for females and six years for males. They can live more than 30 years under favourable conditions.

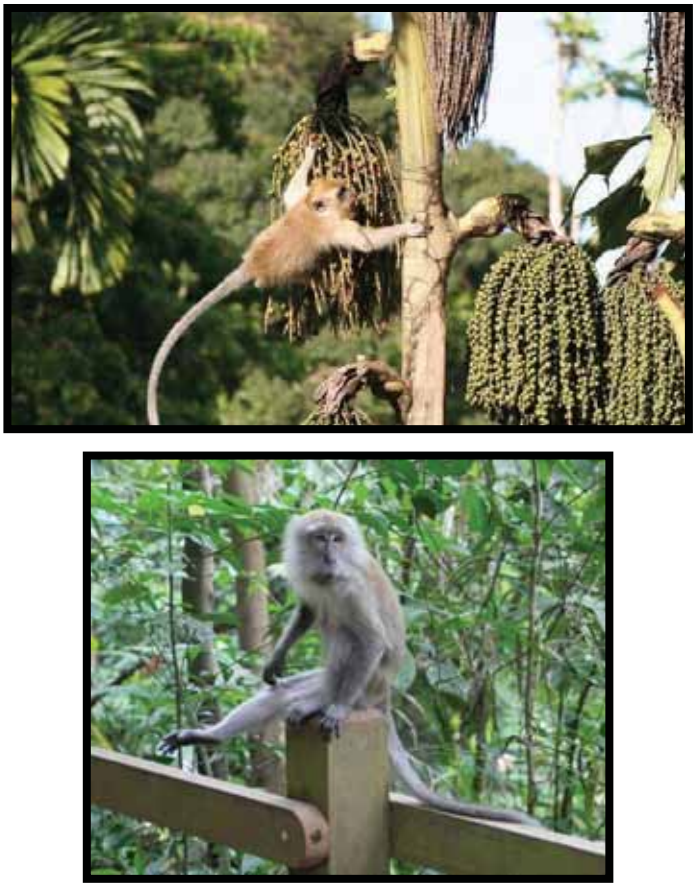


Figure 2.1.1 : Macaca Fascicularis.

Macaques, originated from Africa, but living now mainly in South-East Asia Islands, have colonized more natural environments and possess a distribution area vaster than any other primate, except Homo sapiens, with whom they share a great part of genome (macaca

rhesus, whose genome has been totally sequenced by Gibbs and others in 2007, shares 93% of genes with human).

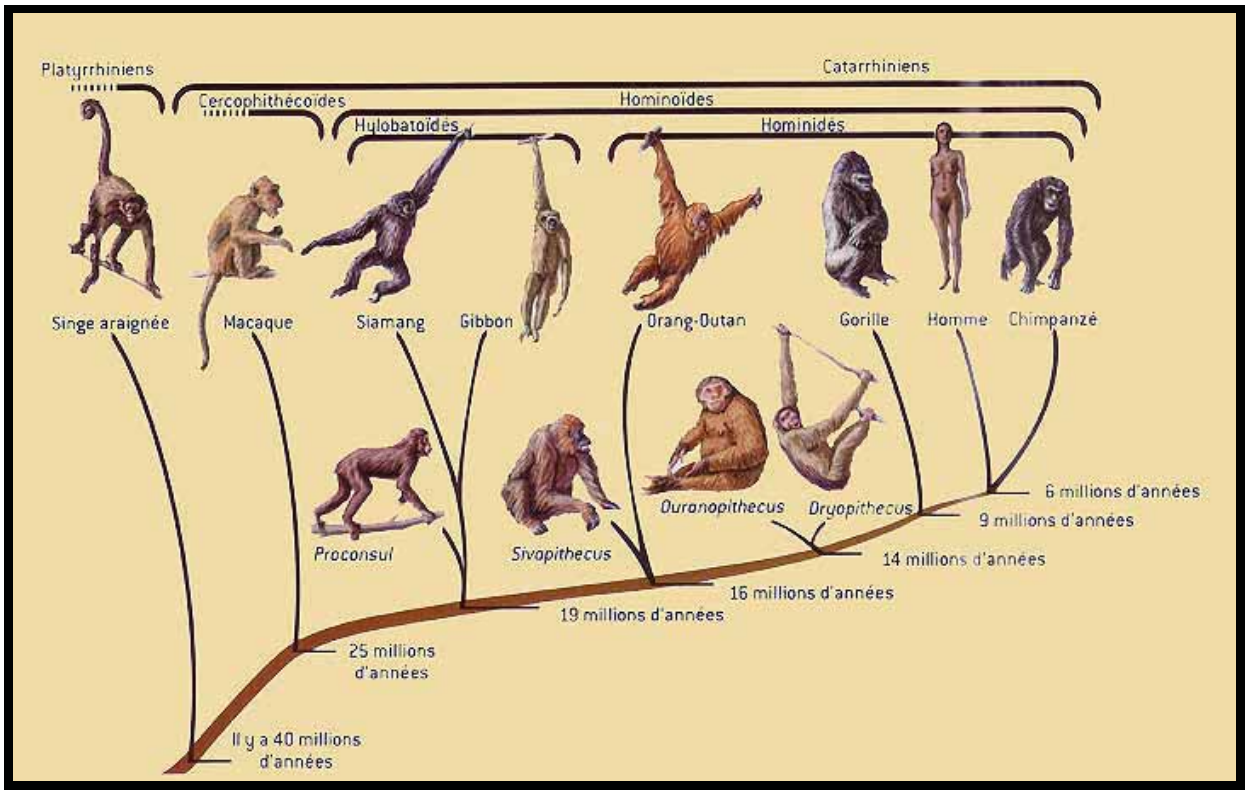


Figure 2.1.2 : Taxonomy of Anthropoidea Primates.

Macaques live in diverse environments such as plains or mountains, tropical or temperated forests, swamps or semi-desert regions, sea or riversides. They are principally frugivores but they also eat crustaceans (mainly crabs), insects and eggs. Macaques have chaps, which are two pockets in their mouth, allowing them to store up food quickly in order to eat it quietly far from their concurrent. Their eyes, frontally placed, give them a three dimensional vision similar to that of human. Macaques have buttock callosities, which are two horned regions on the crupper allowing them to sit during long periods of time. This is their prefered position to have a rest or sleep in trees. Their feet and hands have nails which allow them a tree-dwelling locomotion or an evolution on the ground. One main characteristic of macaques is their capacity, acquired along evolution, to form social groups. Collectivity is an adaptive answer to cope with environment they face (search of food, protection against predators, acquisition of coveted resources). Macaques possess a variate repertory of gestures, shouts and mimics repertory for communication. Their eyes, their mouth, their head and their ears movements are as informative as their shouts.

They live in groups hierarchically organized, implicating cooperation and competition. They wash regularly to stay clean and eliminate parasites, but also to maintain their social relations. A submission or a relief ends generally the conflict; opponents become friends again by embracing, grooming or washing. Macaques follow dominance-subordination relations. Dominant individuals have priority in competition situations, such as feeding. The rank of an individual depends on its birth clan. Females stay for their whole life in their birth group. Most of males leave their origin group at about four to seven years old in order to meet other females and copulate with them. Young males often form little groups travelling together, before settling in another group. Life in group certainly leads animals to develop certain forms of intelligence, such as negociating, having allieds and anticipating what companions or opponents are going to do. Macaques are also able to use tools and exchange informations. They have traditions and some behaviours are transmitted over generations (e.g. to wash potatoes in water, to throw corn in the sea to separate grains from sand, to use a stick to hit a fruit or to put up branches against their park's enclosure). Macaques spend a lot of time searching food: fruits, seeds, buds and leafs, but also insects, flowers and mushrooms. They are active during day and sleep in trees during night, as they feel safer in height to protect against predators. Long-tailed macaques are commercialized for research, mainly in neurosciences, immunology, surgery and pharmacology (from animaldiversity.ummz.umich.edu/site/accounts/information/Macaca_fascicularis.html.; www.gsu.edu/~wwwvir/VirusInfo/macaque.html;en.wikipedia.org/wiki/Macaque;ww.encyclopedia.com;doc/1E1-macaque;pin.primate.wisc.edu/factsheets/entry/long-tailed_macaque).

In our laboratory, macaques are housed with peers of same sex. Generally, four individuals live together in a room of $15m^3$. Monkeys live thus in a very different situation as in their natural environment and as one can imagine, there are certainly consequences on their behaviour. Indeed, they do not have to search food, are forced to live in an artificial and closed environment with peers they would perhaps avoid in the nature, and they are subdued to experimental studies. Nevertheless, as in zoos, there are diverse tentatives to make their life comfortable. Indeed, sawdust is put on the floor of their room, a cylindrical tunnel and sometimes a big tree branch are placed in their room. They have toys and some experimenters play with their monkeys and stimulate them, for example by diversing food and rewards given in the experimental and housing rooms, often by hiding it. Moreover, monkeys are as much as possible housed with peers with who they don't have problematic relationships.

The ethics of the laboratory is to minimize as much as possible the experimental interventions, to prevent pain and to avoid experimental doggedness. As requested by the

Swiss law on animal protection, mental and physical bad treatments are forbidden. Of course, each individual researcher has his own ethical conception and the limits may slitly vary. That's why communication between experimenters, veterinary commission's visits and ethical commissions are very important. To note that in Switzerland, legislation is very strict and controls are severe (more than in other countries).

MK-JO

MK-AV

MK-JA

MK-WI

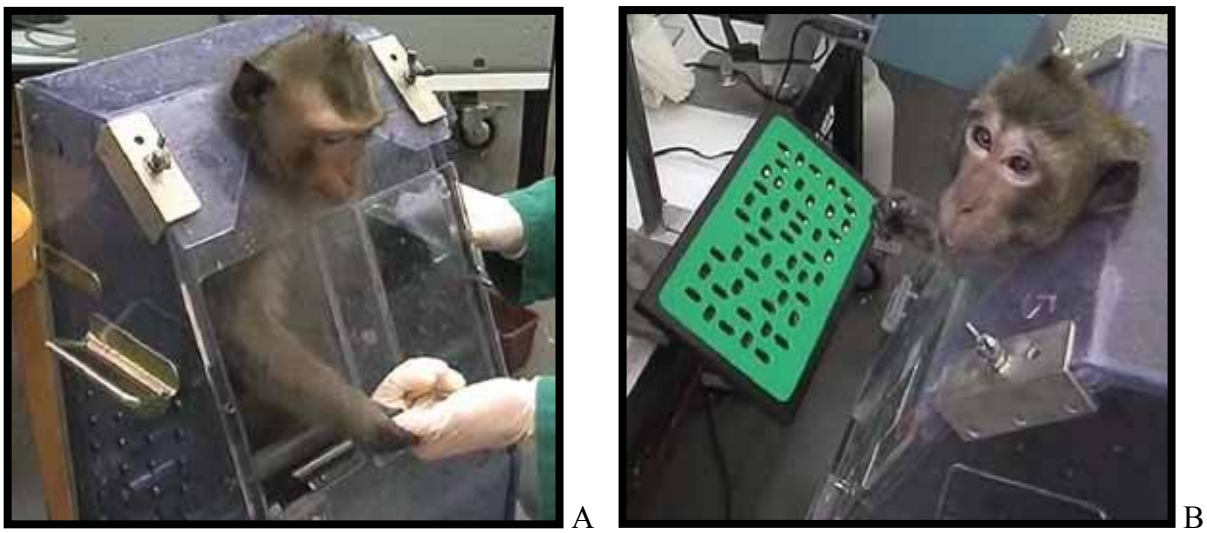
Figure 2.1.3: Macaque subjects of the present study.

For the present study, data were collected in a group of four male long-tailed macaques, Mk-JO, Mk-JA, Mk-WI and Mk-AV (Fig. 2.1.3), originating from China (3) and Vietnam (1). Time course of the events concerning each monkey figures in the Annexes part. Furthermore,

two monkeys (two females, Mk-PU and Mk-PR) took part to as pilot study evaluating the feasibility of autotransplantation (Brunet et al., 2005). Additionnally, various monkeys from other investigations but subjected to the same motor cortex lesion were taken into account for some aspects, in order to increase the inherent to primates studies low number of subjects. The monkeys weighted between 3 and 6 kg and ranged from 2.5 to 3 years old at the time of initiation of motor training sessions. All the behavioural, electrophysiological and surgical procedures were approved by the local ethical committee, in accordance with the Guidelines for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by Swiss veterinary authorities.

2.2 BEHAVIOUR

At their arrival in the laboratory, the monkeys were habituated to their new environment, including the two animal keepers and the experimenter. At seven o'clock in the morning during weekdays, the monkeys were placed by the animal keepers in two cages placed permanently inside their living room. At half past eight in the morning, the experimenter went in their room to work. In a first step, the objective was to establish a trust climate. In a second step, the objective was to train the monkeys to sit into a Plexiglas primate chair especially adapted for them and allowing displacing and restraining them during their work, but allowing free head movement (Fig. 2.2.1). These two steps took about three weeks.



A B

Figure 2.2.1 : Monkeys in a primate chair restraining them but allowing free movements. A. Monkey performing gross motor prehension. B. Monkey performing fine motor prehension.

The monkeys were seated on horizontal bars that can be adapted to their size, making their position comfortable. On the front panel of the primate chair, two sliding doors allowed testing separately the left or the right hand during the performance of a specific task. In a third step, the monkeys were brought in the experimental room (Fig. 2.2.2) to learn different manual dexterity motor tasks. They were not food deprived and had free access to water, but they did not eat before the training sessions, consisting in retrieving little banana pellets from wells (see below "Quantitative Tests" for details). After each task, the monkeys received rewards, such as raisins, almonds, hazelnuts, peanuts, and cereals.

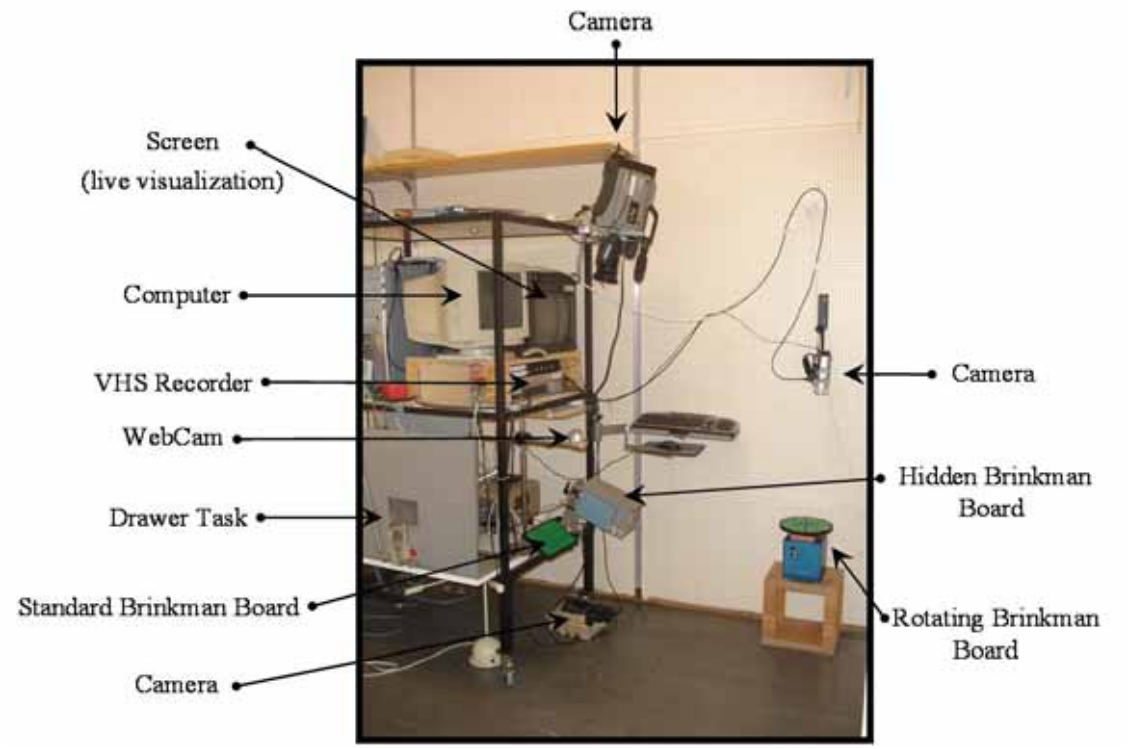


Figure 2.2.2: Experimental chamber.

The monkeys performed the motor tasks from daily to two times per week, with each hand alternatively and with a musical background to avoid as much as possible distractions due to external parasite noise. Each session was video recorded on VHS tapes for off-line analysis. For each monkey, the motor performances for the left and the right hands were analyzed individually. The work sessions were analyzed with a video recorder allowing frame per frame analysis, with a resolution of 25 frames per second. The data were analyzed and coded on analysis protocol sheets (see "Time course of experimental protocol, ICMS, Lesion and Cell reimplantation" in the Annexes part) and databases were then created on Excel. Graphics, panels and statistical analyses were performed using the softwares Excel, GraphPad

Prism, SigmaStat or SPSS. Some graphics were also produced with a home-made program in Matlab.

The behavioral measurements consisted mainly of quantitative tests assessing the hand dexterity of the monkeys. We also collected qualitative data observed during the training sessions. After having worked, the monkeys received additional food (food pellets, banana, apple, carrot, salad, bread…) and were free to move in their room and to interact with their peers. They were also observed in their room, and episodic behavioural observations were also collected, especially when something special happened, such as a change in hierarchy or a new type of vocalization.

2.2.1 QUANTITATIVE TESTS

The following manual dexterity tests were performed:

1) Modified Brinkman Board Task
2) Rotating Brinkman Board Task
3) Hidden Brinkman Board Task
4) Reach and grasp "Drawer" Task

The following parameters were analyzed (see "Analyses protocols" in the Annexes part):

- The number of pellets successfully grasped in 30 seconds. This parameter was analyzed only for the modified Brinkman board task.
- The total time spent to complete the task, and consequently the average time to successfully retrieve a reward by dividing the total time by the number of visited wells. Thus, the number of horizontal and vertical wells successfully visited was noticed. These parameters were not considered for the rotating Brinkman board task.
- The "contact time", which is the time the monkey took to retrieve each food pellet from the well. More precisely, it consisted in the time interval between the moment the monkey entered his finger in the well (T_0) and the moment at which the monkey exited the well with the reward grasped between the thumb and index finger (T_1) (Fig. 2.2.3).

For the modified Brinkman board task, this parameter was analyzed on the 5 first and 5 last vertical and horizontal visited wells (20 wells in total). For the rotating Brinkman board task, this parameter was analyzed on all the wells (32 wells in total).

- The sequence of prehension, which is the order in which the animal took the food pellets.

For the modified Brinkman board task and the hidden Brinkman board task, each well was numerated.

For the rotating Brinkman board task, additional parameters were noticed: absolute well orientation, ring, sector when entering the fingers in the well, sector when exiting the fingers from the well, thus relative well orientation when entering and exiting from the well (for details, see below "Rotating Brinkman Board Task").

- The errors, consisting mainly of the loss of food pellets then falling on the floor, but also pellets displaced to another well or pellets retrieved with the other hand.
- The position of the wrist for the horizontal wells (Radial Abduction / Cubital Adduction) (Fig. 2.2.6). For the hidden Brinkman board task, this parameter was not considered.
- The first finger introduced in the horizontal wells (thumb or index). For the hidden Brinkman board task, this parameter was not noticed.
- If the monkey grasped two pellets at the same time, which was a prehension's strategy used by some monkeys. For the hidden Brinkman board task, this parameter was not noticed.


A


B

Figure 2.2.3 : Contact Time. A. The monkey entered the well at time T_0. B. The monkey exits the well with the pellet between its fingers (index and thumb) at time T_1.

2.2.1.1 Modified Brinkman Board Task

The modified Brinkman board task is a fine motor task evaluating manual dexterity, first elaborated by Brinkman and Kuypers (1973 ; Fig. 2.2.4) and modified in our laboratory by Kermadi et al. (1997), ,Rouiller et al. (1998) and Liu and Rouiller (1999).

Figure 2.2.4 : Brinkman board developed by Brinkman and Kuypers (1973).

The modified Brinkman board task consists in a green Perspex board (20cm x 10cm) containing 50 numerated oval wells, 25 oriented vertically and 25 oriented horizontally (Fig. 2.2.5). The wells measure 14mm x 7mm and are 6mm deep. The width of the wells is slightly larger than the width of the monkey's fingers. The board is inserted within a black plastic frame and the setup is held by an aluminum rod in the experimental chamber in order to keep it in a stable position with respect to the primate chair.

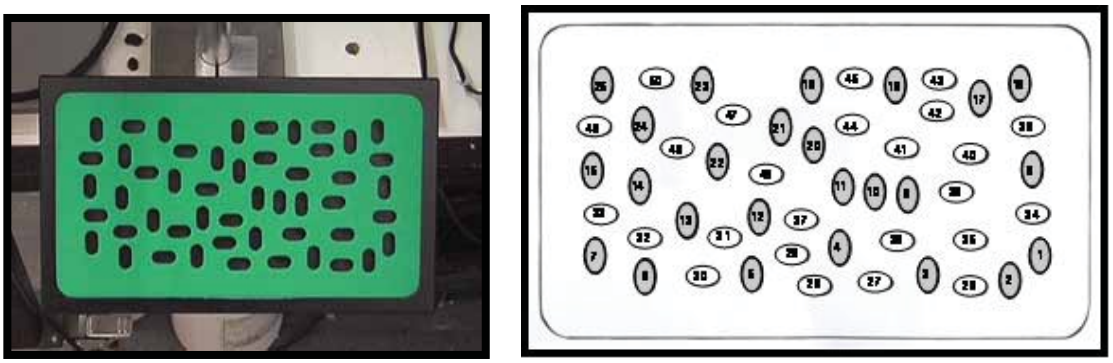


Figure 2.2.5 : Modified Brinkman board used in the present study as seen from above. It contains 25 vertical and 25 horizontal numerated wells.

Before the beginning of a behavioural session, a banana food pellet of 4mm in diameter was placed in each well. The animal was then positioned in front of the 40° inclined board and access was given to one of his hands. The monkey then grasped the food pellets by using the so-called precision grip, which requires the opposition of the thumb and the index

finger. Grasping pellets out of horizontal slots needed in addition a rotation of the wrist (Fig. 2.2.6).

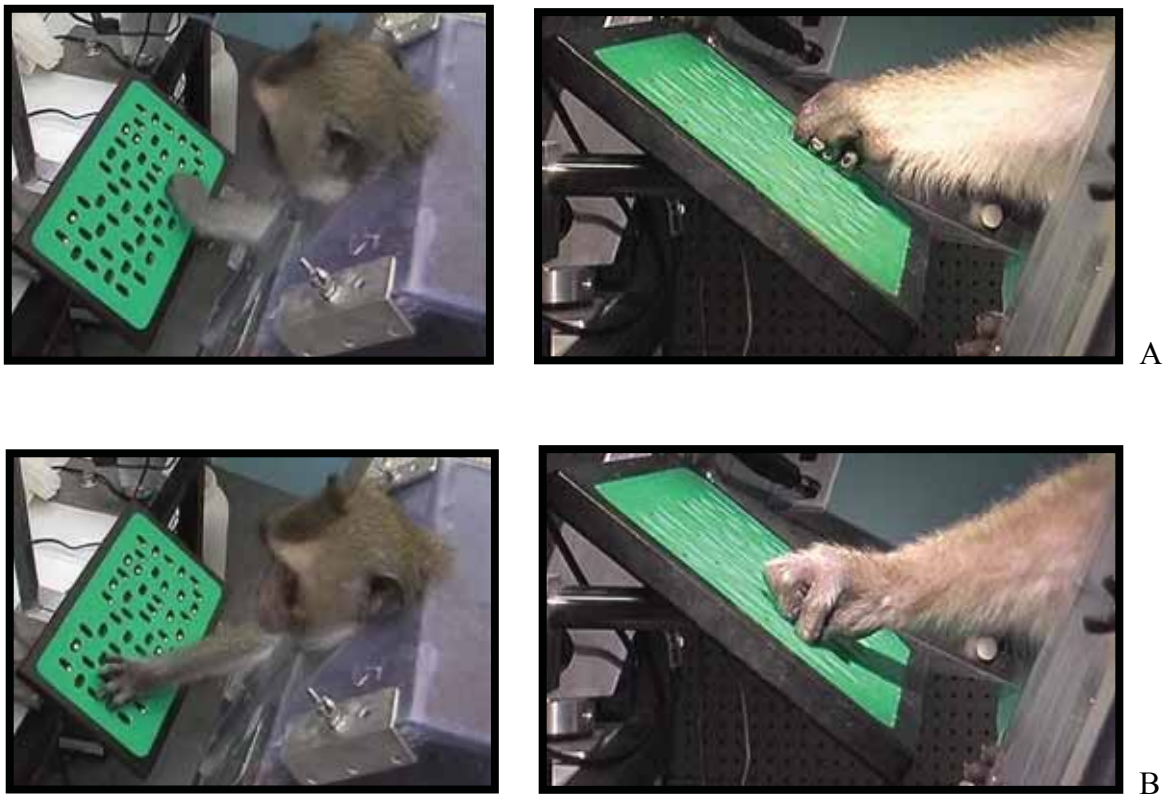
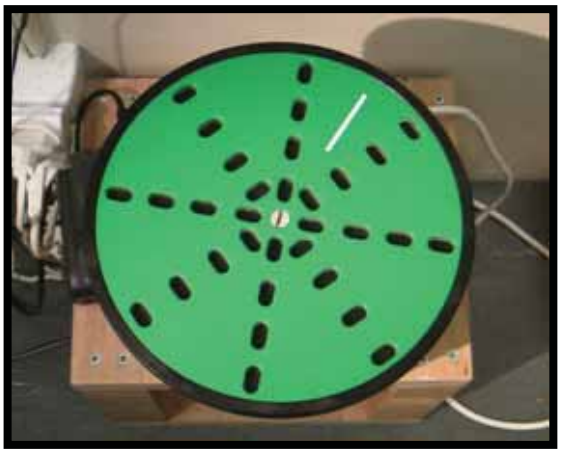


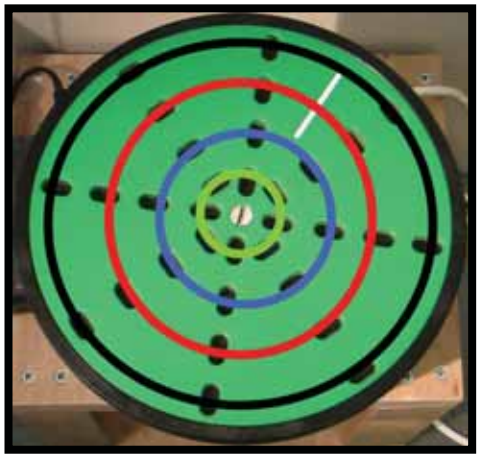
Figure 2.2.6 : Position of the wrist for the horizontal wells. A. Cubital Adduction. B. Radial Abduction.

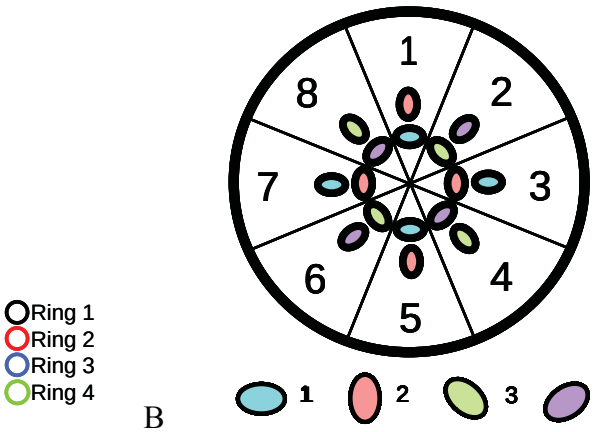
2.2.1.2 Rotating Brinkman Board Task

The rotating Brinkman board task (Fig. 2.2.7A) is an adaptation of the modified Brinkman board task, taking into account dynamic aspects of movements. It consists in a green Perspex circular board (diameter = 21.5 cm), containing 32 oval wells, with orientations corresponding to vertical and horizontal positions when the wells are in front of the monkey, and organized in four rings (Fig. 2.2.7B). The wells measure 14mm x 7mm and are 6mm deep. The board can rotate, either clockwise or counterclockwise. During the board rotation, the wells orientation thus changes. Therefore, four different relative orientations were defined (Fig. 2.2.7C): vertical, horizontal, 45° left, 45° right. Thus, the rotating Brinkman board was divided in 8 sectors.



A





B C

Figure 2.2.7 : Rotating Brinkman board as seen from above (A). It is organized in four concentric rings of wells (B). Four different relative orientations were defined: horizontal, vertical, 45° left, 45° right. Thus, the rotating Brinkman board was divided in 8 sectors (C).

As for the modified Brinkman board task, before the beginning of a behavioural session, a banana food pellet of 4mm in diameter was placed in each well. The animal was then placed in front of the board oriented in the horizontal plane and one of his hands had access to the board. The monkey then grasped the food pellets by using precision grip, but he had to adjust his prehension's movement to the rotation of the board. The velocity of the board rotation could be changed; in the present case, a velocity of 10 turns per minute was arbitrarily chosen.

2.2.1.3 Hidden Brinkman Board Task

The hidden Brinkman board task was developed because the modified Brinkman board task depends mainly on visual control and moderately on feedback from skin mechanoreceptors. Indeed, due to the kind of lesion performed in this study (M1 ibotenic acid lesion by tract penetrations), the primary somatosensory cortex (S1) could also be altered (Liu

and Rouiller, 1999). Furthermore, the primary motor cortex receiving sensory information (Naito, 2004), lesioned monkeys could have not only motor but also sensory deficits. The hidden Brinkman board task should be a tool to distinguish between sensory and motor deficits, as a spread of the lesion to S1 would have a higher impact on performance without visual feedback.

The hidden Brinkman board task consists of a plastic box (Fig. 2.2.8A) containing a board with 20 numerated wells, 10 vertically and 10 horizontally oriented (Fig. 2.2.8B) and having a sliding top panel. When it is open, the monkey has a visual feedback on the board and on his hand; when it is closed, the monkey has no visual feedback and proceeds with his tactile and proprioceptive senses to find rewards. As for the other tasks, the monkey was placed in front of the box and access was given to one of his hands. The width of the entrance of the box was large enough for the monkey to place its hand inside and rotate its wrist easily. The monkey was trained to reach inside the box first with visual feedback and then without visual feedback.

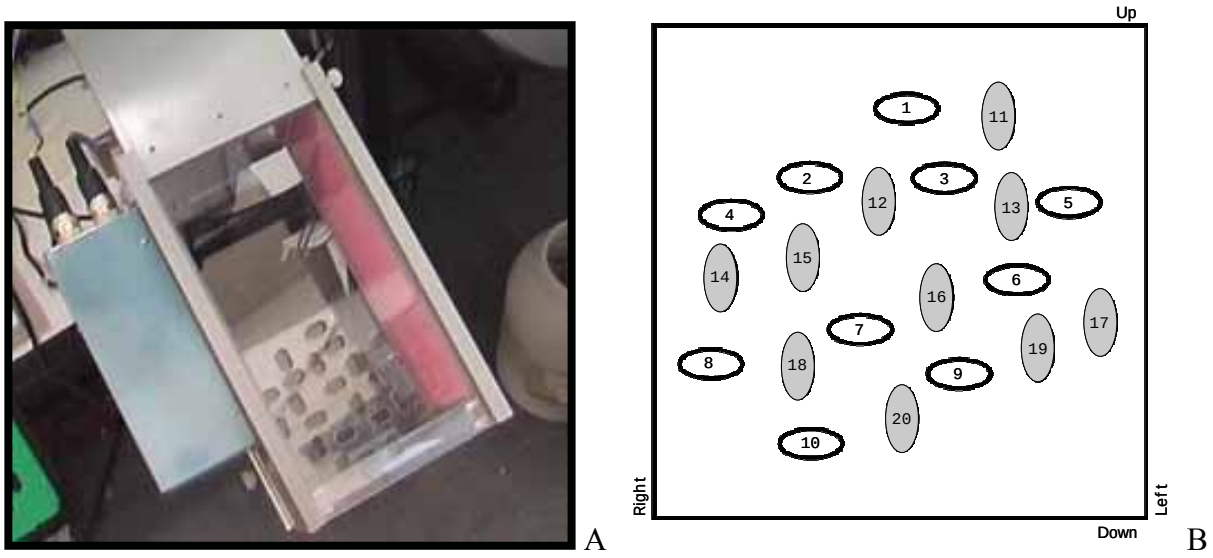


Figure 2.2.8 : Hidden Brinkman board. A. The top, back and side panels were made from non-transparent PVC. In contrast, the bottom of the box was made of transparent Plexiglas, allowing video-recording of the hand from below. B. The Hidden Brinkman Board contains 20 numerated wells, 10 vertically and 10 horizontally oriented.

2.2.1.4 Reach and grasp "Drawer" Task

In the Drawer Task (see Fig. 2.2.9), the monkey performed the task with each of the two hands, for 30 consecutive trials.

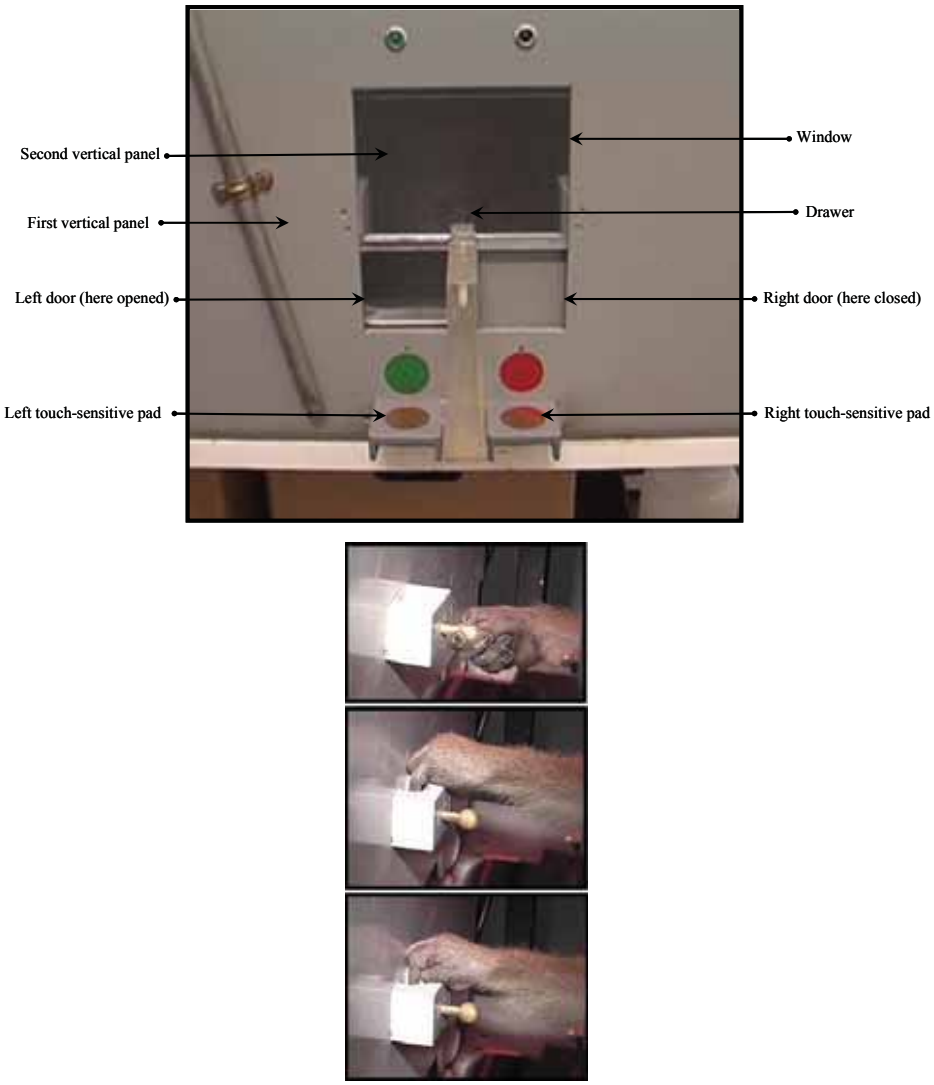


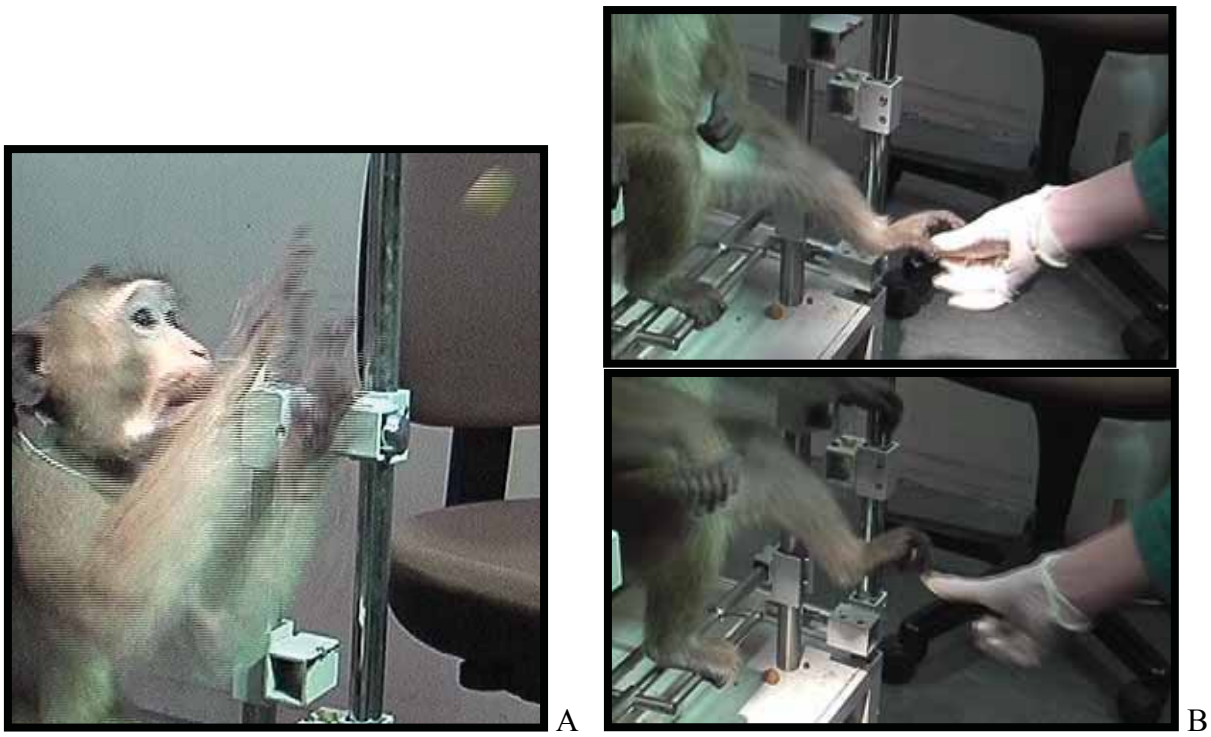
Figure 2.2.9 : "Drawer" Task.

Concretely, the monkey was placed in front of a vertical panel and he had to put his hands on two horizontal touch-sensitive pads, triggering after one second the opening of a vertical door (7x7mm; left or right, depending on the hand tested). Then, he had to pull a drawer placed on a vertical panel (placed 10cm behind the first panel) and which he could see through a window (14x9mm), and to pick up a little food banana pellet using precision grip, the whole with the tested hand, while the other hand remained on the pad. The door then closed, and the monkey restarted. Reaction times data were acquired on a Macintosh Computer using a Mac AD 2.4.2 software.

Unfortunately, due to technical reasons, too much data were lacking after the monkeys were subjected to the motor cortex lesion. Therefore, this task was not taken into account in the analyses of the present study.

2.2.2 QUALITATIVE OBSERVATIONS IN THE PRIMATE CHAIR AND IN THE ANIMAL HOUSE

The qualitative observations were not performed as systematically as the quantitative observations. Indeed, they served as additional information. First, motor capacity was tested further qualitatively using other tests performed on a weekly basis, at the end of a behavioral session, with the monkey only maintained at the neck level. On one hand, the "Ballistic Arm Movements" (BAM) test (Fig. 2.2.10A), evaluating the monkey's capacity to make rapid catching movements, was performed. The monkey had to catch pieces of food thrown by the experimenter from a distance of about 1 meter. In the BAM test, synchrony of the two hands and the pre-shaping ability to open the fingers before retrieving the approaching piece of food were assessed. On another hand, the "hindlimb grasp" test (Fig. 2.2.10B), allowing to assess whether hindlimb motor function was affected by the cortical lesion, was performed. The monkey had to grasp a large piece of food accessible only with one or the other foot, and then to transfer it from the hindlimb to the hand. Note that the aim of performing focal lesions (see below for details) was to avoid an impact on hindlimb performance. For both tasks, video sequences were recorded.



A B

Figure 2.2.10 : A. The "Ballistic Arm Movements" test. B. The "hindlimb grasp" test.

Second, a general observation of the monkey was done each day, consisting in taking time to observe the general behaviour and state of the monkey, such as his movements, vocalizations and behaviour with his partners in the detention room, such as grooming, playing or catching. Third, another kind of observations was done after each medical event -especially after a lesion-, consisting in the same kind of observations as the daily general ones, with a particular focus on the movements and well-being state, and in case of lesion, on the motor capacity: locomotion, limbs affected, gross and fine motor capacity. Some video sequences were recorded. Fourth, some observations were done regularly before and after the lesion, consisting in the assessment by the experimenter of the monkeys' behaviour and state in the experimental room and in the housing room (Table 2.2.1).

	Shivers	**Scratching (self or other)**	**Rotations in the chair**	**Chair exit movements**	**Distress shouts**	**Sum**
Score (0-4)						

A

	Loss of perseverence	Distraction	Refusal to perform the task	Loss of reactivity to pleasant stimuli	Motor immobility Dumbness	Sum
Score (0-4)						

B

	Prostration	Social isolation	Loss of reactivity to pleasant stimuli	Motor immobility Dumbness	Stereotypies	Distress shouts	Bizarre voluntary movements	Flight reaction	Motor excitement	Sum
Score (0-1)										

C

	Observations in the detention room (C)	Anxiety (A)	Humor (B)
Total mean score			
	0-9	0-20	0-20

D

	Banana pellets	**End-task rewards**	**Pellets**	**Banana**	**Apple**	**Other**
Yes-No/Qantity						

E

Table 2.2.1 : Qualitative observations. A. Observations in the experimental room: anxiety. B. Observations in the experimental room: humor. C. Observations in the detention room. D. Total observation scores. E. Observations regarding appetite.

Different criteria were chosen on the basis of bipolar qualifications (Gendre and Capel, 2000) used with humans to quantifiy their anxiety and humor state. Concretely, in the experimental room, two different tables of behavioural observations were used; one concerned anxiety and the other concerned humor. For both, five different criteria were noted from one to four, giving a final score ranging from zero to twenty. In the detention room, another table was used to assess the animals' well-being. It contained 9 dichotomic criteria noted from zero (no) to 1 (yes), giving a final score ranging from zero to nine. Together, the scores in these three tables (anxiety, humor and observations in the detention room) led to three total mean scores indicating eventual changes in the state of the monkey. Another important observation was done on the appetite of the monkey, which is also an indicator of the monkeys' state. Along this line, the weight of the monkeys was checked before every behavioural session.

2.3 SURGERY

2.3.1 ANIMAL CARE FOR SURGERY

After completion of training and once the monkey reached a behavioural plateau, the animal was subjected to the following surgical procedures.

Before taking the monkey to the surgery room, it was prepared by shaving the operative site, cleaning it with betadin and after with alcohol. For all surgeries, the monkey was first tranquilized with ketamine (Ketalar®; Parke-Davis, 5 mg/kg, intramusculary); atropine was injected (0.05 mg/kg, intramusculary) in order to reduce bronchial secretions. Before surgery, the animal was treated with the analgesic Carprofen (Rymadil®, 4 mg/kg, subcutaneously) and the antibiotic Albipen® (Ampiciline 10%, 30mg/kg, subcutaneously). Subsequently, the monkey was anaesthetized with intravenous perfusion of 1% propofol (Fresenius®) mixed with a 5% glucose solution (1 volume of propofol and 2 volumes of glucose solution); ketamine was added to the perfusion solution (65 mg/100 ml). To prevent oedema of the CNS, Methylprednisolone (Solu-medrol, Pfizer ®) was added to the propofol/glucose solution (1 mg/ml). The level of anaesthesia was kept at an optimal level with a perfusion rate of the propofol/glucose mixture of 0.1ml/min/kg. For some surgeries (some lesions and reimplantations; for details, see below), the monkey was anaesthetized with a domitor (medetomidin chlorhydrate: 1 volume) and ketalar (ketamin chlorhydrate: 2 volumes) mixture. After completion of the surgery, an Antisedan® (0.15ml) injection (antagonist of domitor) was made for the monkey to wake up well and rapidly.

In the surgery room, the animal was placed in a stereotaxic framework, with local anaesthetic put on the ear bars in order to reduce pain possibly originating from the ear canals. Before the incision, the operative site was covered with antimicrobial incision drape (Steri-drape 3M™ Ioban™ 2) to thoroughly dry the intact skin. All surgeries were performed in a facility approved by the local cantonal veterinarian, with strict attention to sterile technique. Heart rate, respiration rate, expired CO_2, arterial O_2 saturation and rectal temperature were carefully monitored throughout the surgery. After each surgery, the monkey was under observation until coming out of the anaesthesia, about 30-60 minutes after interruption of the propofol perfusion, and started to eat and drink. The monkey was placed alone in a separate cage for a couple of days to allow better conditions for recovery, and received Carprofen (pills of Rymadil mixed with food) daily and Albipen® (subcutaneously) every two days during one week.

2.3.2 CORTICAL CHAMBER IMPLANT

Each monkey was chronically implanted with a cortical recording chamber for electrophysiological investigations (Fig. 2.3.1).

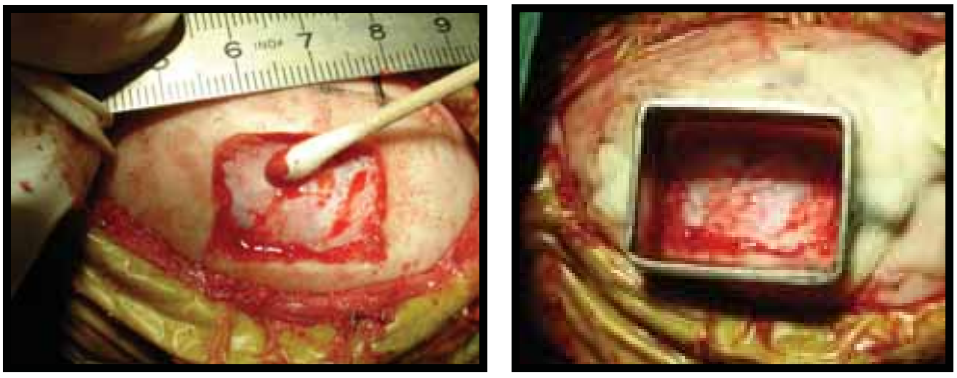


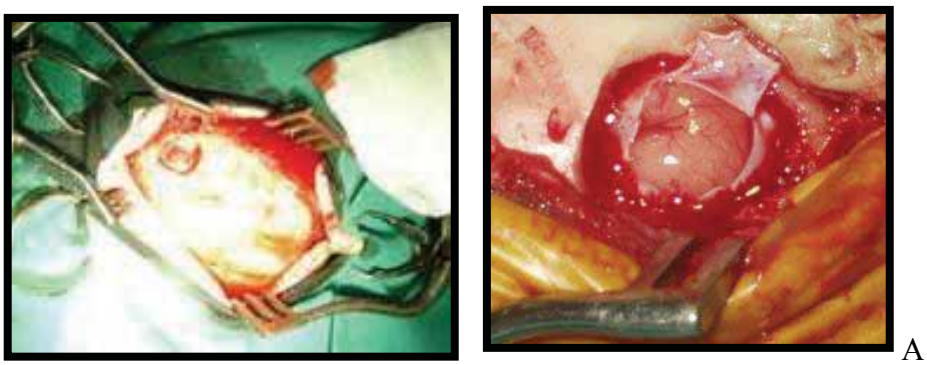
Figure 2.3.1: Implantation of the recording chamber over M1 and S1.

A rectangular stainless steel recording chamber was stereotaxically implanted over the forelimb area on the left hemisphere of each monkey as described in previous studies (e.g. Liu and Rouiller, 1999). The recording chamber was centered at stereotaxic coordinates 15 mm rostral and 15 mm lateral, and its shape was adapted to fit the profile of the monkey's skull allowing for perpendicular penetrations with microelectrodes in the brain. The skull was opened at a location and an area corresponding to the inner dimension of the recording chamber. The recording chamber was anchored to the skull with titanium screws (Synthes®,

Cortex screw). The whole implant was secured to the skull by dental acrylic cement and/or by orthopedic cement (Palacos® 40 Gentamicin 500 mg). The size of the recording chamber was 22 x 17 x 15 mm for three monkeys (Mk-JO, Mk-JA and Mk-AV), and 28 x 19 x 17 mm for one monkey (Mk-WI). At the end, the skin around the chamber was sutured.

2.3.3 BIOPSY OF PREFRONTAL CORTICAL TISSUE

As the treatment used in this study consisted in reimplanting autologous cells in the lesioned site (Brunet et al., 2005), a biopsy in the right dorsolateral prefrontal cortex (dlPFC) was first performed (Fig. 2.3.2) to collect adult progenitor cells.



A

B

Figure 2.3.2 : Biopsy. A. Skull and dura mater opening above the dlPFC. B. Cortical tissue extracted was immediately placed in cold culture medium.

A squared osseous sector of 8 mm x 8 mm was opened above the dlPFC. Then, an approximate volume of 8 mm^3 of cortical tissue was extracted using a surgical blade (no 11, Paragon®) and placed in cold culture medium. The osseous sector was then replaced on the brain and sewed at two sides to the skull. The muscle and the skin were then sutured.

2.3.4 MOTOR CORTEX MAPPING

In order to locate the hand representation in M1, the motor cortex of each monkey (for Mk-WI, only before the fifth lesion; for details, see "Time course of experimental protocol, ICMS, Lesion and Cell reimplantation" in the Annexes part) was electrophysiologically (ICMS) mapped before the lesion. The mapping started when the monkey reached the behavioral plateau and recovered from the cortical surgery for chamber implantation. Tungsten Mylar insulated electrodes with impedances between 0.7-1.5 MΩ (Frederick Haer & Co., Bowdoinham, Maine, USA) were used for ICMS mapping. Two different systems were used. First, for Mk-JO, Mk-AV and four lesions of Mk-WI, a stereotaxic apparatus that held an electrode was placed on a stereotaxic frame and inclined at 30° to be perpendicular to the surface of the dura. Second, for Mk-JA and for the fifth lesion of Mk-WI, a new system, a manual hydraulic microdrive (Narishige group, Japan, model MO-95), attached to the chronic implanted chamber, was used. In this second system, in order to guarantee a perpendicular penetration into the cortex, the needle was secured by a guiding tube. Stimulation was performed at 1 mm intervals, starting from 2 mm below the dura to a maximal depth of 10 mm depth.

A classical intracortical microstimulation (ICMS) paradigm (Asanuma, 1976) was used in awake monkeys. The ICMS consisted of trains of 12 pulses delivered at 330Hz. Individual stimuli were 0.2 ms negative pulse generated by a stimulator and a stimulus isolation unit ("WPI"). The highest intensity was limited to 80μA. The aim of ICMS was to activate corticospinal neurons, consequently motoneurons which activate one or several muscles, thus producing a visible movement or muscle contraction detectable by palpation. At the first stimulation site of a given track, the highest intensity was used until it produced a movement or muscle contraction; after that, the stimuli intensity was reduced to a level below which no effect was observed. This value was considered as the threshold for that given stimulation site. The experimenter held the monkey's arm during the lowest stimulation intensities to have a better feeling of the movement and muscle contraction. Moreover, by manipulating the arm in different positions it was possible to facilitate a joint movement which was not obvious in another arm positions. Depending on the monkey's cooperation, 2 to 5 tracks were made per session. Once the monkey became impatient, the ICMS session was stopped, and the monkey was brought back to the animal room. Generally, the ICMS mapping lasted about 20-30 days.

ICMS data collected were represented in a standard (surface) ICMS map, as described in previous studies (Rouiller et al., 1998; Liu and Rouiller, 1999; Schmidlin et al, 2004, 2005). The representation of the different parts of the forelimb took into account only the lowest ICMS threshold for each electrode penetration that elicited a movement of a particular joint as well as the depth at which the movement was observed. This site of the lowest intensity of stimulation was projected on the surface of the motor cortex in the form of circle. The threshold current intensity of the stimulus used to elicit the movement was also indicated by the size of the circle. In fact, the larger the circle size, the lower the intensity of the stimulation.

2.3.5 PRIMARY MOTOR CORTEX LESION

The cortical lesions, restricted to the hand area of M1 unilaterally, were performed by multiple injections of ibotenic acid (either Fluka 99% or Sigma 95%), as previously reported (Liu and Rouiller, 1999). Ibotenic acid, first isolated from Amanita muscaria in 1964 by Takemoto et al., is thus a substance initially extracted from amanita mushrooms (Fig. 2.3.3), causing motor depression, ataxia and changes in mood, perceptions (such as hallucinations) and feelings. Ibotenic acid is a powerful neurotoxic, an agonist of glutamate, the most frequent released excitatory transmitters by synapses in the mammalian brain (Fig. 2.3.4).

Figure 2.3.3 : Amanita mushrooms providing ibotenic acid. From left to right, Amanita pantherina and Amanita muscaria.

Ibotenic acid causes neuronal death by over-excitation through excessive activation of ionotropic NMDA receptors and neuronal damage of the tissue surrounding the injection site. This lesion technique generates a clearly circumscribed damage at the injection site. Another advantage of ibotenic acid compared to other neurotoxic agents is its selective toxicity for nerve cells in the injected area while axons of passage and nerve terminals of extrinsic origin do not seem to degenerate. A third advantage of ibotenic acid is the rapid degradation into muscimol after intracerebral injection which may lead to minimal general toxicity.

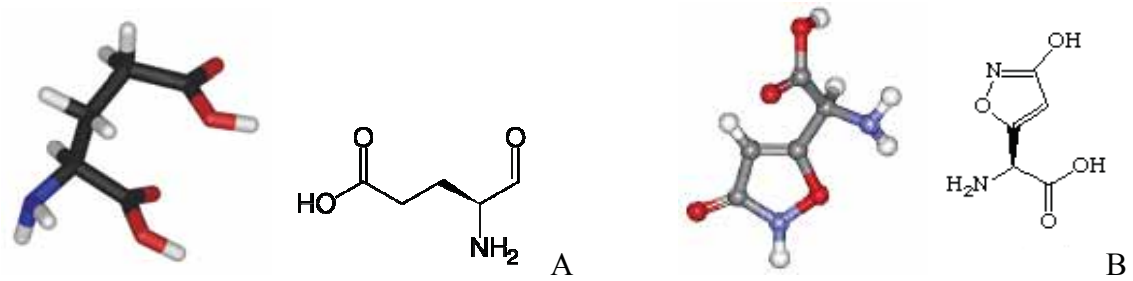


Figure 2.3.4 : Comparison of the chemical structure of glutamate (A) and ibotenic acid (B).

Two types of motor cortex lesions, restricted to the hand representation's area in M1 unilaterally, were performed in this study (Fig. 2.3.5). One type was performed stereotaxically, that is based on the knowledge of the macaque's M1 somatotopy. Concretely, the center of the hand representation is known to be situated at 13-15 mm rostral and 15 mm lateral from the mid-interaural point. This stratregy was used for a monkey without chronic chamber implantation (Mk-WI; three lesions on the right hemisphere, one lesion on the left hemisphere). Another type of lesion was performed on the basis of ICMS mapping, allowing to define precisely the hand territory in M1 (for details, see below "Motor cortex mapping"). For this second type of lesion, which was mainly used in this study, pre-lesion mapping of the forelimb area in M1 of the left hemisphere by ICMS was used to determine the sites of ibotenic acid injections. For each monkey the lesion was made in the hand representation of M1 in the left hemisphere. Injection sites were selected considering the type of movements elicited at each site in ICMS sessions and the stimulation intensity needed to induce a movement in order to cover the hand representation area with a limited number of injections. The sites where ibotenic acid was injected corresponded to the ICMS sites that produced fingers movement. A volume of 1-1.5 µl of ibotenic acid solution (10 µg/µl in phosphate-buffered saline; see Newsome et al. 1985a, b; Merigan et al. 1993; Newsome and Paré 1988; Liu and Rouiller 1999) was injected at each site. The total volume of ibotenic acid injected in each monkey ranged from 15 µl to 60 µl. Ibotenic acid is expected to diffuse approximately 1.5 mm around the center of the injection site. We anticipated that such diffusion distance would cover the target area. Depending on the monkey and on the lesion, 5 to 23 syringe penetrations were made, at 1 to 3 depths.

During the injections of ibotenic acid in the operating room, the monkeys were anaesthetized either with propofol (Mk-JO; Mk-AV; Mk-WI, three lesions in the right hemisphere and first lesion in the left hemisphere) or with a domitor (medetomidin chlorhydrate: 1 volume) and ketalar (ketamin chlorhydrate: 2 volumes) mixture (Mk-WI,

second lesion in the left hemisphere; Mk-JA, two lesions). Two different xy positioning systems, same as for ICMS performing, were used for the lesion, but the tungsten microelectrode used in ICMS sessions was replaced by a 10µl Hamilton syringe. Once the Hamilton syringe was precisely positioned at the aimed location at the surface of the dura, it was then slowly advanced into the cerebral cortex until the tip reached the deepest site in the corresponding tract, where the first injection was made. A volume of 1 µl or 1.5 µl of ibotenic acid solution was slowly injected at each site. The needle was then left in place for at least 1 minute, for the ibotenic acid to diffuse in the adjacent tissue.

Afterwards, monkeys were brought back to the animal room and videotaped regularly in the following hours, in order to exclude or confirm possible effects of ibotenic acid on other regions of M1 than the region originally chosen, and to document the deficits during the first hours after the lesion.

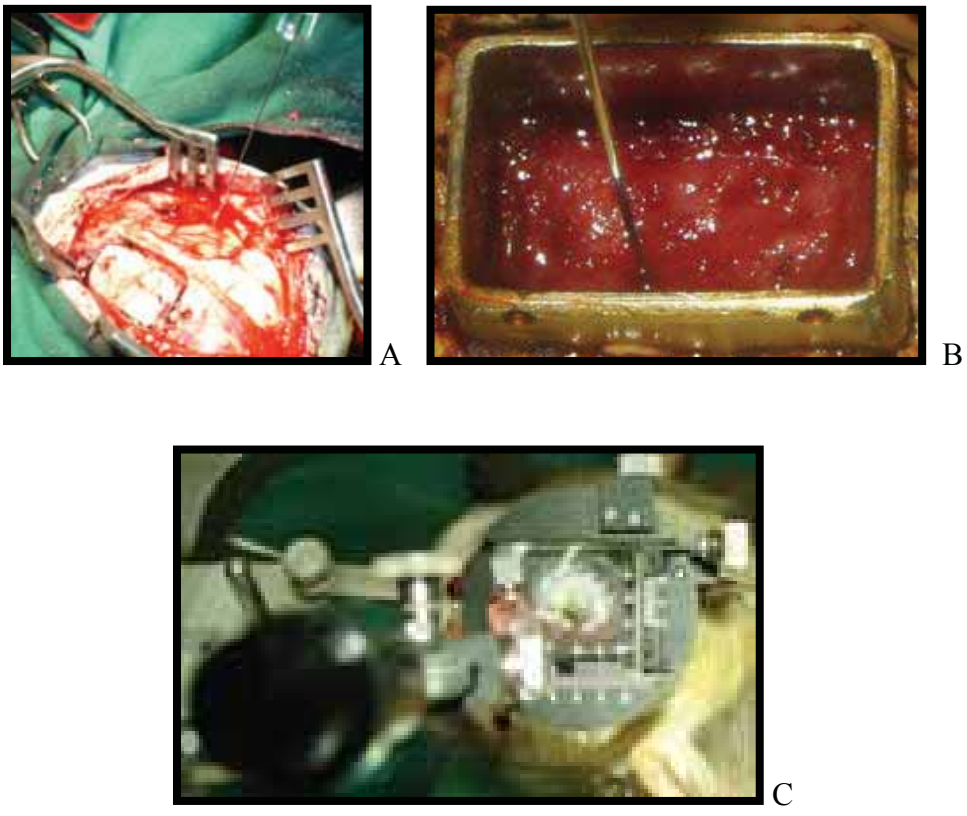


Figure 2.3.5 : Lesion of M1 hand area with a 10 µl Hamilton syringe, performed with a system placed on a stereotaxic frame and inclined at 30° to be as perpendicular as possible to the surface of the dura, either without **(A)** or with a chronic chamber **(B)**. **C.** Lesion performed with a stereotaxic system attached to the chronic implanted chamber. The tungsten microelectrode used in ICMS sessions was replaced by a 10 µl Hamilton syringe.

2.3.6 CELL IMPLANTATION

Once the monkeys reached a spontaneous functional recovery plateau, the treatment was administred. Indeed, the idea was to distinguish the benefit of the treatment from the spontaneous functional recovery. The treatment consisted in reimplanting autologous neural progenitor cells sampled from the dlPFC into the lesioned site area (Fig. 2.3.6).

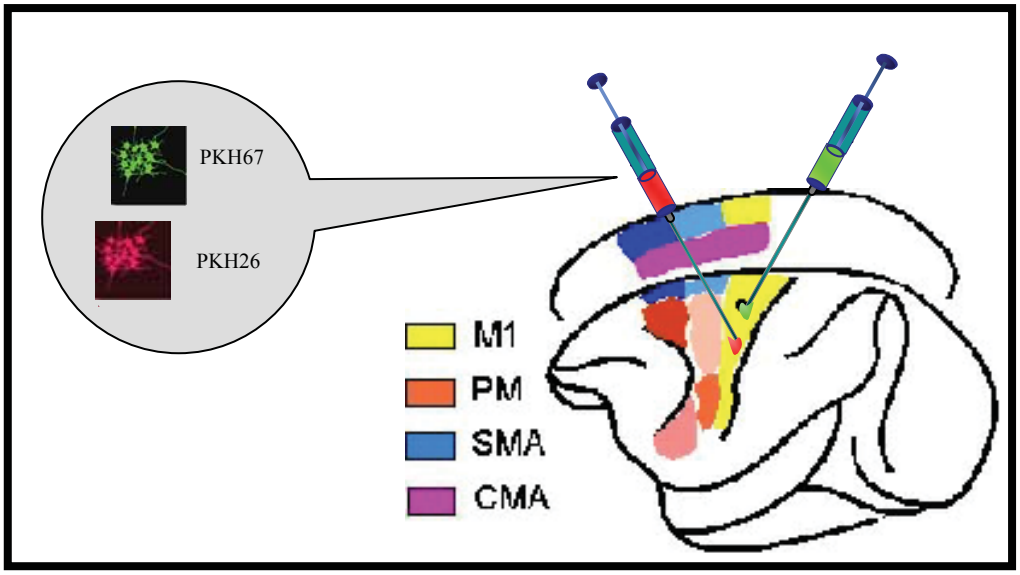


Figure 2.3.6 : Scheme of reimplantation of autologous NPCs performed into and /or near the lesioned area.

The systems to reimplante cells (Mk-JO, Mk-JA) or medium (Mk-AV) were the same as for the lesion. 4 to 8 syringe penetrations were made at 1 to 2 depths. During the reimplantations performed in the operating room, the monkeys were anaesthetized either with propofol (Mk-JA, second implantation) or with a domitor (medetomidin chlorhydrate: 1 volume) and ketalar (ketamin chlorhydrate: 2 volumes) mixture (Mk-JO; Mk-AV; Mk-JA, first implantation). A similar procedure as that used for the lesion was applied. Once the 10µl Hamilton glass syringe was stereotaxically positioned at the aimed location at the surface of the dura, it was slowly advanced into the cerebral cortex until the tip reached the deepest site in the corresponding tract, where the first injection was made. A volume of 5 µl to 25 µl culture medium with or without cells solution was slowly injected at each site. The needle was left in place during about 2 minutes, for the cells to diffuse. The total volume implanted ranged from 20 to 200 µl and contained 250'000 cells in one monkey (Mk-JO), and 600'000 transplanted cells and 150'000 transplanted cells in the two reimplantation time points, respectively, in the other monkey (Mk-JA) (see "Time course of experimental protocol,

ICMS, Lesion and Cell reimplantation" in the Annexes part). To note that for Mk-JO and Mk-AV, the experimenter was blind.

2.4 CELL PREPARATION

2.4.1 CELL CULTURE

Cell culture was described in details by Brunet et al. (2005) and consisted in the following procedure (Fig. 2.4.1).

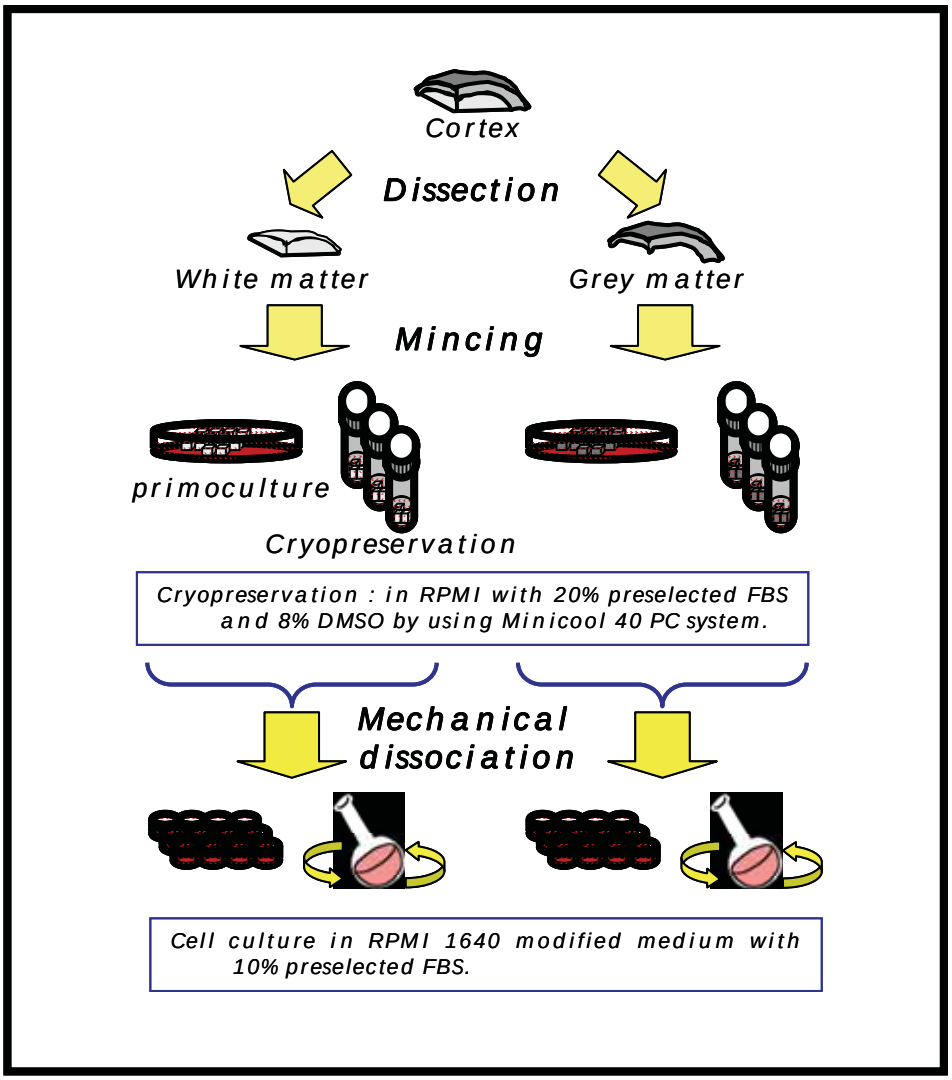


Figure 2.4.1 : Scheme of cell culture (from Brunet et al., 2003).

The cortical biopsies were dissected with a razor blade to obtain enriched fractions of gray matter. Primary cultures were generated by mincing and mechanically triturating the tissue with fire-polished glass pipettes of decreasing diameters under sterile hood.

The resulting cell suspensions were counted by diluting cell suspension 1:10 in trypan blue stain (T9520 Sigma). Cells were resuspended at 50,000 cells/ml in in RPMI (Roswell Park Memorial Institute)-1640 Medium (Gibco) without L-Glutamine (31870-025, Invitrogen AG, Basel, Switzerland) supplemented with NaHCO3 44 mM, 20% preselected Fetal Bovine Serum (pFBS), and an antibiotic/antimycotic cocktail (A7292 Sigma-Aldrich, St Louis, MO, USA) directly into 25 ml-glass Erlenmeyer at 37°C in a water-saturated atmosphere containing 6.5% CO2/93.5% air under horizontal agitation at 70 rotations per minute. A cell suspension was plated on glass coverslips into a 24 well plate in the same incubator conditions. Fifteen days later, the concentration of serum was reduced to 10%. After 20–45 days when cells became confluent on coverslip, cells were cultured in medium without serum and were maintained in these conditions until reimplantation.

2.4.2 TISSUE CRYOPRESERVATION

Paralelly to the cell culture, for each monkey, five tubes were put in cryopreservation. Indeed, Brunet et al. (2003) developed a method to cryopreserve small amounts of adult human and non-human primate brain tissue using a cryopreservation medium containing 20% preselected fetal bovine serum (pFBS). After having obtained enriched fractions of white matter (WM) and grey matter (GM), small pieces (around $1mm^3$) of brain tissue were put in 250 ll of DMEM7a with 20% pFBS and 8% dimethylsulfoxid (DMSO) (D5879 Sigma, Buchs, Switzerland) into a 1 ml conical Nunc CryoTube with wings and with external screw pitch (Nunc No. 375353, Nalge Nunc International, Kamstrup, Denmark). The freezing procedure was accomplished with a MiniCool 40 PC system (AirLiquide, Marne-la-Vallée, France), using liquid nitrogen to cool down to -196°C under programmable condition. This Minicool 40 PC system allows to define the crystallisation point, -8°C in our case, and to control the optimal freezing procedure by cooling down at -1 °C per minute as described by the supplier. Our pieces of brain tissue were frozen in DMEM7a with 20% pFBS and 8% DMSO according to these recommended conditions and tubes were then stored in liquid nitrogen at -196°C, up to 2 years.

To prepare cell cultures from the cryopreserved WM and GM fractions, cryotubes were snap thawed in a water bath at 37°C for less than 1 min. At this step, the design of cryotubes could also play a important role to obtain a rapid and homogenous thawing. As soon as ice has melted, the thawed pieces of WM or GM fraction tissue were rapidly recuperated and rinsed three times in DMEM7a to wash the DMSO. As for primary cultures,

pieces of tissue were minced and mechanically triturated with fire-polished glass pipettes of decreasing diameters. Cells were plated at 250,000 cells/cm2 in DMEM7a with 10–20% pFBS directly on glass coverslip for immunocytochemistry or on plastic dishes at 37°C in a water-saturated atmosphere containing 6.5% CO2/93.5% air. Fifteen days later, the concentration of serum was reduced to 5–10%. Regularly, when a significant acidification is observed according to the color change of red phenol, third of medium was changed with new medium. After 50–60 days when cells became confluent, cells were progressively cultured in medium without serum and were maintained in these conditions up to a maximum of 100 days.

Overall, using optimized conditions (use of preselected fetal bovine serum, preparation of enriched fractions of white or grey matter, mechanical dissociation, seeding on uncoated culture dishes), we succeeded with a success rate of 100% in preparing long-term cultures of human brain cells from cryopreserved adult brain tissue.

Observation of cultures with phase contrast microscopy or with light microscopy after fixation and staining with hematoxylin revealed the same morphological characteristics as prepared primary cultures from the same biological material. Characterization by immunocytofluorescence for glial markers such as glial fibrillary acidic protein (GFAP) (mouse monoclonal G3893 Sigma, Buchs, Switzerland; rabbit polyclonal Z0334 DAKO, Glostrup, Denmark) and S100b (mouse monoclonal M2532, Sigma, Buchs, Switzerland) or for neuro-filament (rabbit polyclonal, gift from B. Riederer, Lausanne, Switzerland), vimentin (mouse monoclonal V9 M0725 DAKO, Glostrup, Denmark), and nestin (rabbit polyclonal AB5922 Chemicon, Temecula, California, USA) also revealed that virtually all cells stained for these different markers in a manner that was indistinguishable from staining obtained on cells prepared from fresh tissue.

2.4.3 CELL LABELING FOR REIMPLANTATION

Cell aggregates from one flask were pooled by centrifugation at 800 rpm, supernatant was recuperated for resuspension. Aggregates were resuspended in 500 µl diluent C with 5µM fluorescent viable dyes (Fig. 2.4.2) PKH26 (red) or PKH67 (green) (MINI67, Sigma-Aldrich, St Louis, MO, USA) for three minutes.

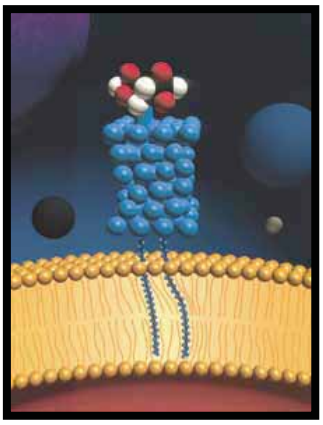


Figure 2.4.2 : Cell labeling with viable membrane fluorescent dyes.

500µl FCS was added for one minute and aggregates washed three times with RPMI medium. After the last wash, aggregates were resuspended with the recuperated medium previously centrifuged at 4000rpm to eliminate cellular wastes. Tubes containing fluorescent dye-stained aggregates were completely filled to be transported. For reimplantation aggregates were settled and supernatant was partially removed until a volume of 100µl remained, in which the aggregates were resuspended at a cell concentration of 12'500 cells per µl in one monkey (Mk-JO) and 3'000 cells per µl in the other monkey (Mk-JA).

To note, concerning PKH labelling, that Haas et al. (2000) showed, in the context of bone marrow transplantation, that the cells do not exchange the fluorescent viable dye PKH that irreversibly bind to cell membranes. It was thus demonstrated that PKH stays in the reimplanted cell.

2.4.4 IMMUNOCYTOCHEMISTRY

As cell cultures were analyzed, coverslips or glass slices were fixed with 4% paraformaldehyde in PBS or with acetone. Cell aggregates in suspension were polled by centrifugation, supernatant eliminated, cells were resuspended in 100 µl of medium and drops with resuspended aggregates were placed on a glass slice, dried in a 37-C dry air incubator for 1h and then fixed with 4% paraformaldehyde in PBS. Coverslips or glass slices were incubated in PBS with 0.1 % casein (C8694, Sigma-Aldrich, St Louis, MO, USA) and then incubated in PBS 0.3 % bovine albumin with antibodies against the following antigens: glial fibrillary acidic protein (GFAP) (monoclonal G3893, 1/1000, Sigma-Aldrich, St Louis, MO, USA and polyclonal rabbit Z0334 1/500, Dako, Glostrup, Denmark), nestin (AB 22 polyclonal rabbit, Chemicon, Millipore, Billerica, MA, USA), doublecortin (guinea-pig

polyclonal DCX AB5910, 1/1000, Chemicon, Millipore, Billerica, MA, USA, Millipore, Billerica, MA, USA), They were then washed and incubated with fluorescent or horseradish peroxidase- conjugated secondary antibodies. After the last washes, they were revealed for peroxidase activity with diamino benzidine or mounted in vectashield® with Dapi to counterstain nuclei before fluorescent microscopic or confocal microscope analysis.

2.5 NECROPSY

Once the entire experiment was completed, the monkeys were sacrified. They were first anesthetized with an intramuscular ketamine injection, and then received a lethal dose of sodium pentobarbital (90mg/kg) by transcardiac perfusion with 0.9% saline (400ml). The perfusion was continued with fixative (3 liters of 4% phosphate buffered paraformaldehyde in 0.1 M phosphate buffer, pH=7.6) and solutions (2 liters each) of the same fixative containing increasing concentrations of sucrose (10, 20 and 30%). The brain was removed, dissected and stored in a sucrose solution (30%) for 1.5-2 weeks.

2.6 HISTOLOGY AND IMMUNOCYTOCHEMISTRY

Frozen sections were then cut in the frontal plane at a thickness of 50 μm. 5-8 series of sections were collected with a cryotome (HM560, MICROM, Volketswil, Switzerland) and were stored at -80°C until used in the cryoprotection 50mM phosphate buffer pH 7.4 solution that contains 25% glycerol (G7893, Sigma-Aldrich, St Louis, MO, USA), 30% ethylene glycol (33068, Ridel-de-Haën, Seelze, Germany). Among these series, one was Nissl-stained. Brain sections were incubated in PBS with 0.1 % casein (C8694, Sigma-Aldrich, St Louis, MO, USA) and then incubated in PBS 0.3 % bovine albumin with antibodies against the following antigens: glial fibrillary acidic protein (GFAP) (monoclonal G3893, 1/1000, Sigma-Aldrich, St Louis, MO, USA and polyclonal rabbit Z0334 1/500, Dako, Glostrup, Denmark), nestin (AB 22 polyclonal rabbit, Chemicon, Millipore, Billerica, MA, USA), doublecortin (guinea-pig polyclonal DCX AB5910, 1/1000, Chemicon, Millipore, Billerica, MA, USA, Millipore, Billerica, MA, USA), S100beta (mouse monoclonal M2532, Sigma-Aldrich, Buchs, Switzerland), vimentin (mouse monoclonal V9 M0725 DAKO, Glostrup, Denmark), NeuN (mouse monoclonal MAB377, Chemicon, Temecula, California, USA), MAP2 (Sigma), MIB1 (Abcam), SMI-32 (Sigma). The immunoreactions were revealed for immunohistochemistry with biotinylated secondary antibodies followed by the immunoperoxydase Vectastain Elite system (PK-6100, Vector Laboratories) and DAB

substrate kit (SK-4100, Vector Laboratories) and counterstained with Mayer's hematoxylin solution (MHS32, Sigma-Aldrich, Buchs, Switzerland). The DAB immunostaining were observed under light microscope (BX40 Olympus) for DAB. The immunohistofluorescence were peformed with fluorescent dye conjugated secondary antibodies and were scanned for 700nm and 800nm infrared fluorescent dyes with Odyssey system (LI-COR, Bad Homburg, Germany) or observed under epifluorescent microscope (IX81 Olympus) equipped with FITC, Cy3, Dapi and infrared 680nm filters for multichannel images.

Histological analyses and reconstruction were performed on Mercator®. Three-dimensional mapping and statistical analyses were then performed on Map3D®. Drawings and cell counting of immunostained sections were made with a Olympus BX40 epifluorescence microscope equipped with a motorized X–Y-sensitive stage and a video CDD camera connected to a computerized image analysis system (ExploraNova, La Rochelle, France). Images were acquired using three software programs (Mosaic, Morpho Expert and Fluo3D, Explora Nova, La Rochelle, France). Slices in the brain section where PKH67 cells were detected were selected to be anatomically analysed. Criteria of green and red fluorescence intensity and cell surface limits from 100-600μm^2 were applied to automatically point all PKH67 and PKH26 cells on eleven to thirteen slices depending on the cell migration (Mercator, ExploraNova, La Rochelle, France). A visual control was done on 20 of 2'500-3'500 fields per slices. The obtained models of the slices with identified cells allowed 3D reconstruction and 3D cell counting through computer based stereological analysis (Map3D, ExploraNova, La Rochelle, France). The total cell counts were compared with the number of implanted cells to obtain a percentage of survival and in parallel the percentage of cells per anatomical structures could be estimated.

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3 RESULTS

The following part was accepted for publication in Neurosurgery.

3.1 AUTOLOGOUS ADULT CORTICAL CELL IMPLANTATION ENHANCED FUNCTIONAL RECOVERY FOLLOWING UNILATERAL LESION OF MOTOR CORTEX IN PRIMATES

3.1.1 INTRODUCTION

Brain lesions in the adult have dramatic consequences as the spontaneous capacity of the brain to functionally recover is limited. Besides existing rehabilitative therapeutic approaches (e.g. physiotherapy), several lines of research aim at developing treatments to promote and refine brain plasticity, in order to enhance functional recovery following brain injury. Stem cells research, which represents a promising strategy to treat several nervous diseases, segregate into two main potential lines of treatment: transplantation of exogenous cells or use of endogenous cells. The implantation of exogenous neural stem cells, either of embryonic or fetal origin, represents a promising approach, although there are serious limitations on ethical, scientific and clinical sides (see e.g. Lindvall et al., 2004; Rosser et al., 2007; Imitola, 2007; Ormerod et al., 2008; McKay & Kittappa, 2008; Kondziolka & Wechsler, 2008; Williams & Keating, 2008; Hess & Borlongan, 2008; Bacigaluppi et al., 2008; see also Locatelli et al., 2009; Preynat-Seauve et al., 2009; Madhavan & Collier, 2009 for review). Another strategy aims at stimulating and/or recruiting endogenous stem cells or precursor cells available in the adult central nervous system, located principally in the forebrain subventricular zone (SVZ) and in the subgranular zone (SGZ) of the dentate gyrus of the hippocampus (see Madhavan & Collier, 2009). These cells however migrate either only to a specific target (olfactory bulb from the SVZ) or only locally in the dentate gyrus (from the SGZ). The possibility to repair post-lesion parts of the central nervous system (cerebral cortex, basal ganglia, spinal cord) remote from these two neurogenic niches is therefore limited. Attempts to enhance experimentally the migration of stem/precursors from the neurogenic niches to distant zones of the brain or to use resident progenitors led to a modest compensatory replacement of lost cells, without clinically relevant functional recovery (see Madhavan & Collier, 2009 for review). Stem/precursors taken from the SVZ or SGZ and transplanted into another brain area may not develop their full potential of cell replacement in a different environment. Stem/precursors resident in the cerebral cortex or basal ganglia may be too few to successfully replace lost cells after a lesion. To bypass these two limitations, the aim of the present study was to extend preliminary experiments to fully test the feasibility and functional relevance of an original endogenous strategy in a non-human primate model of cerebral

(motor) cortex lesion: First, collect adult neural progenitor cells resident in the cerebral cortex of the lesioned subject itself (see Brunet et al., 2002); Second, put the progenitor cells in culture to increase their number (see Brunet et al., 2003, 2005); Third, transplant them into the cerebral cortex, in and/or nearby an experimental lesion and assess the functional outcome.

A crucial step towards feasible, safe and efficient clinical application requires the most appropriate animal model for the corresponding neural pathology, closely mimicking the characteristics of human diseases. The non-human primate model is often mandatory to address safety issues and scientific concerns, especially to include exquisite neural functions present only in primates (see e.g. Courtine et al., 2007; Capitanio & Emborg, 2008). The present study was conducted on macaque monkeys with the aim to provide first quantitative behavioral evidence in primates for a beneficial outcome of cell therapy after cortical injury, using a previously established model of cerebral cortex lesion affecting motor function (Liu & Rouiller, 1999).

3.1.2 MATERIALS AND METHODS

3.1.2.1 Subjects

Data were collected from six adult macaque monkeys (macaca fascicularis; five males and one female) weighting between 3 and 6 kg, ranging from 2.5 to 5 years old at onset of motor training sessions. The monkeys originated from the breeding colony in our own animal facility or were purchased from a certified supplier (Bioprim; 31450 Baziège; France), with the authorization to import delivered by the Federal Veterinary Office (BVET, Bern, Switzerland). All procedures were conducted in accordance to the Guide for Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by local veterinary authorities.

Six adult macaque monkeys were subjected to unilateral lesion of the hand representation in motor cortex, producing a selective paresis of the contralesional hand (Liu & Rouiller, 1999). Post-lesion, two of these monkeys were “treated” (autologous adult brain progenitor cells were re-implanted) for comparison of their functional recovery of manual dexterity with four lesioned, untreated (“control”) monkeys.

Monkeys were housed in our animal facilities in rooms of 12 m^3, in which usually 2-4 monkeys were free to move and to interact among each others. Before behavioral testing in the morning, the animal keeper placed the monkeys in temporary cages for subsequent transfer to a primate chair. The monkeys had free access to water and were not food deprived. The rewards (pellets) obtained during the behavioral tests represented the first daily access to

food. After the tests, the monkeys received additional food (fruits, cereals). The body weight of the animals was monitored before every behavioral session. In case the body weight dropped by 10% or more, the experiment was interrupted until the monkey regained the lost weight.

3.1.2.2 Behavioral task

The six monkeys were trained to perform the modified (static) Brinkman board task (Liu & Rouiller, 1999; Freund et al., 2009), aimed at assessing manual dexterity (right panel in Fig. 3.1.2A). The subjects had to grasp food pellets from small slots oriented either vertically or horizontally by using precision grip (opposition of thumb and index finger). Each monkey was its own control, as the dexterity scores were compared pre- versus post-lesion.

More specifically, the tests were conducted using a Perspex board (10 cm x 20 cm) containing 50 randomly distributed slots, each filled with a food pellet at the beginning of the test (see Freund et al., 2009 for detail). Briefly, twenty-five slots were oriented horizontally and twenty-five slots vertically. The slot dimension was 15 mm long, 8 mm wide and 6 mm deep. The monkeys performed the task daily with each hand until they reached a plateau reflecting a stable pre-lesion performance. The duration of the daily behavioral session was about one hour. After the motor cortex lesion, the behavioral sessions were pursued for a period ranging from 80 to 310 days. The main analysis was focused on the number of pellets retrieved in 30 seconds (retrieval score) from the vertical and the horizontal slots, respectively. A total retrieval score was also considered, consisting of the sum of the pellets retrieved from the vertical and horizontal slots (Fig. 3.1.1).

Besides the retrieval score, reflecting the entire motor sequence (reaching, grasping and withdrawal of the hand), a second parameter was considered (Fig. 3.1.2), namely the contact time, as recently reported (Freund et al., 2009; see also Nishimura et al., 2007). The contact time is defined as the time of contact (in seconds) between the fingers and the pellet. In other words, the contact time is the time interval between the first contact with the pellet in the slot (usually with the index finger) and the precise time of successful grasping when the pellet is removed from the slot. For each session, the contact time was determined separately for each of the first five vertical slots and each of the first five horizontal slots visited by the monkey. In general, the contact time was longer for the horizontal than the vertical slots (Fig. 3.1.2), in line with the notion that the task is somewhat more difficult for the horizontal slots, thus requiring a longer manipulation of the pellet before successive grasping (see Freund et al., 2009 for detail). The contact time can be considered as a more specific readout for the manual dexterity per se (precision grip) than the retrieval score, comprising other components of the

task (including strategy). Comparisons of retrieval scores and contact times pre- versus post-lesion were performed using the non-parametric Mann-Whitney statistical test.

In addition to the modified Brinkman board task, testing the prehension of static objects, another task was introduced to test the ability to grasp moving objects (pellets), presented on a rotating Brinkman board (right panel in Fig. 3.1.2B). The monkey had to anticipate the rotation of the Brinkman board, turning either clockwise (Cl) or counterclockwise (C-Cl). In a given behavioral session, the monkeys performed the rotating Brinkman board once for each direction of rotation, corresponding each to the prehension of 32 pellets in slots distributed in 4 concentric circles (Fig. 3.1.2B). The contact time was measured as described above in the modified Brinkman board, for the first ten slots aimed by the monkey. Comparison of contact times in the rotating Brinkman board were performed using the non-parametric Mann-Whitney statistical test. The two grasping tasks (modified Brinkman board and rotating Brinkman board) can be seen on the following web page: http://www.unifr/neuro/rouiller/motorcontcadre.htm

3.1.2.3 Animal care for surgery

After completion of pre-lesion training, the animals were subjected to the following surgical procedures. The monkeys were first tranquilized with ketamine (Ketalar®; Parke-Davis, 5 mg/kg, intramuscularly); atropine was injected (0.05 mg/kg, intramuscularly) in order to reduce bronchial secretions. Before surgery, the animals were treated with the analgesic Carprofen (Rymadil®, 4 mg/kg, subcutaneously) and the antibiotic Albipen® (Ampiciline 10%, 30mg/kg, subcutaneously). Subsequently, they were anaesthetized with intravenous perfusion of 1% propofol (Fresenius®) mixed with a 5% glucose solution (1 volume of propofol and 2 volumes of glucose solution); ketamine was added to the perfusion solution (65 mg/100 ml). To prevent oedema, Methylprednisolone (Solu-medrol, Pfizer ®) was added to the propofol/glucose solution (1 mg/ml). The level of anesthesia was kept at an optimal level with a perfusion rate of the propofol/glucose mixture of 0.1ml/min/kg. All surgeries were performed under sterile conditions. Heart rate, respiration rate, expired CO_2, arterial O_2 saturation and rectal temperature were monitored throughout the surgery. Each monkey was chronically implanted over the left forelimb area in the primary motor cortex (M1) with a stainless steel recording chamber (22x17x15mm) allowing intracortical microstimulation (ICMS) sessions to map the primary motor cortex and locate the hand representation, as previously described (Liu & Rouiller, 1999; Schmidlin et al., 2004). After surgery, the monkeys received Carprofen (pills of Rymadil mixed with food) daily and Albipen® (subcutaneously) every two days during one to two weeks.

3.1.2.4 Biopsy of prefrontal cortical tissue

In the two treated monkeys (during pre-lesion phase), following the same surgery protocol as described above, a squared osseous sector of 8x8mm was opened above the right dorsolateral prefrontal cortex. The dura-mater was incised and an approximate volume of 8-20 mm^3 of cortical tissue was extracted using a surgical blade (no11, Paragon®) and placed into sterile cold culture medium (Brunet et al., 2005). The osseous sector was then put back in place on the dura-mater and sewed to the skull. The muscle and skin were then sutured. The extraction of brain tissue biopsy from the prefrontal cortex did not affect the capacity to grasp the pellets from the Brinkman board tasks.

3.1.2.5 Cell preparation and implantation

As previously described (Brunet et al., 2005, 2009b), a few hours after the biopsy was performed, the cortical tissue collected was dissected to obtain grey matter cells, placed first in RPMI 1640 medium with fetal bovine serum and an antibiotic/antimycotic cocktail under horizontal agitation, and then kept in culture medium without serum, in suspension and under slow agitation. Prior to re-implantation, the cells were stained for subsequent tracking with fluorescent viable membrane dyes PKH26 (red) for the cells re-implanted into the lesion site and PKH67 (green) for the cells re-implanted near the lesion site in the intact cortex.

Cells contained in culture medium were re-implanted stereotaxically with a Hamilton microsyringe. For one monkey (Mk-JO), the re-implantation was performed into the lesion site 15 days after the motor cortex lesion. A total volume of 20µl with PKH26 labeled cells containing 12'500 cells/µl was injected. For the other monkey (Mk-JA), the first re-implantation was performed into the lesion site (PKH26 labeled cells) and near the lesion site in the intact cortex (PKH67 labeled cells), once the monkey had reached a spontaneous functional recovery plateau. A second re-implantation was performed in the intact cortex, near the lesion site 49 days later (PKH67 labeled cells). The total volume (concentration of 3'000 cells/µl) injected was 100 µl with PKH26 cells and 100 µl with PKH67 labeled cells (first implantation) and 49 µl with PKH67 labeled cells (second implantation), respectively.

3.1.2.6 Primary motor cortex (M1) lesion

The hand area in the left M1 was permanently lesioned in the six monkeys by multiple injections of ibotenic acid (either Fluka 99% or Sigma 95%), as previously reported (Liu & Rouiller, 1999). A volume of 1-1.5 µl of ibotenic acid solution (10µg/µl in phosphate-

buffered saline) was injected at each site by using a Hamilton microsyringe positioned at relevant sites previously defined by intracortical microstimulation. The total volume of ibotenic acid injected in each monkey ranged from 15 to 40µl. More relevant than the volume of ibotenic acid injected is the volume of the actual lesion (volume of gray matter in motor cortex affected by the lesion; see Fig. 3A), as determined for each monkey based on reconstruction of the lesion from consecutive histological sections stained for SMI-32.

3.1.2.7 Necropsy and histology

At the end of the experiment, the monkeys were sacrificed (see e.g. Schmidlin et al., 2004; Wannier et al., 2005) for histological analysis of the lesion and of the implanted cells fate. The monkeys were sacrificed under deep anesthesia (initiated first with an i.m. ketamine injection followed by an i.p. lethal dose of sodium pentobarbital (90mg/kg)) by transcardiac perfusion with 0.9% saline (400ml) continued with fixative (3 liters of 4% paraformaldehyde in 0.1 M phosphate buffer, pH=7.6) and solutions (2 liters each) of the same fixative containing increasing concentrations of sucrose (10, 20 and 30%). The brain was removed, dissected and stored in a sucrose solution (30%) for 1.5-2 weeks.

Frozen sections of the brain were then cut in the frontal plane at a thickness of 50 µm. Eight series of sections were collected with a cryotome (HM560, MICROM, Switzerland). Among these series, one was treated immunocytochemically (SMI-32 antibody), as previously reported (e.g. Wannier et al., 2005). As described earlier (Brunet et al., 2005), other series of brain sections were incubated in PBS with 0.1 % casein and then incubated in PBS 0.3 % bovine albumin with antibodies against GFAP, nestin, MAP2, and DCX. They were then rinced and incubated with fluorescent or horseradish peroxidase- conjugated secondary antibodies. After the last rinces, they were revealed for peroxidase activity with diamino- benzidine (DAB) or mounted in vectashield® with Dapi to counterstain nuclei before fluorescent microscopic or confocal microscope analysis. Histological analyses and reconstruction were performed on Mercator®. Three-dimensional mapping and statistical analyses were then performed on Map3D®. Drawings and cell counting of immunostained sections were made with a Olympus BX40 epifluorescence microscope equipped with a motorized X–Y-sensitive stage and a video CDD camera connected to a computerized image analysis system (ExploraNova, La Rochelle, France). Images were acquired using three software programs (Mosaic, Morpho Expert and Fluo3D, Explora Nova, La Rochelle, France). Sectors of the brain sections where PKH26 and PKH67 labeled cells had been detected were selected for further analysis to be anatomically analyzed.

3.1.3 RESULTS

3.1.3.1 Modified Brinkman board

As a result of unilateral lesion of motor cortex (as seen on surface views of the brain in Fig. 3.1.3B), a total loss of the contralesional hand dexterity was observed, as reflected by a grasping score dropping to zero in the modified Brinkman board task, both in control and treated monkeys (Fig. 3.1.1A,B). The control monkeys (Fig. 3.1.1A) then exhibited a progressive, spontaneous recovery starting after 5 to 50 days, reaching a post-lesion plateau 10 to 100 days post-lesion, depending on the monkey. This spontaneous recovery plateau ranged across the control monkeys from 38 to 98% of the pre-lesion dexterity score (Fig. 3.1.1C). Note that, in the control monkey exhibiting the fastest recovery (Mk-GE), it turned out to be mainly limited to the vertical slots with a poor long-lasting recovery for horizontal slots (Fig. 3.1.1A). The control monkey (Mk-RO) with the best recovery (98%) had the smallest lesion (Fig. 3.1.3A). A statistical comparison of pre- and post-lesion plateaux is given in Figure 1C for the control monkeys, showing that the recovery remained dramatically incomplete (38 to 42%) in three out of four monkeys. The post-lesion plateau then remained stable during several months (Fig. 3.1.1A).

For the treated monkeys (Fig. 3.1.1B), after complete loss of manual dexterity lasting 25 days in Mk-JO and 5 days in Mk-JA, the same progressive spontaneous recovery as seen in control monkeys took place, reaching a first plateau 40 days after lesion in Mk-JO and 80 days after lesion in Mk-JA. In Mk-JO, this first plateau represented 35% of pre-lesion score and lasted 25-30 days. Meanwhile, 15 days after lesion, the autologous adult brain cell transplantation was performed. Starting around 70 days post-lesion, there was an additional enhancement of manual performance, reaching a second plateau 90 days after the lesion (green arrow in Fig. 3.1.1B), respectively 75 days after the autologous transplantation. This second behavioral plateau represented 59% of the pre-lesion score. The difference between the first and second post-lesion plateaux (24%) was statistically significant (Fig. 3.1.1C).

In Mk-JA, the autologous transplantation was performed once the monkey had reached a first performance plateau. Forty-five days after the first plateau (spontaneous recovery), corresponding to day 125 post-lesion, an autologous transplantation was performed, followed by a second one 50 days later, corresponding to day 175 post-lesion. The first, spontaneous behavioral plateau represented 75% of the pre-lesion score and lasted from day 80 to day 185 post-lesion. Then, the same phenomenon as observed in the other treated monkey took place, an additional enhancement (rebound) of performance lasting about 75-80 days, reaching a final plateau 260 days post-lesion, in other words 83 days after the second transplantation

(green arrow in Fig. 3.1.1B). This second manual performance plateau corresponded to a complete recovery (100%): the enhancement of recovery presumably related to the transplantation was 25%, a statistically significant difference between the first and second post-lesion plateaux (Fig. 3.1.1C).

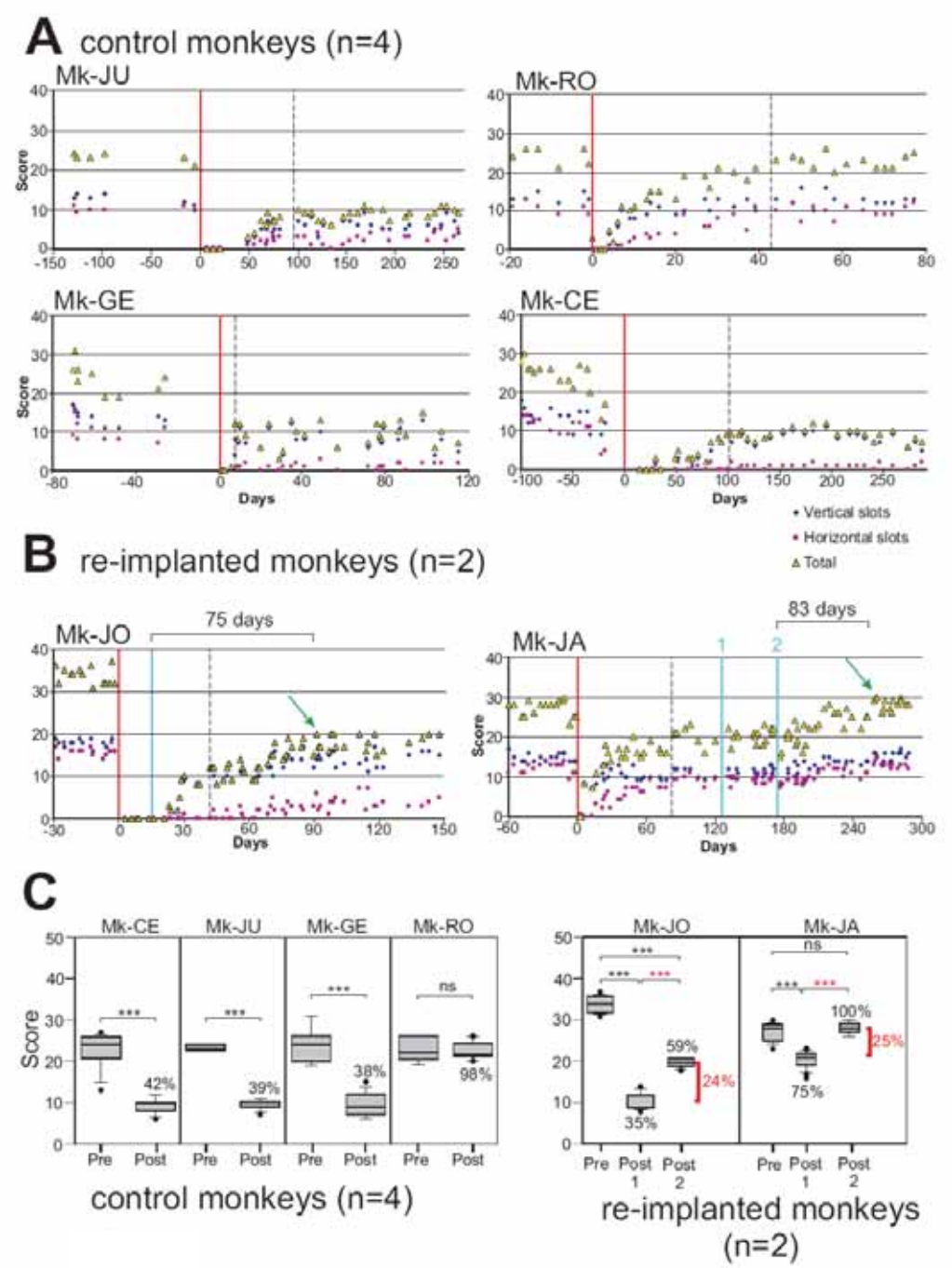

Figure 3.1.1: Behavioral data (retrieval score).
Panels A and B: Time course in days of manual dexterity performance (score), pre- and post-lesion, as derived from the modified Brinkman board task in four control monkeys (**panel A**) and in two treated monkeys subjected to cell transplantation (**panel B**). The score is given by the number of pellets successively retrieved by the monkeys during 30 seconds. Yellow triangles represent the total score, blue diamonds the score for vertical slots and pink squares the score for horizontal slots. The vertical red line indicates the time of the lesion (day zero). In **panel A**, the vertical dashed line shows the time point at which a spontaneous plateau of recovery was reached. In **panel B**, the vertical dashed line represents the first (spontaneous) plateau of recovery. In the two treated monkeys (**panel B**), the vertical blue lines show the time of autologous cell implantations. The green arrows

point out to a second behavioral plateau, at the end of a rebound in the recovery curve (see text), time-locked to the cell implantation after a delay indicated in days (ranging between 75 and 83 days).
Panel C: The behavioral data are represented by box and whisker plots, comparing the pre-lesion and post-lesion retrieval scores at plateau. In the four control monkeys, there was only one post-lesion plateau whereas, in the two treated (re-implanted) monkeys, in addition to the first plateau of spontaneous recovery (Post1) there was a rebound in the recovery curve leading to a second plateau (Post2) of manual dexterity scores. The different plateau scores were statistically compared (Mann-Whitney test) with $p<0.001$ (***) whereas "ns" is for $p>0.05$. The percentage values in black next to each post-lesion plateau represent the percentage of functional recovery as compared to the pre-lesion score. The percentage values in red are for the magnitude of the behavioral rebounds as compared to the first plateau of spontaneous recovery. In box and whisker plots, the horizontal line in the box corresponds to the median value and the top and bottom of the box are for the 75th and 25th percentile values respectively. The top extremity of the whisker above the box is the largest data point included in the range going from the 75 percentile up to a level defined as the sum of the 75 percentile plus 1.5 times the inter-quartile distance. The bottom extremity of the whisker below the box is the smallest data point included in the range going from the 25 percentile down to a level defined as the 25 percentile minus 1.5 times the inter-quartile distance.

To investigate more specifically whether the second plateau observed exclusively in the two treated monkeys corresponds to an enhancement of manual dexterity per se, a more detailed analysis of the modified Brinkman board data was conducted, based on the contact time (see methods). The contact time was determined in the sessions included in time windows corresponding to the first and second plateaux in Mk-JO (40-68 post-lesion days and 90-164 post-lesion days for plateau 1 and plateau 2, respectively) and in Mk-JA (80-180 post-lesion days and 252-300 post-lesion days for plateau 1 and plateau 2, respectively). The distribution of the contact times observed at plateaux 1 and 2 is shown in the form of box and whisker plots in Figure 3.1.2A, separately for the vertical and horizontal slots, for each of the two treated monkeys.

In Mk-JO, irrespective of the slot orientation, the contact times were significantly shorter at plateau 2 than at plateau 1, reflecting an improvement of manual dexterity when the second plateau was reached. In Mk-JA, a same conclusion can be drawn for the vertical slots, whereas there was no statistically significant difference for the horizontal slots. Nevertheless, the contact times for the horizontal slots in Mk-JA were less variable at plateau 2 than plateau 1. In agreement with the retrieval score data, the extent of decrease of median contact times going from plateau 1 to plateau 2 in Mk-JO (both slot orientations) and Mk-JA (vertical slots) ranged from 24 to 30%, representing an enhancement of manual dexterity comparable to the improvement of performance reflected by the retrieval score (24-25%; see Fig. 3.1.1C).

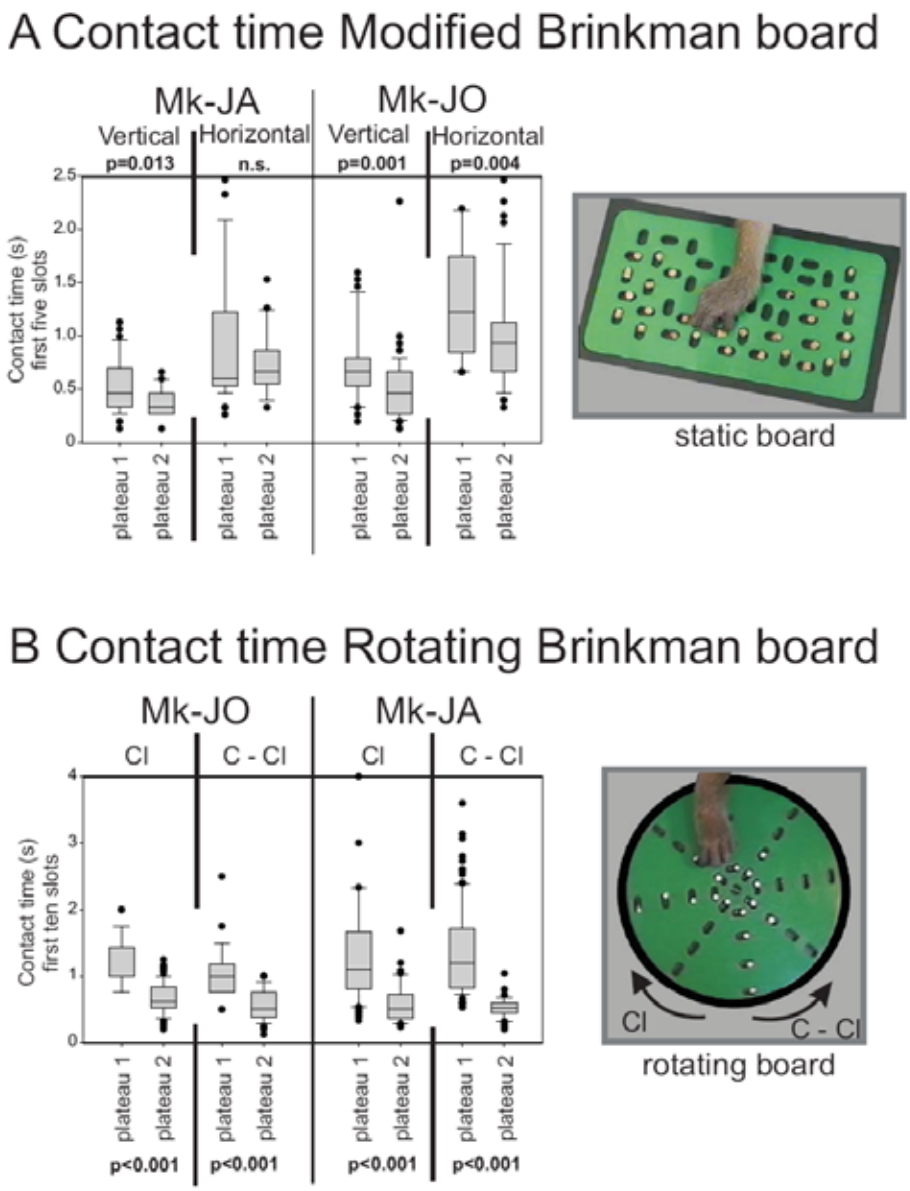

Figure 3.1.2: Behavioral data (contact time). The contact times, measured during the post-lesion plateaux 1 and 2, are plotted in the form of box and whisker plots, for the modified (static) Brinkman board task (panel A) and the rotating Brinkman board task (panel B). The shorter contact time the better is the manual dexterity as less time of contact between the pellets and the fingers was needed to successfully grasp the pellet. In Mk-JA, data were collected from 9 behavioral sessions during the first plateau and from 4 sessions during the second plateau. In Mk-JO, data were collected from 6 behavioral sessions during the first plateau and from 12 sessions during the second plateau. n.s.=non-significant difference between plateau 1 and plateau 2 ($p>0.05$). The statistics refer to the comparison of contact times between the plateau 1 and the plateau 2 (Mann and Whitney test). Same conventions for the box and whisker plots as in Figure 1C.

3.1.3.2 Rotating Brinkman board task

To assess further in the two monkeys (Mk-JO; Mk-JA) subjected to implantation of autologous adult cortical cells that the second recovery plateau represents a significant improvement of manual dexterity as compared to the first plateau of recovery, the contact times in the rotating Brinkman board was compared between the sessions taken within the time windows corresponding to the 2 distinct plateaux (see above). As shown in Figure 2B, irrespective of the direction of rotation of the board, there was a statistically significant

decrease of contact time going from post-lesion plateau 1 to post-lesion plateau 2 ($p<0.001$ for both monkeys), indicative of an enhanced manual dexterity. Comparing the median values, there was a decrease of contact time from plateau 1 to plateau 2 ranging from 42% to 57%. The enhancement of manual performance was thus more pronounced for the rotating Brinkman board than for the modified Brinkman board.

3.1.3.3 Relationship between lesion size and recovery

There was a substantial variation of post-lesion recovery across monkeys (Fig. 3.1.1). To assess the effect of lesion size, the degree of functional recovery in percent was plotted as a function of lesion volume, corresponding to the extent of gray matter in motor cortex affected by the lesion (Fig. 3.1.3A).

As expected, a small lesion in a control monkey (Mk-RO) is followed by a nearly complete spontaneous functional recovery. In contrast, still in control monkeys, above a lesion volume of about 40 mm^3 and up to a volume of about 100 mm^3, the extent of spontaneous recovery remained fairly constant near 40% (Fig. 3.1.3A). The two treated monkeys showed a tendency towards slightly better recovery, as compared to control monkeys, taking into account the lesion size. Indeed, Mk-JA showed a slightly better manual performance recovery than Mk-RO, which both had a small lesion, and Mk-JO had a 20% better recovery than Mk-GE, which had a fairly comparable lesion size. Nevertheless, a dramatic difference between the two subgroups of monkeys is the presence of a rebound in the post-lesion recovery curve in the two treated monkeys (second plateau of an extent represented by the vertical dashed line in Fig. 3.1.3A), a phenomenon completely absent in the four control monkeys (Fig. 3.1.1).

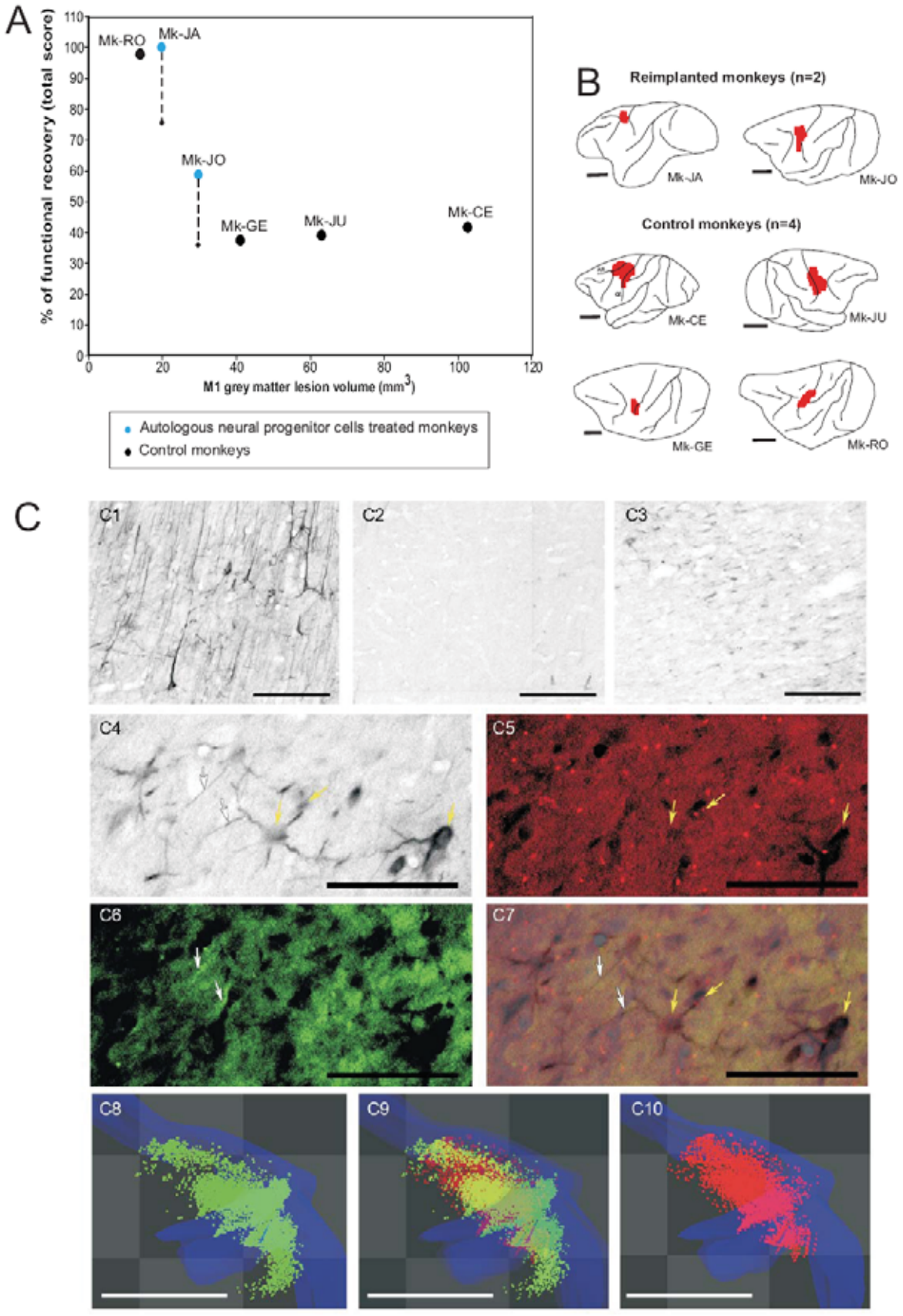

Figure 3.1.3: Relationship with morphological data
Panel A: Plot of the percentage of post-lesion functional recovery of the contralesional hand (total score) as a function of the volume of the primary motor cortical lesion. Black filled circles represent the control monkeys and blue filled circles the monkeys treated with autologous brain cells. For the two treated monkeys, the vertical

dashed line represents the additional recovery observed (rebound), presumably in relation to the cell re-implantation (see text).
Panel B: Lateral views of the projection on the cerebral cortex surface of the approximate location and extent of the left M1 grey matter lesion (red) as observed on frontal histological sections of the monkeys' brain. Theses reconstructions do not take the depth of the lesion into account. The precise extent of the lesion is given by its volume in panel A. At the bottom, the brain of the four control monkeys are shown, whereas the brain of the two monkeys re-implanted with autologous brain cells are represented at the top. AR and CE mean arcuate and central sulci, respectively. Scale bar = 10 mm.
Panel C: Histological data in transplanted and control lesioned motor cortex area.
C1-C3: Brain DAB-immunohistology for SMI-32 neurons. In panel C1, SMI-32 positive neurons as seen in the intact part of the motor cortex (at some distance from the lesion): SMI-32 positive neurons are present in layer III and layer V. The lesion territory is shown in panels C2 and C3 for a control monkey and a treated monkey, respectively: in the control monkey, no SMI-32 staining is visible (C2); in contrast, there are some positively stained cells in the lesion of the treated monkey (C3). Scale bar = 200μm.
C4-C7: High magnification of SMI-32 positive cells in the lesion site of the treated monkey (C4 and C7). These SMI-32 positive cells (yellow and white arrows in C4 and C7) correspond to PKH26 labeled cells (yellow arrows in C5) or to PKH67 labeled cells (white arrows in C6). Scale bar = 200μm.
C8-C10: 3D reconstruction of the motor cortex lesion territory showing the distribution of PKH67 labeled cells (green dots in C8 and C9) and PKH26 labeled cells (red dots in C9 and C10). Note that the two sets of cells migrated in the same lesion territory in the overlay reconstruction (C9). Scale bar = 1 cm.

The two treated monkeys of the present study, as well as the three monkeys of our previous report also subjected to autologous transplantation (Brunet et al., 2005), did not exhibit any sign of pain or discomfort related to the autologous cell implantation, indicating that this procedure does not produce major undesired, secondary effect.

3.1.3.4 Cell analysis

When looking at SMI-32 stained brain sections in motor cortex in an intact territory, there were darkly stained SMI-32 positive neurons in layer III and layer V, both in control and treated monkeys (Fig. 3.1.3C, panel c1). In contrast, in the lesion territory, there was no SMI-32 staining in the control monkeys as expected (Fig. 3.1.3C, panel c2), whereas lightly stained SMI-32 positive neurons were present in the lesion territory of the treated monkeys (Fig. 3.1.3C, panel c3). When considering more closely these SMI-32 positive cells located in the lesion site of the treated monkey (Fig. 3.1.3C, panel c4), it appeared that they sometimes corresponded to PKH26 (red colored, designed by yellow arrows) and PKH67 (green colored, designed by white arrows) positive cells that were transplanted in the monkey's brain (Fig. 3.1.3C, panels c5-c7). In a treated monkey, a 3D reconstruction of the motor cortical area shows the distribution of PKH26 (red) and PKH67 (green) labeled cells (Fig. 3.1.3C, panels c8-c10), implanted respectively in the lesion and at the vicinity of the lesion. Finally, PKH67 positive cells were present in the lesion site, indicating that they migrated from the intact cortex towards the lesion territory. The histological observations did not reveal the presence of teratoma or tumor formation.

3.1.4 DISCUSSION

To the best of our knowledge, the present study is the first report in non-human primates supporting the notion that implantation of autologous adult brain progenitor cells leads to significant enhancement of functional recovery after brain injury. The enhancement of recovery is not directly reflected by a difference of post-lesion behavioral plateau between "treated" and "control" monkeys: indeed, the recovery was quite variable across monkeys, due to unavoidable inter-individual variability of lesion volume and precise position of the lesion. The evidence for enhancement of functional recovery in relation to the treatment is rather based on the presence of a rebound (second plateau of recovery) in the post-lesion recovery curve, present only in the two treated monkeys and not in the four control monkeys (Fig. 3.1.1). The manual performance obtained at the second plateau was significantly higher than the performance reflected by the first plateau of recovery (Figs. 3.1.1 and 3.1.2). Furthermore, the rebounds observed in the treated monkeys turned out to be time-locked to the corresponding cell transplantation with a delay of 75-85 days (Fig. 3.1.1B).

This delay may reflect to two possible mechanisms: cell replacement and/or endogenous neurogenesis induced by factor delivery. First, the re-implanted cells may integrate the neuronal network after differentiation into mature neurons that express MAP2 (Brunet et al., 2005) and SMI-32 (Fig. 3.1.3C). As shown previously (Brunet et al., 2005), one month after transplantation, the cells still expressed progenitor markers such as nestin and were differentiated into neurons at three months post-re-implantation. Furthermore, Koch et al. (2009) demonstrated that human embryonic neural stem cells can functionally integrate with and receive synaptic input from host brain tissue after 18-24 weeks. Such integration may occur in our study, where the autologous strategy is expected to facilitate the migration and integration of the transplanted cells. In line with this ability to colonize the entire lesion, the re-implanted cells express doublecortin (DCX), a migrant neuroblast marker that participates to the regulation of microtubule dynamics and stability during neuronal morphogenesis and migration (Horesh et al., 1999). Second, the cells may have a bystander effect through factor delivery and influence the endogenous brain modeling (Zhang & Chopp, 2009). Indeed, preliminary results (data not shown) indicate that, in vitro, these adult primate brain cells secrete neurotrophic factors, such as BDNF, LIF and CNTF. It is known that, after lesion of M1 in monkey, the ipsilesional premotor cortex (PM) plays a significant role in the "spontaneous" functional recovery (e.g. Liu & Rouiller, 1999; Frost et al., 2003; Plautz et al., 2003; Dancause et al., 2006). Among possible mechanisms, it was shown that the normal projection from PM to M1 is redirected to the post-central gyrus (somatosensory cortex; Dancause et al., 2005). It may be that the transplanted autologous adult cortical cells secrete

factors favorable for such redirection of relevant projections originating from intact adjacent motor cortical areas.

Both the absence of rebound in the recovery curve of the four control monkeys and the consistent delay of about 80 days between autologous cells implantation and the end of the rebound leading to a second plateau argue in favor of a positive effect of the cell transplantation on the functional recovery of manual dexterity following lesion of the hand area in M1 in macaque monkeys. In line with such interpretation, the autologous cell implantation took place at very different time points with respect to the lesion in the two treated monkeys. Indeed, if the rebounds would be due to factors independent from the autologous cells' implantation, then a consistent delay of 80 days would most likely not have been observed in the two treated monkeys. Another important observation derived from Mk-JA is that the rebound in the post-lesion recovery curve occurred even though the autologous cells' implantation took place a long time after the lesion (more than 3 months). The magnitude of the rebound of post-lesion recovery curve in the two treated monkeys was close to 25%, as assessed in the modified Brinkman board task, added to the "spontaneous" post-lesion recovery score (Fig. 3.1.1C). In the second behavioral test (rotating Brinkman board), the effect even reached about 50% improvement of manual performance. In the control monkeys, there was no sham treatment (for instance, infusion of vehicle only). However, it is unlikely that the absence of rebound is due to the lack of cortical penetration in the peri-lesional territory with a syringe, as numerous cortical penetrations with electrodes took place pre-lesion to establish the somatotopic map of the motor cortex.

A similar rebound effect as in the two monkeys with cell transplantation was found in another group of monkeys subjected to a comparable lesion of M1, but treated with anti-Nogo-A antibody (Wyss et al., 2008), a therapeutic strategy known to also enhance functional recovery after cervical cord lesion in monkeys (Freund et al., 2006, 2009). The time course of rebound observed in the anti-Nogo-A antibody treated monkeys subjected to lesion of M1 was comparable to the rebound delay of about 80 days observed here in the monkeys subjected to autologous transplantation. This observation suggests that both treatments, although based on different mechanisms, exert their effect following a comparable time course, which may represent a favorable situation to tentatively combine the two treatments with the aim to obtain an additive, if not synergistic effect.

Nearly all pre-clinical studies of cell therapy on animal models of cerebral infarct were conducted on rodents as host animal (see Locatelli et al., 2009 for review), with the exception of a recent study (Roitberg et al., 2006), in which human neural stem cells were transplanted

in a monkey model of ischemic stroke. Evidence for cell replacement was found but the functional outcome was not tested. In this domain (animal model of cerebral injury), the present observation of enhancement of functional recovery after cell therapy is an original contribution in primates. In a monkey model of another neurological disease (Parkinson), the transplantation of DA neurons generated from embryonic stem cells in monkeys into MPTP monkeys was followed by an attenuation of neurological symptoms (Takagi et al., 2005). The evidence was based on a neurological score testing a wide repertoire of functions but it is less quantitative than the present study focused on the recovery of manual dexterity, a prerogative of primates, not investigated in the MPTP monkeys (Takagi et al., 2005). Still in the context of Parkinson's disease in a monkey model, using the same auto-transplantation strategy of autologous adult progenitor cells, there is also evidence for a neuroprotective effect (Brunet et al., 2009b) and a significant enhancement of functional recovery as compared to control monkeys (Brunet et al., 2009a). Taken together, the latter study and the present report on non-human primates based on re-implantation of autologous adult cortical cells either in models of cortical injury or Parkinson's disease both provide new and encouraging results by demonstrating a significant functional recovery related to the treatment (to be confirmed on a larger sample of animals and in combination with other strategies). These studies also confirm that the re-implanted autologous brain cells did not form teratoma or tumor over time. Considering that the autologous strategy does not involve as complex ethical and/or immunosuppression concerns as in heterologous strategies, there is reasonable hope to successfully translate this approach into the clinics in a reasonably close future. On a more general perspective, the present study provides additional evidence in favor of a strategy aimed at recruiting endogenous stem cells or progenitor cells, associated in synergy with exogenous stems cell actions, as recently advocated (Madhavan and Collier, 2009).

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3.2 ADULT BRAIN PROGENITOR CELLS

3.2.1 IN VITRO CELL FATE

3.2.1.1 Introduction

As previously described (Brunet et al., 2002), observation of adult human brain cell cultures with phase contrast microscopy or with light microscopy after fixation and staining with hematoxylin revealed particular morphologic characteristics. From the beginning, cultured human adult brain cells appeared as a heterogeneous population and first presented two distinct morphologic patterns: flat cells with a large nucleus and large cytoplasm or stellate cells with a small nucleus and long processes (Fig. 3.2.1.1, a and b).

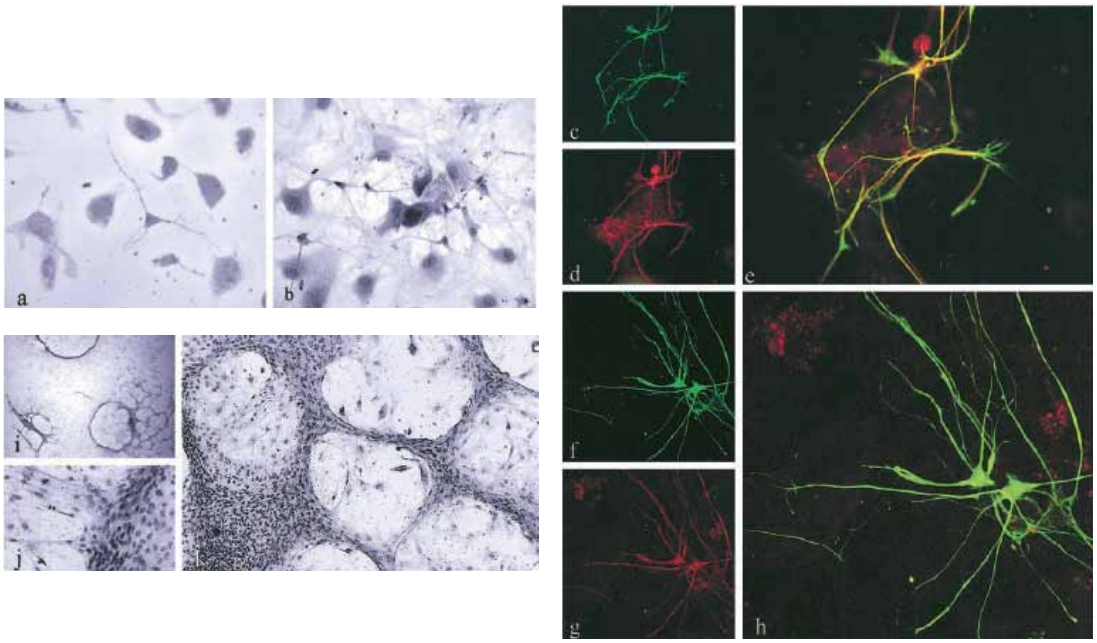


Figure 3.2.1.1: Cytology of adult human brain cells. a, b, and i to k, hematoxylin staining of the same mechanically dissociated culture at different times: a, 15 days in vitro (DIV); b, 38 DIV; i to k, 67 DIV. c to e, glial fibrillary protein (GFAP) (green) and beta tubulin III (red) colocalization in stellate cells in the mechanically dissociated culture at 38 DIV. f to h, GFAP (green) and O4 (red) colocalization in stellate cells in the enzymatically dissociated culture at 71 DIV. Original magnification: a to h, j, x40; i, x10; k, x20.

After cells reached confluency, bundles of closely associated cells formed and evolved into a dense network (Fig. 3.2.1.1, i to k) on top of flat cells. Characterization by immunocytofluorescence revealed that virtually all cells stained for GFAP, with stellate cells being more intensely labeled than flat cells (Fig. 3.2.1.1, c). Interestingly, we observed that isolated stellate cells that expressed GFAP also coexpressed beta-tubulin III (Fig. 3.2.1.1, c to e) and the oligodendrocyte marker O4 (Fig. 3.2.1.1, f to h). Before confluency (up to 30 to 40 days in vitro (DIV)), neither vimentin- nor nestin- positive cells were observed (data not shown). As the culture evolved and bundles formed, three distinct phenotypes could be

distinguished: flat cells that slightly expressed GFAP, small closely packed cells that constitute the core of the bundles and express vimentin and/or nestin, and finally stellate cells that present long GFAP-positive processes oriented longitudinally and that are associated with bundles (data not shown). In relation to the presence of GFAP-positive cells, their ability to transport glutamate by measuring the uptake of 3H-D-aspartate was functionally tested as previously described for neonatal mouse astrocyte (Debernardi et al., 1999): a small, active transport of glutamate was reliably detected, which represents another feature associated with mature glial cells (Danbolt, 2001).

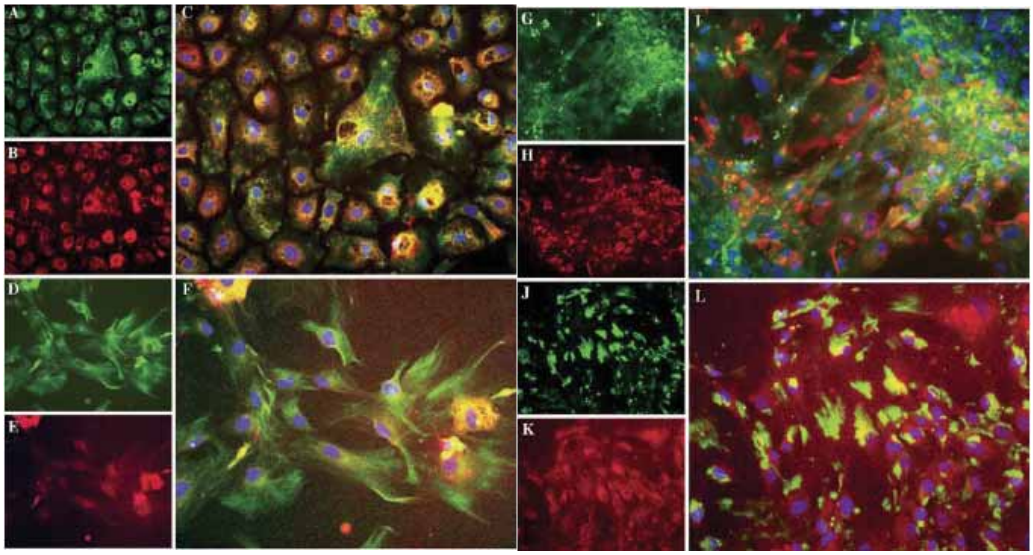


Figure 3.2.1.2: Cytology of adult human brain cells in vitro from fresh or cryopreserved brain tissue. (A–C) Nestin (green) and vimentin (red) expression in cell culture from fresh brain tissue at 106 days in vitro (DIV). (D–F) Nestin (green) and GFAP (red) expression in cell culture from cryopreserved tissue at 36 DIV. (G–I) GFAP (green) and Vimentin (red) expression in cell culture from cryopreserved brain tissue at 42 DIV. (J– L) Neurofilament (green) and S100beta (red) expression in cell culture from cryopreserved brain tissue at 42 DIV. Original magnification: a-c x40, d-l x20. (C, F, I, and L). Nuclei were stained with DAPI (blue). Cells shown on c were at a sub-confluent stage while all the others are representative of a confluent stage.

Furthermore, cells from cryopreserved adult human brain tissue revealed the same morphological characteristics (Brunet et al., 2003). Characterization by immunocytofluorescence for glial markers such as glial fibrillary acidic protein (GFAP) and S100beta or for neurofilament, vimentin and nestin also revealed that virtually all cells stained for these different markers in a manner that was indistinguishable from staining obtained on cells prepared from fresh tissue (Fig. 3.2.1.2). Thus, cells obtained in these cultures turned out to be immature neuroectodermal cells that express glial and neuronal markers such as glial fibrillary acidic protein (GFAP), S100beta and neurofilament (Figs. 3.2.1.2F–L). They appear to arise from an ongoing process of de-differenciation since the expression of progenitor markers such as vimentin or nestin is also observed (Figs. 3.2.1.2A–I).

The main purpose here was to observe and characterize in vitro adult monkey brain cells removed from the dorsolateral prefrontal cortex (dlPFC) and then reimplanted into a lesioned cortical area, M1 in the present context, where they demonstrated to survive over time after reimplantation and differentiate into neurons (Brunet et al., 2005), as well as to enhance functional recovery (see chapter "Autologous adult cortical cell implantation significantly enhanced the functional recovery of manual dexterity after unilateral lesion of motor cortex in non-human primates").

3.2.1.2 Methods

All procedures are described in the "General Material and Methods" under "Cell Preparation".

3.2.1.3 Results

Analyses revealed that, as observed in advanced adult human brain cultured cells, the cultures of adult monkey brain cell were constituted by aggregates of 2000-4000 cells, among which about 10-50 cells were GFAP positive with long processes, surrounding a set of DCX positive cells, DCX-Nestin positive cells and DCX-GFAP positive cells (Fig. 3.2.1.3).

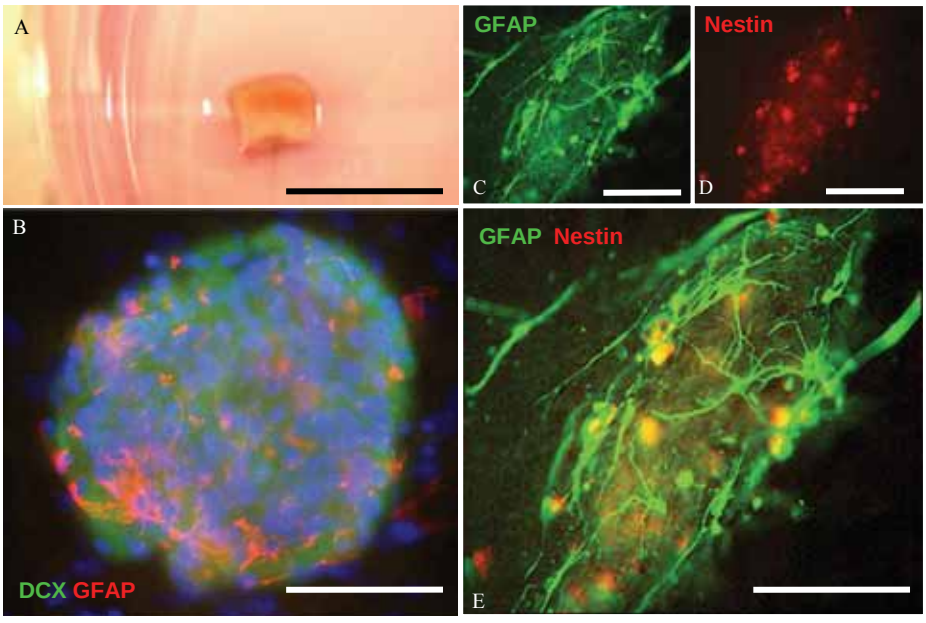


Figure 3.2.1.3: A: Adult monkey brain tissue removed from right dlPFC. In culture, these cells form aggregates (B and E) constituted by GFAP cells (C) surrounding Nestin cells (D), here at 67 DIV. Nuclei were stained with DAPI (blue). Black bar=1cm; white bar=100µm.

In these aggregates, some DCX and GFAP positive cells were able to proliferate, as observed by their ability to incorporate BrdU (Fig. 3.2.1.4).

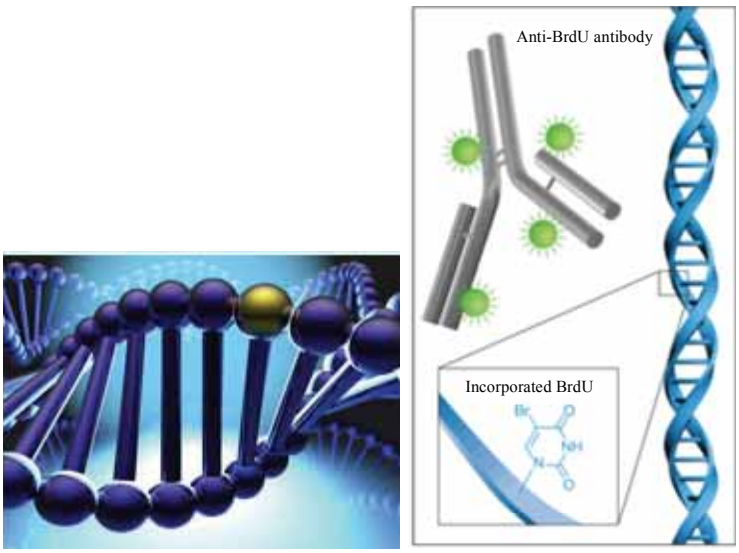


Figure 3.2.1.4: Incorporation of Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU). BrdU is an analog of the DNA base thymidine. During DNA synthesis, BrdU can replace thymidine and be incorporated into the newly synthesized DNA of dividing cells. From www.abdserotec.com/uploads/brdu12main.jpg and modified from http://www.invitrogen.com/etc/medialib/en/images/ics_organized/other/probesonline/Issue0708.Par.83926. Image.-1.0.1.gif

Therefore, in these cultures, a cell ecosystem was recreated, namely the narrow link between astrocytes -GFAP positive cells that do not divide-, and small DCX positive cells that were progenitors and of which a subpopulation of progenitor cells was able to divide. As explained more extensively in the next chapter treating of cell origin, only a small part of the cells, the DCX-GFAP positive ones, preserved their proliferation ability, whereas those that became only DCX and then DCX-Nestin positive stayed at a quiescent progenitor stage. As shown in Table1 and in Figure 3.2.1.5, a given proliferative cell did not divide into two proliferative cells, but probably rather into a DCX-GFAP cell that would proliferate and into a cell that became DCX-Nestin and that stayed quiescent.

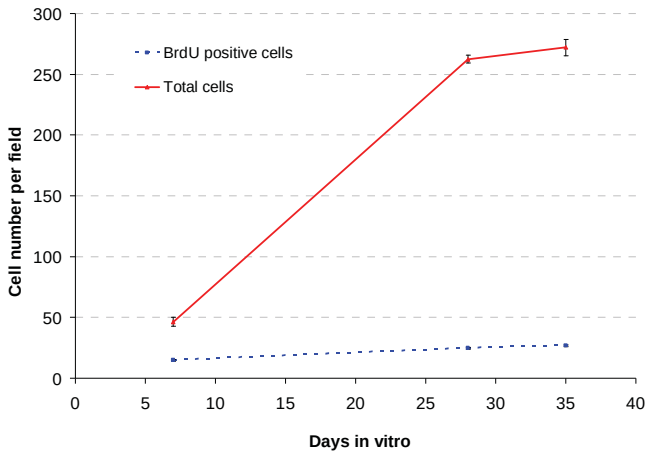


Figure 3.2.1.5 : Quantification at three time points of BrdU+ cells compared to total cells. Results were expressed by means and standard deviations of cell number per field (n = 3 cases, five fields were counted per case, *** $p<0.001$, the two last points compared to the first one). Note that near all BrdU+ cells were DCX and GFAP positive.

	OBSERVATIONS			"PROGENITOR" MODEL		"STEM CELL" MODEL	
DIV	BrdU positive cells per field	Total cells per field	%brdU	If p → p + q		If p → 2p	
				Nb of cycles	Doubling time (Hrs)	Nb of cycles	Doubling time (Hrs)
7	12.67	19.67	64.41				
28	25.00	218.67	11.43	15.711	32.08	3.974	126.84
35	28.00	277.00	10.11	2.333	72.00	1.222	137.44

Table 1: Verification of the two models of division in regard to the countings of BrdU positive cells compared to total cells. Progenitor cell is expressed by p and quiescent cell by q; → means division. Thus, the number of cycles as well as the doubling time between DIV 7 and 28, and DIV 28 and 35 differ according to the model.

The counting of BrdU positive cells, namely cells that proliferated, compared to total cells showed that the number of BrdU positive cells stayed quite always identical: it doubled between the 7^{th} and the 28^{th} day in vitro, and then, between the 28^{th} and the 35^{th} day in vitro, it stayed quite identical, whereas the total number of cells increased. Thus, on 35 days, a model of a progenitor giving rise to a progenitor (DCX and GFAP) and a quiescent cell (DCX) seems to fit well (Fig. 3.2.1.6b), explaining that the number of cells able to incorporate BrdU (and thus to proliferate) was quite the same as at the beginning. In this model, the number of total cells would be equal to $N*(1+n)$ where N is the number of initial cells and n the number of cycles. If a progenitor would give rise to two progenitors (Fig. 3.2.1.6a), as proposed by the stem cell standard model, the number of cells would be equal to $N*2^n$. By applying BrdU during the last 24hours, half of the cells would be able to incorporate BrdU, as observed with the tumoral cell line LN308 (Fig. 3.2.2.S2 of the next chapter about cell origin), whereas the present counting showed only a small subpopulation of BrdU positive cells, suggesting an asymetric proliferation. Furthermore, the doubling time in a model of a progenitor cell dividing into two progenitor cells is aberrant (more than 125 hours), whereas this same parameter in the model integrating a division giving rise to a quiescent cell and a progenitor cell fits with the knowledges about cell division time (24-30hrs), although the mean doubling time between DIV 28 and 35 is higher than it could be expected.

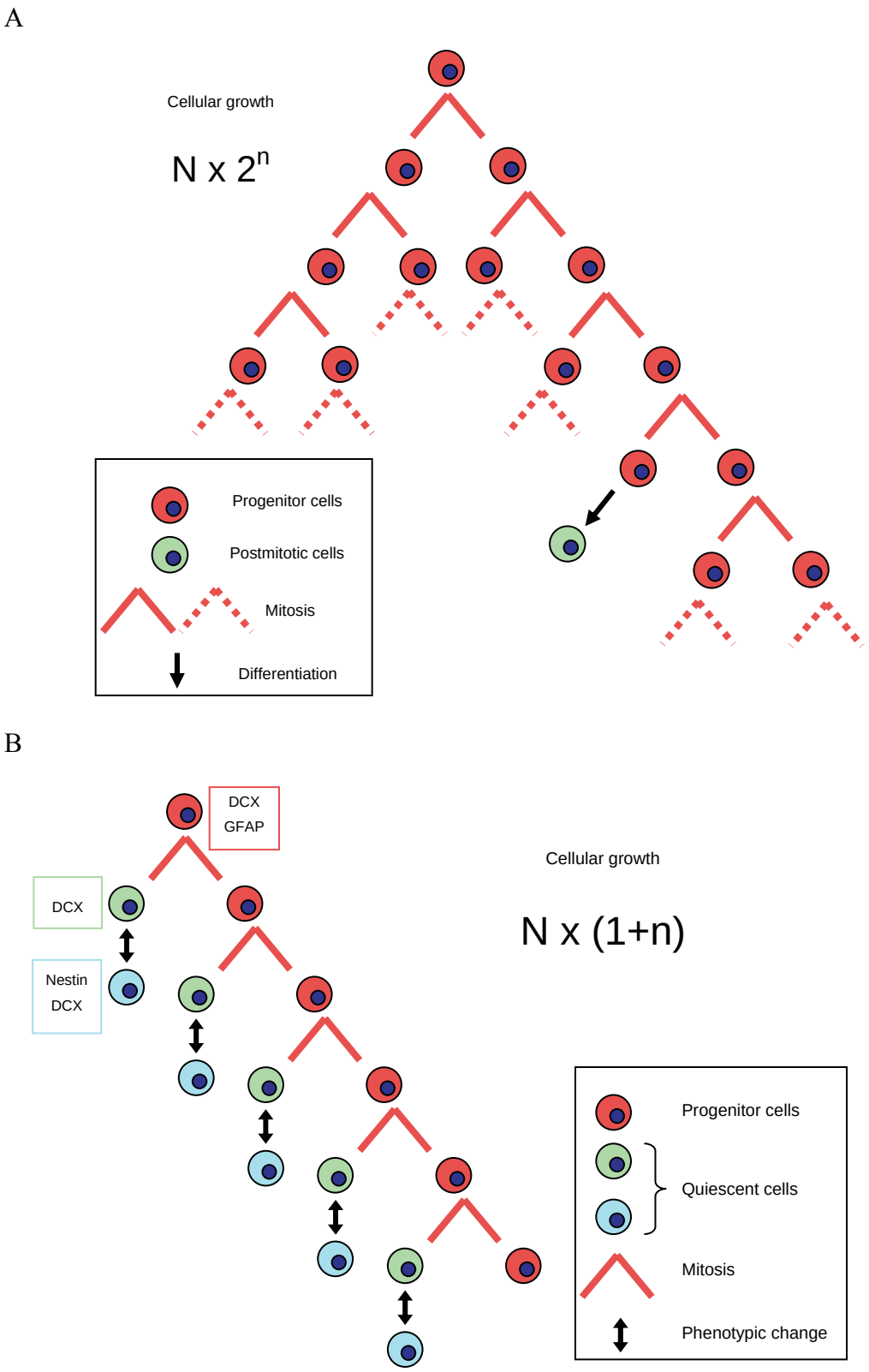


Figure 3.2.1.6: Two models of cell division. A: Classical model of stem cell exponential division, with a single progenitor cell giving rise to two progenitor cells able to divide or differentiate. B: Our alternative model of adult neural progenitor cell division, with a single progenitor cell giving rise to a new progenitor cell able to divide and a quiescent cell that will not divide but able to change phenotypically. N = initial number of GFAP-DCX cells ; n = number of cycles.

3.2.1.4 Discussion

The analyses of adult monkey brain cells in vitro revealed a creation of aggregates evoking a cell ecosystem constituted by neural progenitor cells (DCX positive) and astrocytes (uniquely GFAP positive) that did not proliferate but assured a support to the progenitor cells. Indeed, in the layers where only GFAP cells were present, the culture went out very quickly and these mature astrocytes degenerated. Furthermore, in cultures where only small DCX cells were present, the culture had difficulty to settle, even was not able to build up, as a collaboration between the two cell populations seemed to be needed, namely between progenitor cells and astrocytes that are a feeder support and that form a protection. Therefore, it seems that a cell ecosystem was recreated that could be related to stem cell niches (Scadden, 2006) and that mimicked what can be observed in vivo.

Progenitor cells are often associated to the vascular system, which constitutes the hemato-encephalic barrier, formed by vascular cells and astrocytar feet. One can thus suppose that in vivo, these astrocytes surrounding the vessels play a role to transport the glucose, transform it into lactate at the level of the neurons, assure the supply in energy elements, in metabolites for the cerebral tissue, and protect the progenitors, which consume a lot of energy, as they have an important metabolic activity to divide and differentiate.

In our model of autologous cell therapy, the quiescent cells are a good candidate to play a role once reimplanted in the lesioned area, as they are comparable to post-mitotic neuroblasts that, after division, stay at a precursor stage and are able to differentiate either into astrocyte or into neuron. In our case, they probably differentiated into neuron, as along time they expressed MAP2 (see chapter “In Vivo Cell fate and distribution”). To note that at the moment, the cells able to incorporate BrdU were not detected in vivo over time. We cannot affirm that they did not divide anymore, but they do not divide massively when they are reimplanted. Indeed, they would otherwise form tumors and PKH cells would express some proliferation markers, which was not the case, except in Mk-JO (MIB1 marker; see chapter “In Vivo Cell fate and distribution”). In Mk-JO, no tumoral formation was observed as the MIB1 labeling remained under the limit of 5%; further analyses could be led on the presence of microglias or infiltrating lymphocytes that would be in proliferation phase.

Overall, these adult brain cells, which seemed to organize in vitro similarly to what can be observed in vivo at a histological level in the colocalization between DCX staining and GFAP staining, namely by recreating an optimal and viable cell ecosystem constituted by a partnership between progenitor cells and astrocytes, represent an attractive source for cell therapy, especially for autologous transplantation.

The following part was accepted for publication in Journal of Comparative Neurology.

3.2.2 CELL ORIGIN: DOUBLECORTIN CELLS OF THE PRIMATE CEREBRAL CORTEX ARE AT THE ORIGIN OF IN VITRO ADULT NEURAL PRECURSORS.

3.2.2.1 Introduction

Doublecortin (DCX) was first described through its mutations involved in X-linked lissencephaly and "double cortex" that are allelic human disorders mapping to Xq22.3-Xq23 associated with arrest of migrating cerebral cortical neurons (des Portes et al., 1998; Gleeson et al., 1998). Briefly, DCX was identified as the microtubule-associated protein expressed by migrating neuroblast during a limited phase of their development in both developing and adult mammals (Brown et al., 2003; Francis et al., 1999; Gleeson et al., 1999; Matsuo et al., 1998). DCX plays a crucial role for microtubule stabilization (Gleeson et al., 1999) and nuclear translocation during neuronal migration (Koizumi et al., 2006) as well as in growth cone dynamics (Burgess and Reiner, 2000). Detailed analysis demonstrated that in the rodent nervous system, DCX expression was already induced in fast dividing neuronal precursors, persisted for approximately 30 days and was terminated thereafter as a consequence of neuronal maturation (Brown et al., 2003). During the neuronal development, the cell migration being of the most important step for the layering of the cortical brain structures, DCX participates to the regulation of microtubule dynamics and stability during neuronal morphogenesis (Horesh et al., 1999). In the adult brain, it is now commonly accepted that DCX is expressed in the areas of neurogenesis, indeed in the subventricular zone as well as the hippocampus. Outside these neurogenic niches, DCX was previously thought to be almost inexistent (Brown et al., 2003; Omori et al., 1998). However, more recently, the presence of DCX+ cells was described in the cerebral cortex of guinea pig, cat and primate, suggesting that these cells might be developing interneurons (Cai et al., 2009; Xiong et al., 2008). Considering the animal studies it is now conceivable that some precursor cells in the human cortex proceed to the neuronal lineage (Jessberger et al., 2005). As a consequence, cells expressing the marker of immature neurons (DCX) might be detectable in adult human neocortex and play a role not as a neurogenic marker but in glia-to-neuron signalling mediating synaptic or metabolic plasticity (Verwer et al., 2007).

In human brain, another argument in favor of the crucial role of DCX in neuronal migration is its expression in cytomegalic neurons and balloon cells in the cortical lesion found in tuberous sclerosis and focal cortical dysplasia that show disturbance in laminar architecture and cell differentiation (Mizuguchi et al., 2002). Since its pattern of expression is observed in different neuronal developmental contexts, DCX is considered as a reliable

internal indicator for neurogenesis that may replace bromodeoxyuridine (BrdU) incorporation experiments (Brown et al., 2003). Evidence from animal models indicates that during injury such as ischemic events or seizure induction, the neurogenic response is associated with the transient increased expression of DCX in adult rodent brain (Arvidsson et al., 2002; Brown et al., 2003; Couillard-Despres et al., 2005). Observations in rodents during increased physical activities, environmental enriched conditions or ageing, demonstrated that DCX was closely correlated with the neurogenesis in two specific brain structures: the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (DG) (Brown et al., 2003; Couillard-Despress et al., 2005; Rao et al., 2005). Moreover, DCX continued to be expressed in many cells of the adult rat telencephalon apart from the rostral migratory stream, especially in the corpus callosum and the piriform cortex, suggesting that continuous migration of neuronal precursors may still occur in these areas during adulthood (Nacher et al., 2001). More recently, the presence of newly generated neurons has been described in the adult rat paleocortex layer II (Pekcec et al., 2006; Shapiro et al., 2007a,b). These cells appeared to be generated during embryonic development mainly at E15.5 and persisted in the paleocortex layer II over time (Gomez-Climent et al., 2008). DCX-positive cells have also been described in the cortex layer II of the adult guinea pig (Xiong et al., 2008). In non-human primate piriform cortex and in the human olfactory bulb, newly generated neurons were also observed by immunostaining with other neurogenesis markers such as polysialylated neural cell adhesion molecule (PSA-NCAM), beta-tubulin-III, collapsin response mediator protein-4, neuronal nuclear protein (Bernier et al., 2002) and DCX (Bedard et al., 2002; Bedard and Parent, 2004; Cai et al., 2009). The presence of newly generated neurons was also described in the primate striatum after a single injection of brain-derived neurotrophic factor (BDNF) producing-adenovirus (Bedard et al., 2006). In human brain, a recent study based on autopsy and surgically resected tissue from 60 patients has reported for the first time the presence of DCX-positive cells in the neocortex, where a minority of them also expressed the astrocytic marker GFAP (Verwer et al., 2007).

The aim of this report is to determine the DCX expression in primate neocortex, the cell distribution in the cerebral cortex and more importantly to show their potential link with the progenitor adult brain cells obtained in culture from neocortical biopsies (Arsenijevic et al., 2001; Brunet et al., 2002; Brunet et al., 2003), a technique that we have been developing for more than a decade. This technique represents a new source of cells for transplantation since these progenitors were demonstrated to survive over time after re-implantation and differentiate into neurons (Brunet et al., 2005). The potential therapeutic impact is actually

under evaluation for functional recovery and brain repair in non-human primate models of brain injury or neurodegenerative disease.

3.2.2.2 Methods

This report is based on human and non human primate brain tissues. The pieces of cerebral cortex and brains were obtained from three adult cynomolgus monkeys (*Macaca fascicularis*), in accordance to the Guide for Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by local (Swiss) veterinary authorities, including the ethical assessment by the local (cantonal) Survey Committee on Animal Experimentation and a final acceptance delivered by the Federal Veterinary Office (BVET, Bern, Switzerland) and two adult St Kitts green monkeys (Chlorocebus sabaeus) from St-Kitts Biomedical research foundation (St-Kitts-and-Nevis) with the authorization of CITES importation (agreement 38925). The pieces of six brain resection from human neurosurgery were obtained from trauma (n=4) and epilepsy neurosurgeries (n=2) in accordance with the local ethical committee of Lausanne Hospital University.

3.2.2.2.1 Production of the in vitro adult human and non-human primate brain cells

This report is based on three human cortical biopsies and six cases, two cercopithecus, three macaca, and according to the decision of the local ethical committees from Lausanne, Fribourg and St-Kitts-and-Nevis, the 1 cm^3 piece of resected cortex was washed three times in PBS supplemented with 33 mM glucose, 60 mg per liter penicillin and 100 mg per liter streptomycin. If there was more than two hours delay before seeding cells in culture, the brain samples were kept in Hibernate A Medium (BrainBits LLC, UK) at room temperature as the cold conditions were shown to be disadvantageous for viable culture. Under a sterile environment, cortical tissue was washed another three times in sterile PBS-glucose with antibiotics and well orientated from the cortical surface to the white matter, sliced with a razor blade to obtain enriched fractions in the different GM and WM layers as shown in scheme 1.
For each slice, primary cultures were generated by mincing and mechanically triturating the tissue with fire-polished glass pipettes of decreasing diameters. Cells were plated at 250,000 cells/cm^2 in RPMI 1640 medium (Gibco BRL) supplemented with glucose 25 mM, Glutamine 2 mM, 10-20% preselected Fetal Bovine Serum (pFBS) and an antibiotic/antimycotic cocktail (A7292 Sigma-Aldrich, Buchs, Switzerland) directly on glass coverslip for

immunocytochemistry or on plastic dishes at 37°C in a water-saturated atmosphere containing 6.5% CO_2/93.5% air.

For each fraction, primary cultures were generated by mincing and mechanically triturating the tissue with fire-polished glass pipettes of decreasing diameters. Cells were plated at 250,000 cells/cm^2 in RPMI 1640 medium (Gibco BRL) supplemented with glucose 25 mM, Glutamine 2 mM, 10-20% preselected Fetal Bovine Serum (pFBS) and an antibiotic/antimycotic cocktail (A7292, Sigma-Aldrich, Buchs, Switzerland) directly on glass coverslip for immunocytochemistry or on plastic dishes at 37°C in a water-saturated atmosphere containing 6.5% CO_2/93.5% air**.**

Observation of cultures with phase contrast microscopy or with light microscopy after fixation and staining with hematoxylin revealed the same morphological characteristics in human and non human primary brain cell culture. Characterization is done by immunocytofluorescence for glial markers such as glial fibrillary protein (GFAP) (mouse monoclonal G3893 Sigma-Aldrich, Buchs, Switzerland; rabbit polyclonal Z0334 DAKO, Glostrup, Denmark), S100 beta (mouse monoclonal M2532, Sigma-Aldrich, Buchs, Switzerland), vimentin (mouse monoclonal V9 M0725 DAKO, Glostrup, Denmark) and neural stem cell/progenitor markers such as nestin (rabbit polyclonal AB5922 Chemicon, Temecula, California, USA) and DCX (rabbit polyclonal ab18723 Abcam Cambridge UK and guinea pig polyclonal Chemicon, Temecula, California, USA).

3.2.2.2.2 Immunohistochemistry

Immunohistological stainings were performed on 50-µm thick formalin-fixed cryosections or 4-µm thick formalin-fixed paraffin-embedded sections. Immunohistological characterization was done for glial markers such as glial fibrillary protein (GFAP) (mouse monoclonal G3893 Sigma-Aldrich, Buchs, Switzerland; rabbit polyclonal Z0334 DAKO, Glostrup, Denmark), S100 beta (mouse monoclonal M2532, Sigma-Aldrich, Buchs, Switzerland), vimentin (mouse monoclonal V9 M0725 DAKO, Glostrup, Denmark), neuronal markers such as NeuN (mouse monoclonal MAB377, Chemicon, Temecula, California, USA) and neural stem cell/progenitor markers such as nestin (rabbit polyclonal AB5922 Chemicon, Temecula, California, USA) and DCX (rabbit polyclonal ab18723 Abcam Cambridge UK, guinea-pig polyclonal Chemicon, Temecula, California, USA and goat polyclonal sc-8066 Santa Cruz Biotechnology, California, USA).

The immunoreactions were revealed for immunohistochemistry with biotinylated secondary antibodies followed by the immunoperoxydase Vectastain Elite system (PK-6100, Vector Laboratories) and DAB substrate kit (SK-4100, Vector Laboratories) and

counterstained with Mayer's hematoxylin solution (MHS32, Sigma-Aldrich, Buchs, Switzerland). The DAB immunostaining were observed under light microscope (BX40 Olympus) for DAB. The immunohistofluorescence were peformed with fluorescent dye conjugated secondary antibodies and were scanned for 700nm and 800nm infrared fluorescent dyes with Odyssey system (LI-COR, Bad Homburg, Germany) or observed under epifluorescent microscope (IX81 Olympus) equipped with FITC, Cy3, Dapi and infrared 680nm filters for multichannel images.

3.2.2.2.3 Histological observation and quantification.

Drawings and cell counting of immunostained sections were made with a Olympus BX40 epifluorescence microscope equipped with a motorized X–Y-sensitive stage and a video CDD camera connected to a computerized image analysis system (Explora Nova, La Rochelle, France). Images were acquired using three software programs (Mosaic, Morpho Expert and Fluo3D, Explora Nova, La Rochelle, France). Representative 50 μm slices separated by 5 mm were used, that mean 7 sections per animal (n=2). Per section, 5 cortical columns were drawn and the layers were outlined for each column. The threshold of DAB staining and the limit of cell surface from 50-500 μm2 were applied to automatically identify all DCX+ cells. The threshold of Mayer nuclei staining and the limit of nuclei surface from 10-80 μm2 were applied to automatically identify all cells (Mercator, Explora Nova, La Rochelle, France). A visual control for these DCX+ cell and nuclei identifications was done on 20 of 260-300 fields per column. The obtained countings allowed to acquire the percentage of DCX+ cells compared to total cells in layers and columns.

3.2.2.2.4 Western blot

Total homogenates were obtained in RIPA lysis buffer (Santa Cruz Biotechnology, California, USA) from human cortex cryosections. The protein concentration was quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Waltham, Massachusetts, USA). 10 μg were loaded per well on Nupage 4-12% Bis Tris gel (Invitrogen, Life technology, Carlsbad, California, USA), run at 200V constant and transferred on PVDF membrane (PerkinElmer, Zaventem, Belgium). The membranes were first incubated in Odyssey Blocking Buffer (OBB) (LI-COR, Bad Homburg, Germany) then with antibodies against DCX (same as for immunohistochemistry) and Actin (Mouse AC74, Sigma-Aldrich, Buchs, Switzerland), all diluted in OBB at 1/2000. The membranes were then washed three times in PBS for 10 minutes and incubated with appropriate secondary antibodies conjugated

with AlexaFluor 680 dye (Invitrogen, Life technology, Carlsbad, California, USA) or with IRDye 800 (Rockland Immunochemicals Inc, Gilbertsville, Pennsylvania, USA). After three washes with PBS, the membranes were scanned with the infrared system Odyssey (LI-COR, Bad Homburg, Germany).

3.2.2.2.5 RT-PCR

Total RNA from fresh slice fractions and pellets of cells was extracted with Trizol (Invitrogen) and 200 ng total RNA was used for first strand synthesis using MulVRT-M0253S (New England Biolabs, Inc.) as described by the supplier. PCR were performed with Polymerase Kit (Qiagen GmbH) with 5 pmol of specific primers per reaction. The specific primers for DCX and nestin were designed to amplify the mRNA 1021-1494 (accession AJ003112) and 714-1433 (accession X65964) human sequences respectively and were homologous for the corresponding 1021-1494 (accession XM_001100519) and 850-1567 (accession AY650322) monkey sequences. For actin mRNA as control, two primer panels were used to amplify the 375-1154 (accession X00351) human sequence and the 197-906 (accession AB004047) monkey sequence. All specific forward and reverse primers for RT-PCR were designed on different exons to avoid any genomic contamination. All PCRs were performed in the Personal Thermocycler (Biometra, GmbH) with one first denaturation step (3 min, 94°C), followed by 35 cycles of denaturation (94°C, 30s), annealing (60°C, 30s) and elongation (72°C, 60s) and a final extension step (72°C, 10 min). The infra-red fluorescent nucleic acid dye Syto60 (Molecular Probes, Eugene, Oregon, USA) was incorporated within the loading buffer (1/6000) in order to detect and quantify the amplicons with the Odyssey infrared imaging system (LI-COR, Bad Homburg, Germany) after migration on a 0.8% agarose gel. To confirm the specificity of the mRNA RT-PCR amplification, amplicons were subcloned with pGEM-T easy vector system I (Promega, Madison WI, USA) and sequenced.

3.2.2.2.6 Incorporation of BrdU

The proliferation marker bromodeoxyuridine (BrdU; Sigma-Aldrich, Buchs, Switzerland) was used to investigate the specific phenotype of the proliferating cells. As proliferating positive control, glioblastoma cell lines (LN308) was used in the same conditions for BrdU incorporation as human and non human primate adult brain cell primoculture. At the three time points 6 days in vitro (DIV), 28 DIV and 35 DIV of the cell culture, the adult brain cells were incubated 24 h before PAF fixation with a 5µM final concentration of BrdU added to the culture medium. The BrdU was detected after a pretreatment with HCl 2N for 2 min. The immunocytofluorescence was done with mouse anti

BrdU antibody (744, DAKO, Glostrup, Denmark), rabbit anti DCX antibody (ab18723 Abcam, Cambridge UK) and chicken anti GFAP (AB5541 Chemicon, Temecula, California, USA) and the appropriate fluorochrome conjugated secondary antibodies against mouse Ig (alexa 488, A-11032 Invitrogen, Life technology, Carlsbad, California, USA), rabbit Ig (alexa 594, A-11034 Invitrogen, Life technology, Carlsbad, California, USA) and chicken Ig (IRDye 700, 603-130-126, Rockland Immunochemicals Inc, Gilbertsville, Pennsylvania, USA). The nuclei were counterstained with DAPI. Immunostained cells were observed under epifluorescent microscope IX81 (Olympus).

3.2.2.2.7 Cell counting of BrdU positive cells.

The cell counting per coverslip was determined by the sum of 4 fields on one coverslip. The total cell number was determined by the counting of DAPI counterstained nuclei. The co-localization of BrdU with other markers such as GFAP or DCX were done by co-immunocytofluorescence before counting. The mean of cell number was established with the six independent cell cultures from three patient biopsies.

3.2.2.3 Results

All results obtained from non human primate brains were comparable from both species cynomolgus monkeys (*Macaca fascicularis*) and St Kitts green monkeys (Chlorocebus sabaeus) and were commonly described belong.

3.2.2.3.1 DCX-positive cells are present in non-human primate neocortex.

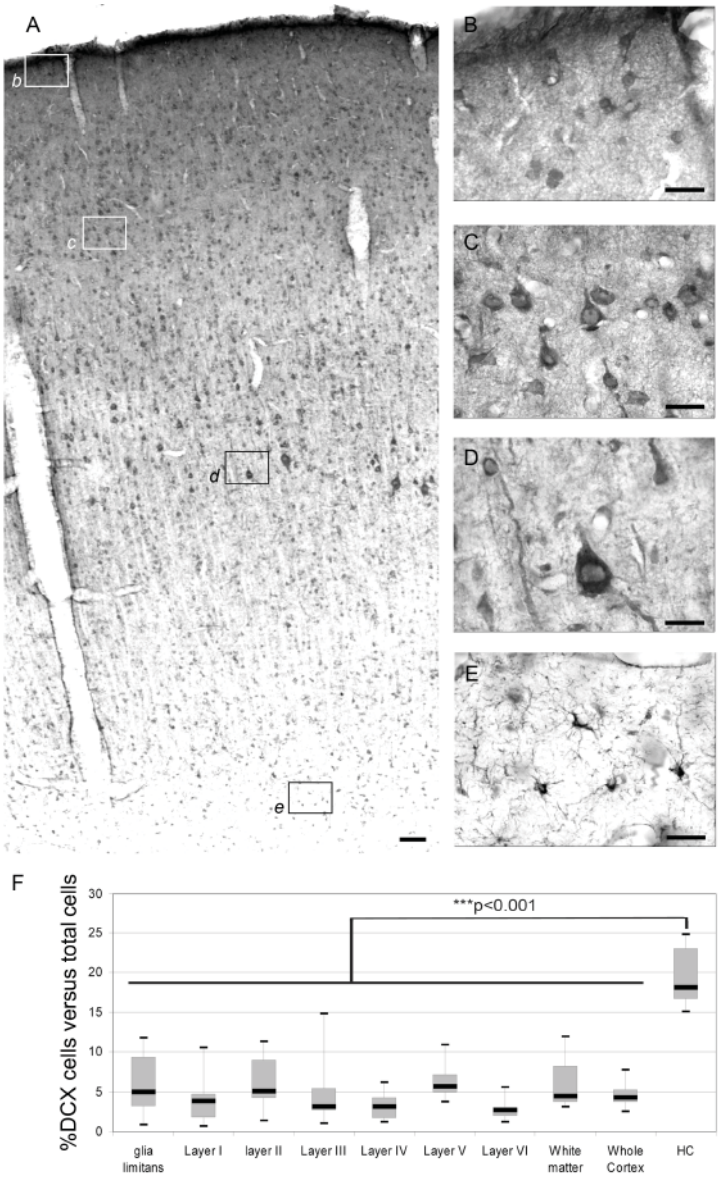


Figure 3.2.2.1: DCX immunohistochemistry done on macaca brain cryosection with guinea pig antibody against DCX (Chemicon). The mosaic reconstruction (A) is a part of cerebral cortex in the right precentral gyrus at the level of superior precentral sulcus. B-E correspond to boxes in A: DCX is expressed in non human primate cortex, in cells with long processes in the glia limitans and layer I (B), in pyramidal cell bodies in the layer II (C) and in the layer V (D) and in small stellar cells at the border between GM and WM (E). The nuclei were counterstained with Mayer's hematoxylin solution (MHS32, Sigma-Aldrich, Buchs, Switzerland). Note that this DCX distribution is through all the neocortex with no significant difference in intensity or density. Scale bars A= 100 μm, B-E= 25 μm. F: Distribution of DCX positive cells in the cortical layers. The countings for DCX and total nuclei were done in 35 cortical columns (5 columns per section, 7 sections per animal (n=2)) and in 12 areas of hippocampal subgranular zone (HC) (3 areas per section, 4 sections). The first eight boxplots represent the distribution in the glia limitans, the six cortical layers and the white matter next to the grey. The "whole cortex" boxplot corresponds to the pooling of countings in all layers. The results are expressed in percentage of

DCX positive cells versus total nuclei. Note that there is no significant difference between cortical layers, whereas there is significantly less DCX positive cells (near 4 fold less) in cortical structures compared to sugranular zone of hippocampus (Anova analysis, Bonferroni multiple comparison *** $p<0.001$).

In the non-human primate cerebral cortex, the immunohistochemical analysis showed that the expression of DCX was not restricted to the subgranular zone of the hippocampus and to the subventricular zone but DCX was also found in the cerebral cortex (Figure 3.2.2.1A). This DCX expression was highly located at the glia limitans, in layer II and layer V of the cortex (Figure 3.2.2.1B-E). The cell counting on DAB immunostained sections showed that 4.55±1.58 % of total cells expressed DCX in whole neocortex. In terms of percentage of positive cells, no significant differences were observed between cortical structures or between cortical layers (Figure 3.2.2.1F). Furthermore, this percentage of DCX positive cells is significantly lower (near 4 fold) in cortical structures than in the hippocampal subgranular zone where 19.62±3.74 % of cells were DCX positive. Higher magnifications showed that the cell expressing DCX were not shaped homogeneously, indeed DCX cells with long processes were observed in the glia limitans and in the layer I (Figure 3.2.2.1B), DCX pyramidal cell bodies with neuronal shapes were stained in layer II (Figure 3.2.2.1C) and V (Figure 3.2.2.1D) and small stellar DCX cells were located at the limit between gray matter and white matter (Figure 3.2.2.1E). The immunohistofluorescence to detect DCX with Odyssey infrared scanner showed that additionally to the expression in the subventricular zone (Figure 3.2.2.2C) and the subgranular zone of the hippocampus (Figure 3.2.2.2D), the presence of DCX was observed in the whole cerebral cortex (Figure 3.2.2.2).

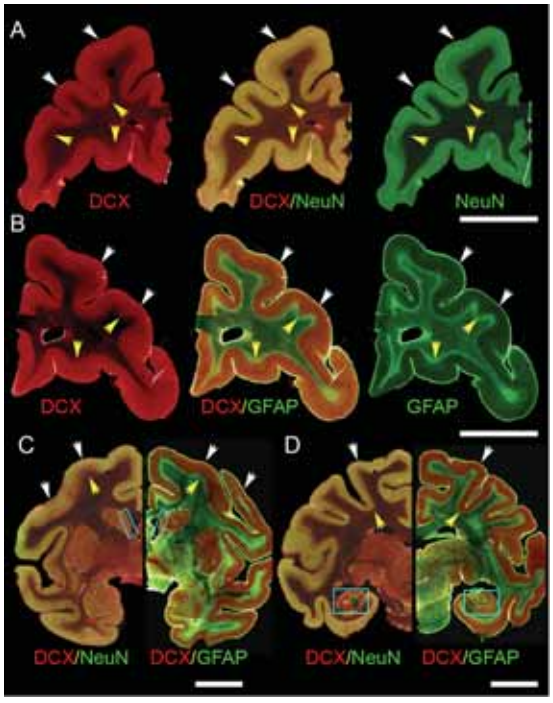


Figure 3.2.2.2: Coronal sections of St Kitts green monkey brain infrared immunostained for DCX (guinea pig antibody Chemicon) and GFAP or DCX and NeuN detected with Odyssey (LICOR, Germany). Each fixed monkey brain sections were cut in two half-sections one incubated with guinea-pig anti-DCX and mouse anti NeuN antibodies and the second one incubated with guinea-pig anti-DCX and rabbit anti-GFAP antibodies followed by the appropriate infrared fluorochrome conjugated secondary antibodies (see Methods). The panel A present the left frontal cortex half-section immunostained for DCX (in red pseudo color) and NeuN (in green pseudo color) with the overlay, the panel B presents the right frontal cortex half-section immunostained for DCX (in red pseudo color) and GFAP (in green pseudo color) with the overlay. The panels C and D only present the overlays obtained as in A for more caudal section, the blue squares boxed the subventricular zone in C and the hippocampus in D, respectively. Note that if the main DCX immunostaining is co-localized with NeuN, the glia limitans (white arrows) and the limit between white and gray matters (yellow arrow) also present co-localization. Also note that DCX is detectable in the whole cortex from frontal to occipital lobes. Scale bars = 1 cm.

It confirmed the presence of DCX from glia limitans to the limit between gray and white matters in the non human primate neocortex. Similar results were obtained on the cryosections derived from the five normal monkeys (3 *macaca fascicularis* and 2 *chlorocebus sabaeus*). The main cortical expression of DCX was localized in the gray matter of the frontal section (Figure 3.2.2.2A and 3.2.2.2B). At this resolution rate of 21 μm, a colocalization with NeuN could be observed at a macroscopic histological level in the cortical neuronal layers (Figure 3.2.2.2A), but also with GFAP in the glia limitans and at the limit between gray and white matters (Figure 3.2.2.2B). This pattern of non-human primate cortical expression of DCX has been observed through all the neocortex even though the expression was stronger in the subventricular zone and in the subgranular zone of the hippocampus (Figure 3.2.2.2C and 3.2.2.2D). DCX was expressed in the whole cortex and the comparison of the infrared DCX-immunolabeling intensities revealed no significant difference between the different cortical areas. The expression of DCX was also confirmed by western blot (Figure S1A) done with four adult human cortical homogenates and two primitive neuroectodermal tumours as controls, and the same bands were revealed with the rabbit immunoglobulin against DCX (Abcam) (Figure 3.2.2.S1A, note that B represent the actin expression as control for westernblot loading) and the goat one (Santa Cruz).

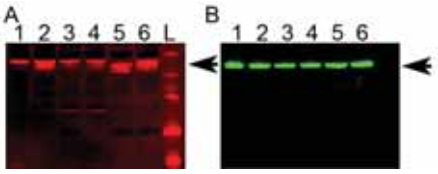
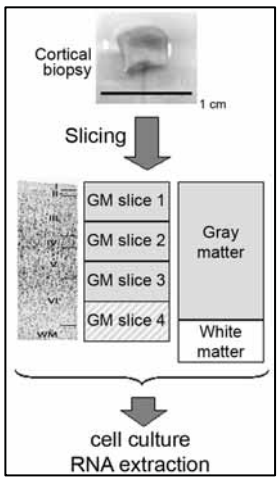


Figure 3.2.2.S1, **supplemental information to figure 3.2.2.2**: Western blot done with goat polyclonal antibody against DCX (sc-8066 Santa Cruz Biotechnology, California, USA) (A) and Actin (Mouse AC74, Sigma-Aldrich, Buchs, Switzerland) (B). The western blot was done with 4 normal adult human brain homogenates (lanes 1-4), with to PNET tumour extracts (lanes 5-6) as control. L correspondent to the SeeBlue ladder (Invitrogen).
Note that the same results were obtained with rabbit polyclonal ab18723 Abcam Cambridge UK).

By RT-PCR done with the "slicing" procedure (scheme 3.2.2.S1), the DCX mRNA was detected in the human neocortex, with a significant higher expression in slices 3, 4 and 5 that mainly corresponded to the topography of layer IV and to the limit between gray and white matter (Figure 3.2.2.3).



Scheme 3.2.2.S1 supplemental information for experimental procedure: Principle of the cortex slicing method. As described in the text, the cortical biopsy is orientated from glia limitans to white matter. It is then sliced parallel to the cortex surface with a razor blade to obtain layer enriched fractions. Each fraction is then separately minced and dissociated to obtain cell cultures or to extract total RNA. In parallel, grey matter and white matter fractions were also minced and dissociated as described in a previous study.

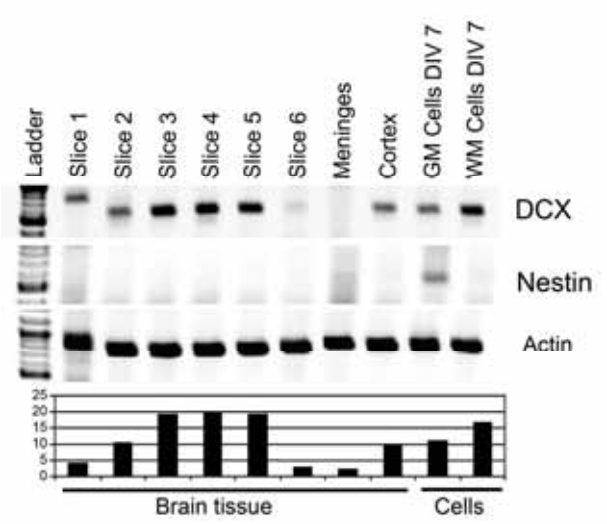


Figure 3.2.2.3: RT-PCR done with total RNA extracted from human neocortical layering slice enriched fractions and from adult human brain cell cultures. DCX mRNA is present in all fractions from human cortex and in cell cultures at the first days in vitro (DIV7) except in the meninges and in the deeper white matter fraction (slice 6). Nestin mRNA is not detectable in the same RT-PCR conditions except slightly in the GM cell culture (DIV7). Actin mRNA is used as control to establish the relative ratio DCX/Actin of Syto60 infrared intensity presented in the graph (this experiment is representative of the 6 slicing experiments done with four human and two non-human primate biopsies).

The specific forward and reverse DCX primers were designed on exons 3 and 7 of the human gene sequence respectively, the size corresponded to the DCX mRNA sequence. For three RT-PCR samples (one human and two monkeys), the amplicons were subcloned, sequenced and confirmed the complete homology with the DCX sequence. With the same samples, in the same RT conditions, the PCR for nestin did not allow to reveal the presence of nestin mRNA in cortical slices. However a slight nestin expression was detected in the GM cell culture after 7 days.

3.2.2.3.2 DCX is expressed in astrocyte and neuron in non human primate cortex.

To identify the cell subtypes of DCX positive cells revealed by immunohistochemistry, co-immunofluorescence labelings were performed by using the rabbit antibody against DCX (Abcam). The same pattern was observed with this rabbit anti-DCX antibody compared to the guinea-pig one (Figure 3.2.2.4).

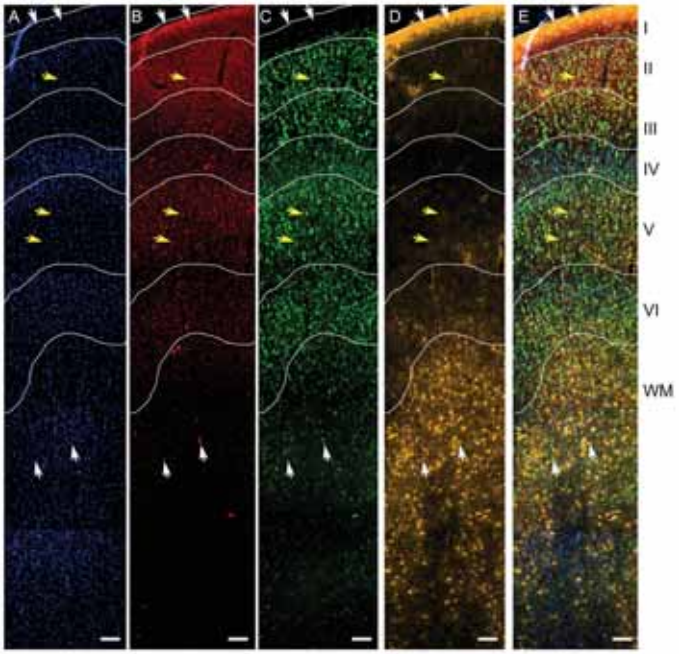


Figure 3.2.2.4: A. mosaic reconstruction of the cercopithecus neocortex triple immunofluorostaining for GFAP, DCX (rabbit antibody, Abcam), and NeuN detected with alexa 594, alexa 488 and IRDye 700nm conjugated secondary antibodies, respectively, with a counterstaining of nuclei with DAPI. DCX (in green) was mainly shown in layer I (white arrows), co-localized with GFAP (in red), in layer II and V (yellow arrows) co-localized with NeuN (in orange pseudocolor) even if it is distributed from the glia limitans (white arrows) to the limit between gray and white matters. Scale bars = 100 μm.

The mosaic reconstruction of the cortex exhibited the same layer distribution of DCX. Cell counting on triple immunofluorescence stained sections gave the same percentage 4.33±1.48 % of DCX positive cells versus total cells in a cortical column as shown above with DAB staining. Higher magnification demonstrated the presence of four subpopulations of DCX positive cells (Figure 3.2.2.5).

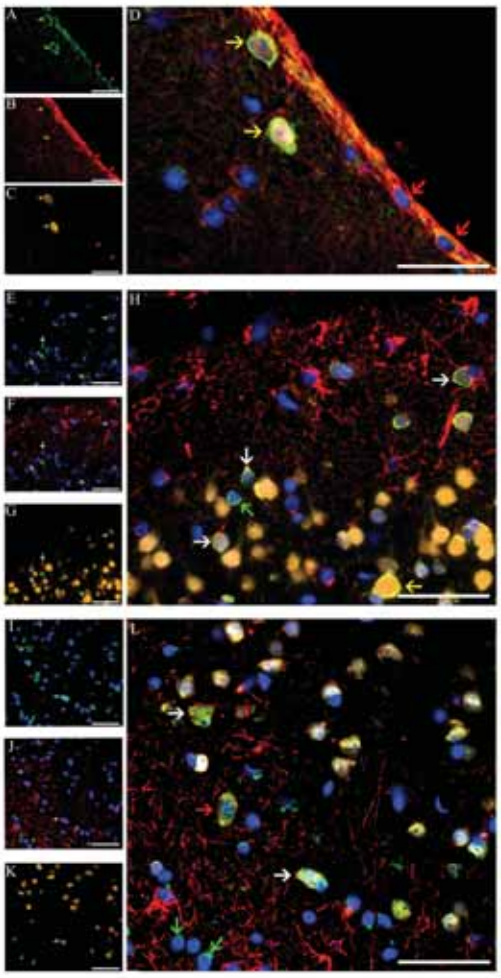


Figure 3.2.2.5: Higher magnification of the cercopithecus neocortex triple immunofluorostaining for co-localization of DCX (rabbit antibody, Abcam), NeuN and GFAP on 5 µm paraffin sections. A subpopulation of DCX positive cells co-expressed NeuN (yellow arrows) in layer I (A-D), in layer II (E-H) and layer V (I-L), another one co-expressed GFAP (red arrows) in the glia limitans (A-D) and at the limit between gray and white matters (I-L), a third one co-expressed NeuN and GFAP (white arrows) and a fourth subpopulation do not express NeuN nor GFAP (green arrow) in layer II (E-H) and in layer V (I-L). Scale bars = 50 µm.

First, small cells only expressed DCX and represented 17.85±4.32 % of the total DCX positive cells. The cells that only expressed DCX presented long processes and small nuclei. No co-localizations were observed with Iba1 (microglia/macrophage marker) nor nestin (neural progenitor/stem cell marker) nor vimentin (glial progenitor marker) (data not shown). Second, 26.24±3.31 % of the DCX positive cells co-expressed NeuN and represented 2.89±1.18 % of the total NeuN positive cells. The DCX positive cells that co-expressed NeuN in the layer I (Figure 3.2.2.5A-D) presented the typical shape of the Cajal-Retzius cells. In the layers II and V, the DCX-NeuN positive cells exhibited a pyramidal neuronal shape (Figure 3.2.2.1 and 3.2.2.5E-H). Third, 26.44±6.03 % of the DCX positive cells co-expressed GFAP and represented 11.66±4.06 % of the total GFAP positive cells. The DCX-GFAP positive cells also presented two types of shape. In the glia limitans, these DCX-GFAP positive cells had long processes but, at the border between gray and white matters, they were small stellar

cells (Figure 3.2.2.5I-L). Fourth, 29.47±7.07 % of DCX positive cells surprisingly co-expressed NeuN and GFAP; to note that 92.85±5.80% of the total GFAP and NeuN positive cells expressed DCX (Figure 3.2.2.5).

3.2.2.3.3 DCX-positive cells are present in adult brain cell cultures from human brain biopsies.

The DCX mRNA was detected in adult human brain cells since the first days *in vitro* when nestin was slightly or not detectable at that time (Figure 3.2.2.2). The immunocytofluorescence also revealed the presence of DCX in cells since DIV 10. At different time points, the progression of the cell culture and the expression of DCX were investigated. The cell quantification by infrared Syto 60 nucleus labeling showed that only enriched cell suspensions that contained DCX positive cells, the cortical slices 1 and 3 and the white and grey matter fractions (WM and GM respectively) were able to survive over time (Figure 3.2.2.6J).

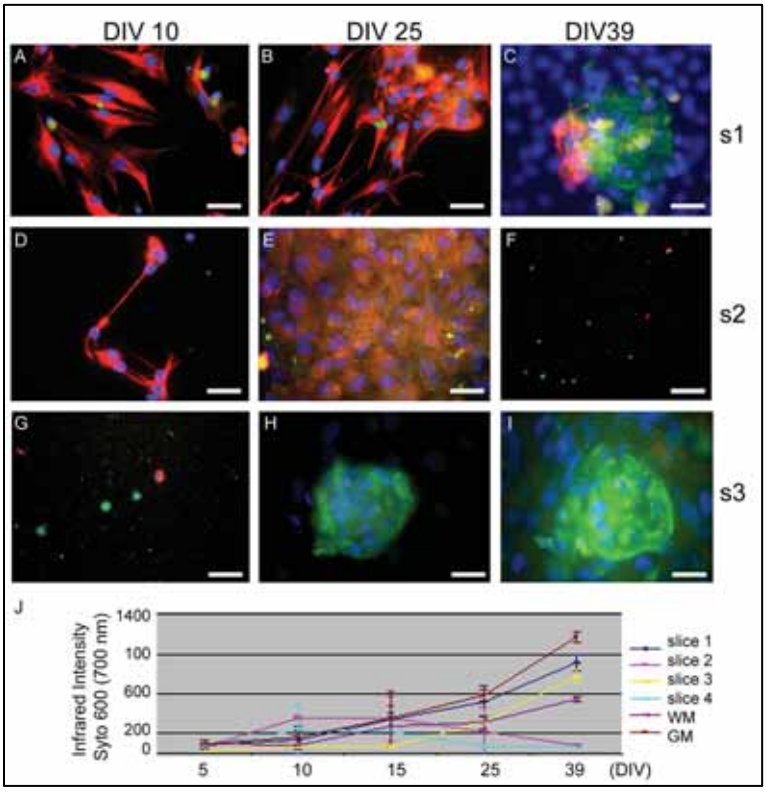


Figure 3.2.2.6: Cell culture from neocortical layering slice enriched fractions immunostained for GFAP (in red) and DCX (guinea pig polyclonal antibody, Chemicon) (in green) with a counterstaining of nuclei with DAPI (in blue).
A-C: slice 1 culture, D-H: slice 2 culture and G-I: slice 3 culture at three different time point DIV 10, 25 and 39. In the slice 1 and 3, the presence of DCX positive cells is detected at all time points and the DCX population increase overtime. After DIV 25, GFAP is just detected in very few cells. J: The cell quantification done with the infrared DNA dye Syto60 showed that only enriched cell suspensions from slice 1, slice 3, WM and GM fractions are able to survive over time. Note that these fractions contain DCX positive cells in the early stage of culture. Scale bars = 50 µm.

The slice 4 that included deeper cortical layer and the limit between gray and white matter did not present viable cells over time. The slice 2 that presented only few DCX positive cells at DIV 10 rapidly degenerated with no survival over time (figure 3.2.2.6D-F). The slices 1 and 3, the enriched GM fraction and the enriched WM fraction that presented DCX positive cells maintained a substantial population of cells that survived over time and formed adult brain cell aggregates (figure 3.2.2.6A-C and 3.2.2.6G-I).

The incorporation of BrdU revealed that a few populations of cells were in S phase over 24 hours (figure 3.2.2.7). Less than 25 positive cells per field were detected for BrdU from normal WM and GM fractions. This number did not change over time but the population continued to increase to a total number of 45 cells per field at DIV 7, of 280 cells per field after 28 days. At 35 days, the percentage of BrdU positive cells only represented 9.8% despite the 24 hours of exposure to BrdU (figure 3.2.2.7).

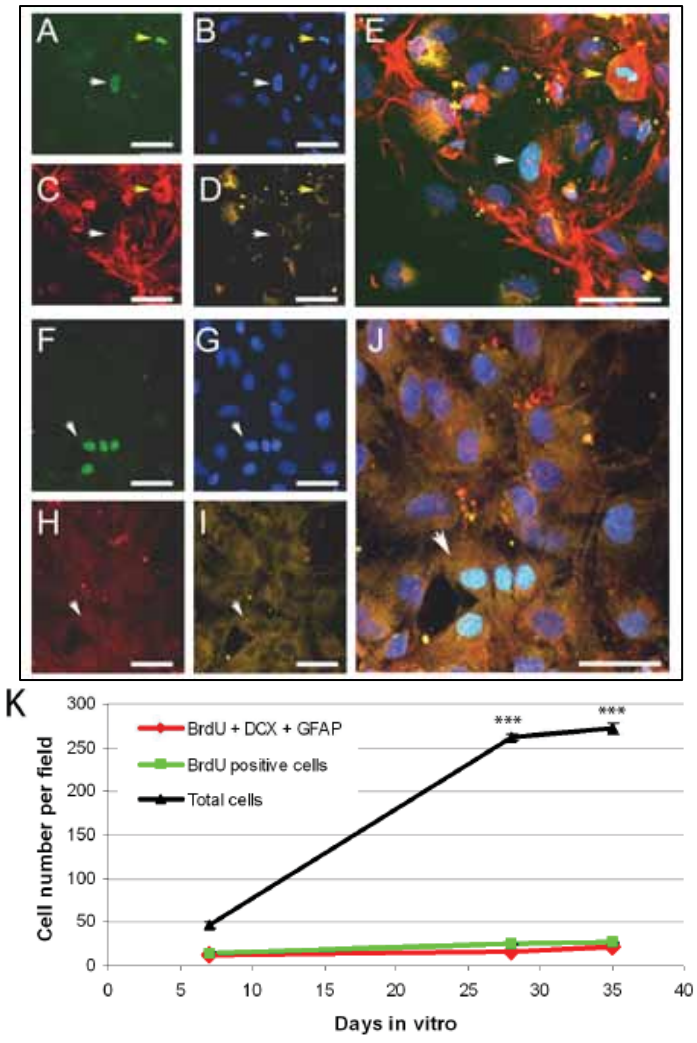


Figure 3.2.2.7: Immunostaining for BrdU (in green), GFAP (in yellow pseudocolor) and DCX (rabbit antibody, Abcam) (in red) with a counterstaining of nuclei with DAPI (in blue).
A-E: WM cells in vitro (DIV35), F-J: GM cells in vitro (case 173 DIV28). These two panels present the detection of BrdU (A, F), DCX (C, H), GFAP (D, I) and Dapi (B, G) and the overlay of the four color pictures (E, J). The BrdU positive cells from normal WM and GM fractions expressed DCX and GFAP (white arrow)

even if they were at the metaphase stage of mitosis (yellow arrow). K: Quantification at three time points of DCX+GFAP+BrdU+ cells compared to BrdU+ cells and total cells. Results were expressed by means and standard deviations of cell number per field (n = 3 cases, five fields were counted per case, *** $p<0.001$, the two last points compared to the first one). Near all BrdU+ cells were DCX and GFAP positive. Note that the number of BrdU+ cells did not significantly increase over time contrarily to the total cell number. Scale bars = 50 µm.

Nearly all the BrdU positive cells expressed DCX and GFAP (white arrow, figure 3.2.2.7) although they were at the metaphase stage of mitosis (yellow arrow, figure 3.2.2.7A-E). LN308 glioma cell line was used as experimental positive control for BrdU incorporation (Figure 3.2.2.S2) and showed that near 70% per field of nuclei incorporated BrdU over 24 hours.

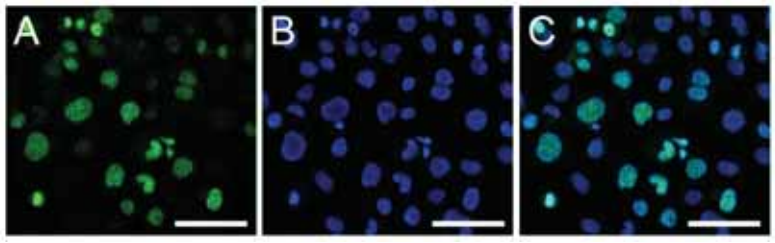


Figure 3.2.2.S2, supplemental information to figure 3.2.2.7: LN308 glioma cell line as positive control for BrdU incorporation detected for BrdU (A, in green) and Dapi (B, in blue) with the overlay (C). Note that more than 70% of cells incorporated BrdU during 24 hours.

3.2.2.4 Discussion

In the present study, we demonstrated that the expression of DCX is present in the cortex of adult humans, Macaque and Green monkeys. We newly found out four different populations of DCX+ cells. Indeed, the DCX immunoreactivity was localized in all the cerebral cortical layers from the glia limitans and around the limit between white and gray matters. The DCX positive cells had different shapes: in layer I, they presented the typical shape of the Cajal-Retzius cells; in the layers II and V, they exhibited a pyramidal neuronal shape. In the glia limitans, they presented long processes whereas at the border between gray and white matters, they were small stellar cells. We also demonstrated using mRNA and protein quantification that DCX was detectable in the whole human neocortical tissues. The detection of DCX mRNA was performed by RT-PCR that is more sensitive than Northern blot (Omori et al., 1998).

Moreover, we could demonstrate that the four DCX cell populations were colocalized with different neuronal and glial markers depending on their location in the cortex: in layers I, II and V, the DCX positive cells expressed NeuN, whereas in the glia limitans and around the limit between white and gray matters, the DCX positive cells co-expressed GFAP. In the glia limitans, the DCX-positive cells with long processes expressed GFAP and in the border

between gray and white matters, the small stellar DCX-positive cells also expressed GFAP. The cells that only expressed DCX presented long processes and small nuclei.

DCX cells were clearly described in the neurogenesis niches of adult rodents, but they were never detected in their cortex except in the rat piriform cortex where DCX was colocalized with NeuN, suggesting an involment in axonal or synaptic plasticity (Nacher et al., 2001). Comparatively to the total cell density, DCX positive cells were significantly less numerous in the cortex than in the neurogenic subgranular zone of the hippocampus. Nevertheless, DCX positive cells in the cerebral cortex represent a significant population amounting to around 4-5% of all cortical cells. Furthermore, they were evenly distributed among the four identified populations of DCX cells. This large distribution would be related to the greater ability in evolved brain to elaborate interconnectivity between cortical structures. It is now more obvious that DCX cells are also observed in the whole cortex and subcortical areas of the more evolved species like simian and human adult brains. We hypothesize that this observation may have a close relation to mammalian evolution, as brain size has gradually increased (for review see Bradbury, 2005), in particular the cerebral cortex of the more evolved species (for review see Levitt et al., 1997).

We now think that the DCX cortical cells are not stem cells, but they may have a neural progenitor potential (Arsenijevic et al., 2001; Bjorklund and Lindvall, 2000; Brunet et al., 2002; Brunet et al., 2005; Goldman et al., 1997; Palmer et al., 1999). These cells are certainly at the origin of the adult brain cell culture we obtained in vitro with simian and human cortex; however, this could never be reproduced with rodent brains where no DCX cortical cell could ever be detected. In this report we clearly demonstrate that the cells obtained in vitro expressed DCX at the beginning of the culture. When the cortex was sliced in layers, only the layers containing DCX cells were able to give viable cells over time. The BrdU incorporation showed that only a few cells were dividing and they expressed DCX and GFAP. The number of dividing cells did not increase over time that suggests an asymmetric division. A dividing cell would generate a quiescent progenitor cell that still express DCX but not GFAP and a new dividing cells that express DCX and GFAP. These proliferating cells were closely associated with cells that have long GFAP positive processes. This essential association for the development of the culture over time should recreate the stem cell-like niche *in vitro*. This niche with few astrocytes that surround numerous DCX and DCX/GFAP progenitors represents an interesting tool for cell therapy since the astrocytes protect through cell contact and certainly factor delivery. We have already demonstrated the important rate of survival of such cells after autologous reimplantation and their ability to migrate and to differentiate into neuron in motor cortex lesion in monkeys (Brunet et al., 2005) or to limit the

TH depletion in asymptomatic MPTP Parkinsonian monkeys (Brunet et al., 2009). This post-transplantation abilities would also be in relation with the cell-cell interaction and the progenitor character of these cells described in this report.

In conclusion, the presence of DCX positive cells and the ability to produce precursor cells from the primate cortex were demonstrated to be closely linked. This specificity of adult primate DCX expression in the cortex is likely to be associated with the evolution of primate cortex. The roles of these DCX cells have to be investigated in the synaptic and metabolic plasticity but also in the neuropathological context. In the future, these cells would represent an important role for brain repair as endogenous tools or as autologous transplanted tools after in vitro cell culture.

3.2.3 IN VIVO CELL FATE AND DISTRIBUTION

3.2.3.1 Introduction

As described in the preceding chapters, adult monkey brain cells obtained from a dlPFC biopsy could survive in culture and recreate a cellular ecosystem constituted by astrocytes surrounding progenitor cells expressing DCX and GFAP as well as DCX and/or Nestin. Furthermore, once reimplanted in the lesioned tissue, these cells showed a capacity to migrate towards the lesioned area and to differentiate into mature neurons, as demonstrated in a previous feasibility study (Brunet et al., 2005; see also the following Results part). In the present investigation, observations carried on autologous cells reimplanted in two monkeys subjected to an unilateral M1 lesion and who were also assessed on their manual dexterity. Results on this behavioural side (see chapter entitled “Autologous adult cortical cell implantation enhanced functional recovery of manual dexterity after unilateral lesion of motor cortex in monkeys”) showed an enhancement of recovery of manual dexterity, in the order of 25% additionally to the “spontaneous” recovery, which was probably promoted by autologous adult brain progenitor cell implantation.

3.2.3.2 Methods

All procedures are described in the “General Material and Methods” under “Cell Preparation”.

3.2.3.3 Results

3.2.3.3.1 Control monkeys

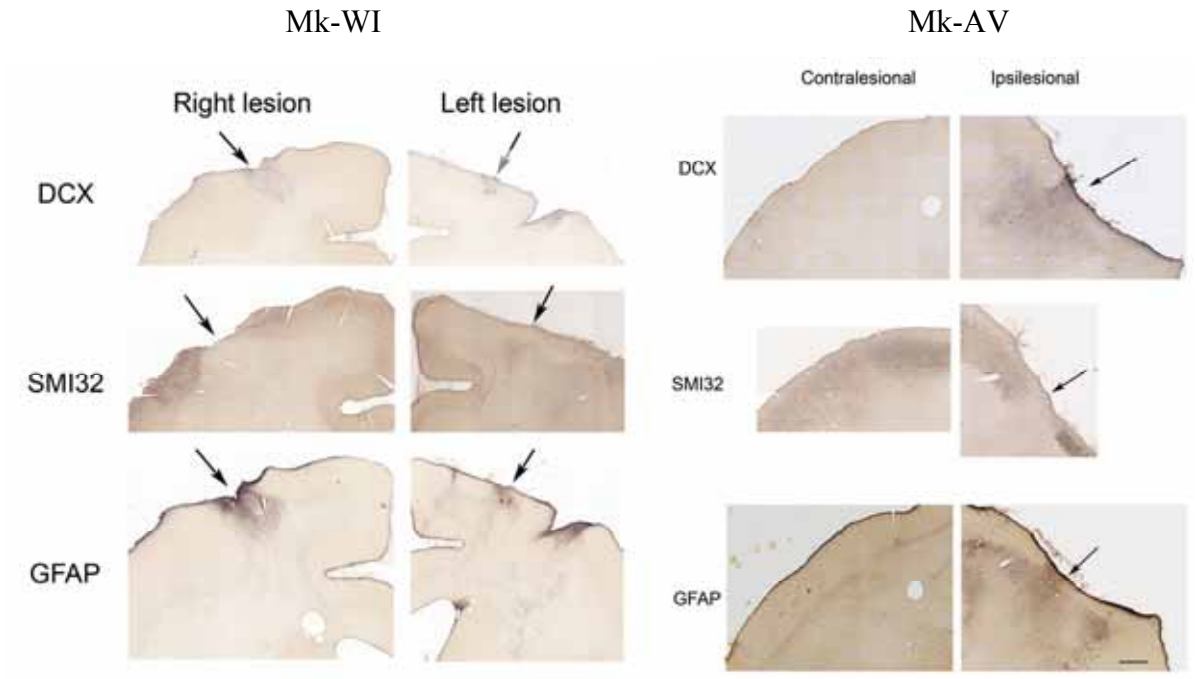


Figure 3.2.3.1: Histological observation of control lesioned motor cortex area in two monkeys. Mk-WI (left side) was lesioned in M1 bilaterally, whereas Mk-AV (right side) had a unilateral left lesion affecting mainly PM. Hematoxylin staining was performed for DCX, SMI-32 and GFAP. Black arrows indicate the lesion site. Scale bar=500µm.

As shown in figure 3.2.3.1, in the two control cases, an increase of the DCX signal could be observed in the lesioned area. There was thus a recruitment of DCX cells, which did not seem to be organized in layers, but that rather seemed to occupy the lesioned area in a quite diffuse way.

This recruitment seemed to be always conjugated with zones of gliosis, expressed by an increase of GFAP staining at the level of the lesioned area and of the white matter, where fibers were degenerating. Indeed, as expected, astrocytes reacted to the cell degeneration.
On the contrary of these increases of recruitments, there was no expression of SMI-32 in the lesioned area of these two non-reimplanted monkeys.

3.2.3.3.2 Treated monkeys

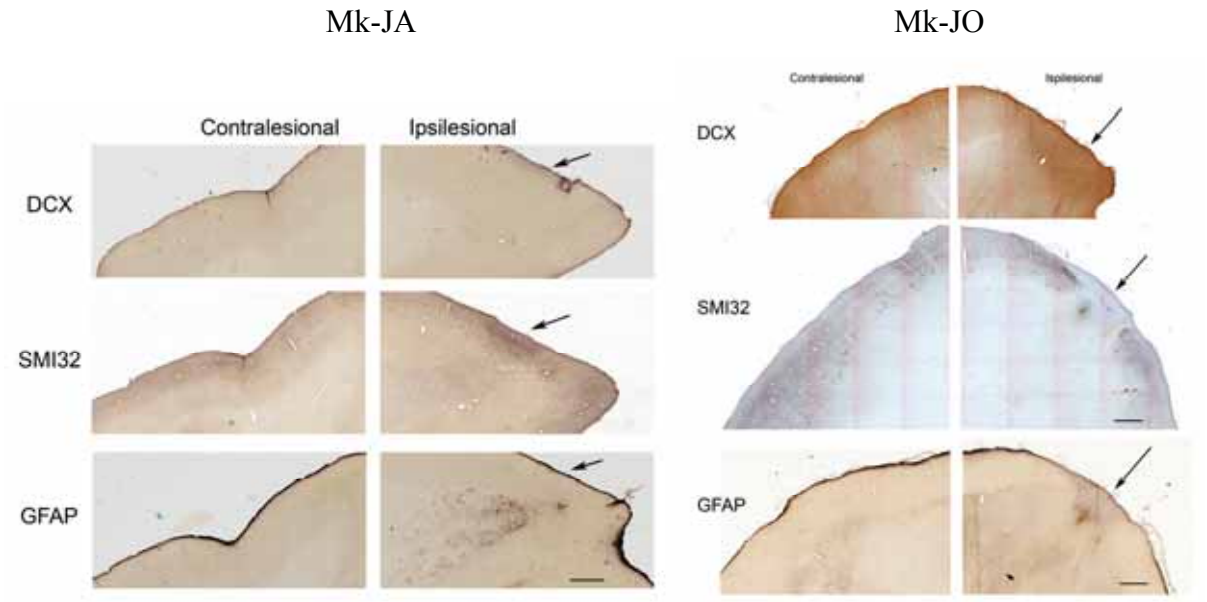


Figure 3.2.3.2: Histological observation of treated lesioned motor cortex area in two monkeys, Mk-JA (left side) and Mk-JO (right side), lesioned in left M1. Hematoxylin staining was performed for DCX, SMI-32 and GFAP. Black arrows indicate the lesion site. Scale bar=500µm.

In the reimplanted monkeys (Fig. 3.2.3.2), there were two different patterns, probably consequent to the two different time points of reimplantation, namely 15 days after the lesion in Mk-JO and 130/170 days after the lesion in Mk-JA, a parameter that seemed to play an important role on the in vivo development of the reimplanted cells and that will be discussed further.

In Mk-JO, in spite of the passed time, a lot of DCX cells stayed in the lesioned area, in a quite vast manner. There was probably an activation of the endogenous DCX cells, as there was more DCX cells in the ipsilesional side and as they were not all PKH positive. The main reimplanted cells stayed relatively grouped and at a progenitor stage (see Fig. 3.2.3.6 below), with the gliosis flooding and surrounding them, limiting the cell migration, as shown in GFAP staining. In the SMI-32 staining, on the contrary to the control monkeys, some cells were present in the lesioned area, but were agglomerated.

In Mk-JA, in whom cells were reimplanted after the whole peri-lesional phenomenon, practically no DCX cells were found, but an increase of SMI-32 cells occurred, probably arose from the two reimplantations, with a diffusion in the lesioned area. Indeed, we could observe that some SMI-32 cells were PKH26 and other PKH67 (see Fig. 3.2.3.3 below). This SMI-32 staining was much more present than in Mk-JO, suggesting that the reimplantation was apparently more favourable in Mk-JA. These SMI-32 cells in Mk-JA were less organized and with a cell body localization more variable than in a non-lesioned territory, but the fibers seemed well oriented with regard to the surface. A more extensive study, for example with

imaging, could reveal a reorganization of the fibers in a reimplanted zone in contrast to a non-reimplanted zone. In Mk-JA, some GFAP was found in the white matter, where fibers were probably reforming and progressing, which justified that a reactional gliosis was present even several months after these reimplantations.

There was thus probably a participation of the endogenous astrocytes, as no GFAP positive cells were observed in the PKH26 and PKH67 cells, and these astrocytes seemed to represent important partners to the phenomena of cell recruitment, either endogenous cells or reimplanted cells.

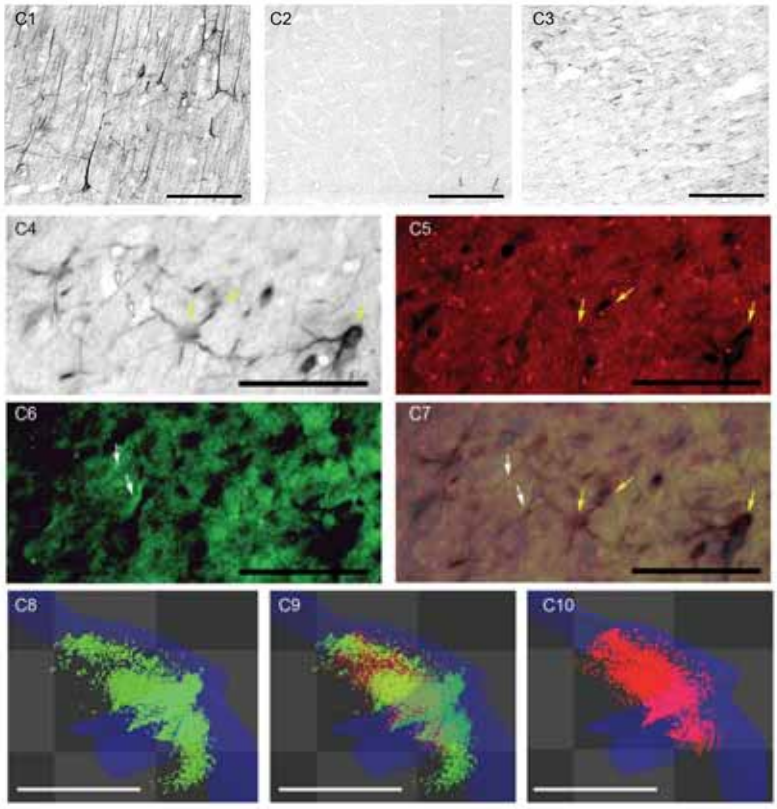


Figure 3.2.3.3: Histological and stereological observation of transplanted and control lesioned motor cortex area. C1-C3: Brain DAB-immunohistology for SMI-32 neurons. The proximal non-lesioned area (C1) and the lesioned motor cortex (C2) of a control monkey and the lesioned area with reimplanted cells of treated monkey (C3) illustrated that SMI-32 positive cells are presented in the treated monkey's lesioned site. Scale bar = 200µm. C4-C7: high magnification of SMI-32 positive cells in the lesioned area of the reimplanted monkey. These SMI-32 positive cells (yellow and white arrows in C4 and C7) correspond to PKH26 labelled cells (yellow arrows in C5 and C7) or to PKH67 labelled cells (white arrows in C6 and C7). Scale bar = 200µm.
C8-C10: 3D reconstruction of the motor cortex lesioned area that showed the distribution of PKH67 labelled cells (green points in C8 and C9) and PKH26 labelled cells (red points in C10 and C9). Note that the two sets of cells migrated in the same lesioned area in the overlay reconstruction (C9). Scale bar = 1 cm.

Figure 3 illustrates three cases: a non-lesioned cortex (Fig. 3.2.3.3 C1), a cortical area of a non-reimplanted lesioned monkey (Fig. 3.2.3.3 C2), were there was a total loss of fibers, and a cortical area of a lesioned monkey reimplanted (Mk-JA; Fig. 3.2.3.3 C3) were a reorganization of the fibers occurred. In this last case, the fibers and the cell bodies

distribution were less organized, but at least, there were SMI-32 fibers that reformed and that seemed oriented, thus "searching" their right way. From these two reimplantations performed in Mk-JA, localized differently, the two groups of cells mixed well (Fig. 3.2.3.3 C8-C10), went into the lesioned area, and a differentiation of these cells into SMI-32 positive cells occurred (Fig. 3.2.3.3 C4-C7).

In Mk-JA, the cells that did not express SMI-32 expressed mainly MAP2, as observed previously in two monkeys participating to a feasibility study (Brunet et al., 2005).

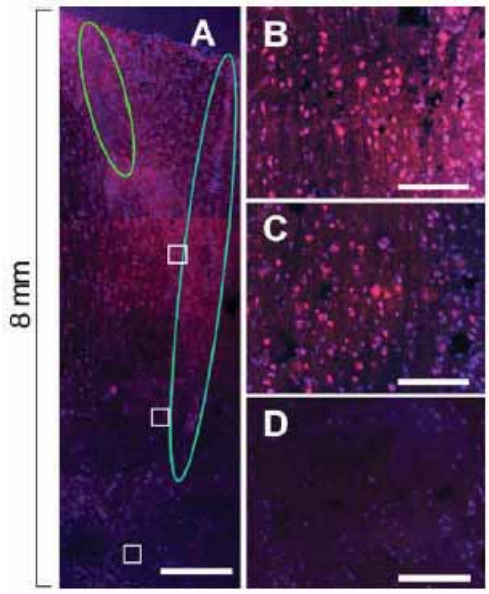


Figure 3.2.3.4: A.Brain histology 3 months after adult brain cell reimplantation. All these sections were performed in the ibotenic acid lesioned motor cortex. A.: Migration of PKH26 (red)-labeled monkey autotransplanted brain cells toward the ibotenic acid induced lesion. The green ellipse corresponds to the reimplantation site and the blue ellipse represents the ibotenic acid track. B-D. : Magnification of the small squares in panels A, B and C, respectively, in the middle and at the bottom of ibotenic acid induced lesion, and panel D, in a deeper areau without PKH26 reimplanted cells. Nuclei are counterstained with Dapi (blue). (A: scale bar=1mm; B-D: scale bar=75µm).

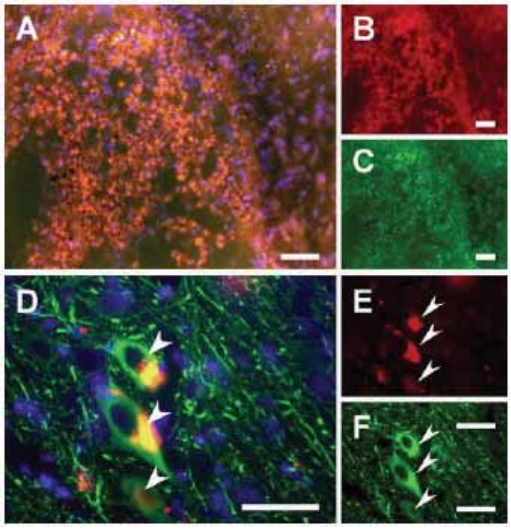


Figure 3.2.3.5: Immunolabeling of brain section in the ibotenic acid lesioned motor cortex, 3 months after adult brain cell reimplantation. A-C: Merged image (A) of PKH26 (red) reimplanted cells (B) that express nestin (green) (C) in the reimplantation site area. D-F: Merged image (D) of PKH26 (red)-labeled cells (E) that express MAP2 (green) (F) after migration toward the lesioned area. White arrows show three PKH26-positive cells that express MAP2. Nuclei are counterstained with Dapi (blue) (A-F: scale bar=15µm).

Indeed, in these monkeys sacrified three months after reimplantation, PKH26-labeled cells were observed up to 1 cm away from the injection site, surrounding the ibotenic acid lesion (Figs. 3.2.3.4.A–D). At the implantation site, PKH26-labeled cells still expressed low level of nestin (Figs. 3.2.3.5A–C), whereas in the lesioned cortical area, many PKH26-labeled cells expressed the neuronal marker MAP2 and presented a neuronal morphology (Figs. 3.2.3.5D–F).

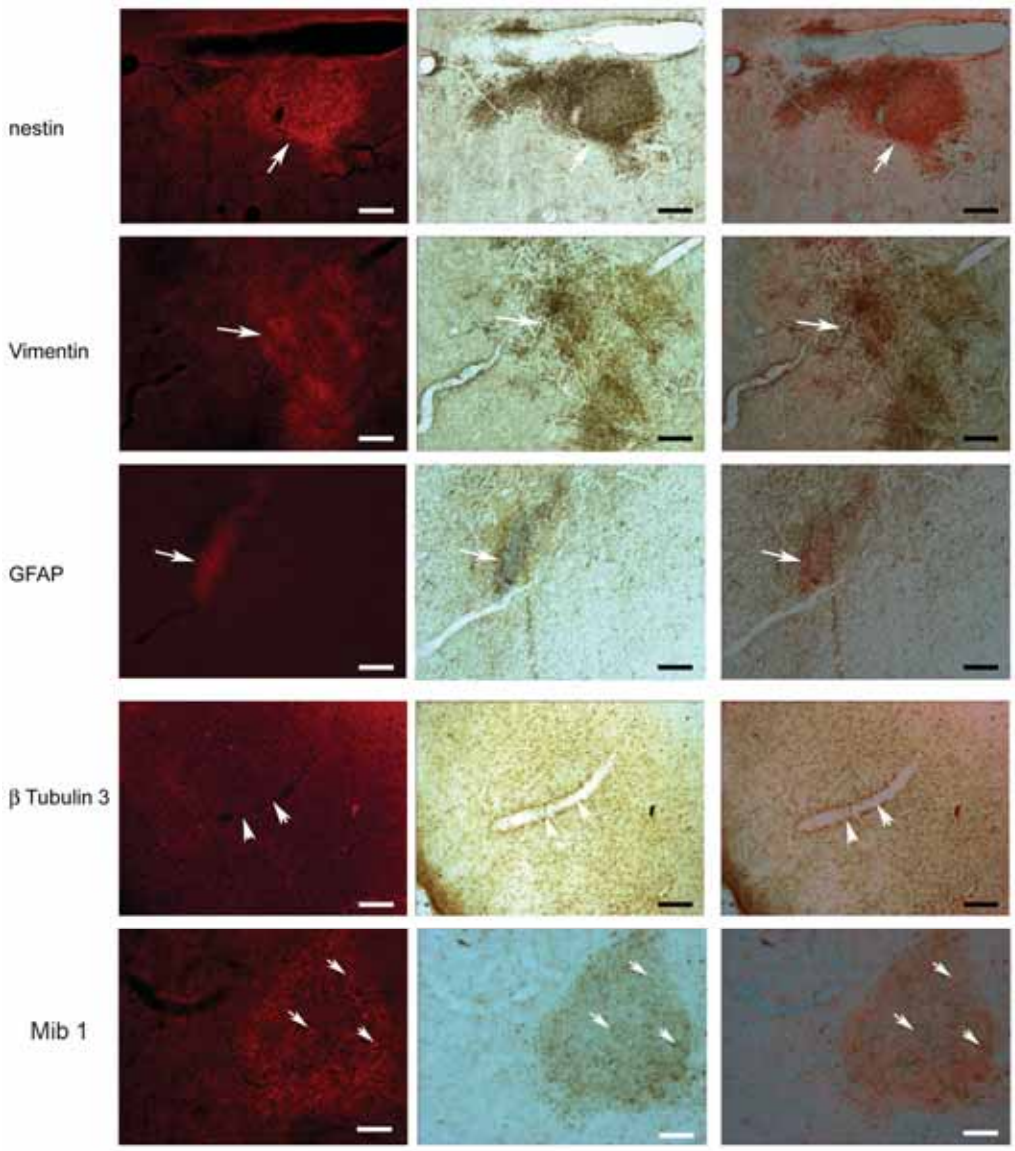


Figure 3.2.3.6: Histological observation of treated lesioned motor cortex area in Mk-JO. PKH26-labeled cells (red; left side) were merged (right side) with hematoxylin staining (center) for nestin, vimentin, GFAP, β-tubulin 3 and MIB1. White arrows indicate PKH-labeled cells. Bar = 50µm

On the contrary, in Mk-JO, a lot of reimplanted cells stayed Nestin positive, some were Vimentin and beta-tubulin 3 positive, whereas no much were GFAP positive; there was thus a poor differentiation (Fig. 3.2.3.6). Some cell agglomerates stayed very long after the reimplantation, which means that the cells survived well, but were not in a favorable context allowing migration and neuronal differentiation such as observed in the two monkeys of the feasibility study and in Mk-JA. As shown by the GFAP staining, the reimplanted cells were surrounded by endogenous GFAP positive cells. Finally, the MIB1 positive cells observed here were probably a consequence of the reactional gliosis. They were not tumoral, as their cell body did not present a tumoral morphology and as they would otherwise be more massive, especially since a lot of time passed.

3.2.3.4 Discussion

In the control monkeys, the increased DCX positive cells were quiescent precursor cells, thought to be recruited and then migrating toward the lesioned area in order to enhance the capacity of plasticity of the lesioned target region. The recruitment of DCX cells was conjugated to the gliosis, expressed by an increase of GFAP staining. It would be interesting to demonstrate that neurogenesis occurred by showing that these cells just divided. We did not show that these endogenous cells differentiated into neurons, but when DCX positive cells were reimplanted in the lesioned area, they showed an ability to differentiate into MAP2, letting us suppose that the recruitment of these cells could result in the formation of new neurons that would form new circuits and thus allow neuronal plasticity, resulting in recovering a part of the activity, which would lead to the so-called "spontaneous" recovery.

In this sense, the belief that if there were too much motor movements in rehabilitation, glutamate discharges would occur that had toxic aspect, would have to be tempered. Indeed, a well measured stimulation in order to induce movements that would finally maintain the activity and recruit neuronal circuits could be a good way of doing. It would thus be interesting to study the peri-lesional plasticity itself. What would constitute the main elements of this natural recovery? Elements are known that prevent the natural recovery (glial scare, NOGO…); it would be interesting to investigate what constitutes the positive aspects, such as probably the recruitment of these cells that present abilities of progenitor cells.

Concerning the gliosis, it has been shown (Brunet et al., 2004) that these astrocytes expressing strongly GFAP consumed much glucose. Therefore, this reactional gliosis probably enhanced the metabolism in order to manage the problem of fibers degenerescence and to participate as sustain to the DCX cells. Indeed, as shown in the previous chapter "In vitro cell fate", when the cells were put in culture, these astrocytes protected the progenitor cells, which themselves consume much glucose too.

In the treated monkeys, especially in Mk-JA, there was evidence that PKH-labeled cells, either 26 or 67, were able to differentiate and co-express SMI-32. It is thus probable that once the DCX cells were recruited in this lesioned motor cortex environment, they differentiated and expressed a marker very specific to pyramidal neurons, namely SMI-32.

In Mk-JO, reimplantation was performed probably too early after the lesion (15 days) in a peri-inflammatory time window, in which factors were secreted that activated the gliosis and the proliferation, and that were not favourable for the migration and the differentiation. Indeed, in such a peri-inflammatory zone, the rate of LIF, CNTF, EGF is known to be

increased (for review, see Linker et al., 2009). Thus, in Mk-JO, the migration did not occur as well as in the previous cases or in the case of Mk-JA.

It seems thus that in terms of participation to a better functional recovery, there was mainly a cell replacement in Mk-JA, whereas in Mk-JO, there was rather a facilitation of the reorganization that occurred around, namely a stimulation of the surrounding structures to make synapses and have a greater activity. In terms of electrophysiology, a more extensive study could have shown if the tissues surrounding the lesion were more reactive in terms of formation of new neuronal circuits than in control monkeys. Nevertheless, not all the cells stayed at a precursor cell stage in Mk-JO, as some PKH-labeled cells co-express Beta-tubulin 3, which is a marker of neuronal precursors, thus engaged in a differentiation process to become neurons and which apparently followed the vessels in order to be fed in energy, but were delayed or even diverted from their vocation to differentiate into neurons, as they had to cope with the inflammatory process, and thus stayed at this stage. Furthermore, some SMI-32 cells were PKH, but far not all; one cannot exclude that the other SMI-32 cells were endogenous DCX cells that differentiated, perhaps with the help of the reimplanted cells acting by liberating factors that potentiated the environment to have a good recovery.

Thus, in Mk-JO, as there were SMI-32 positive cells that were not PKH in the lesioned area, the endogenous plasticity was probably increased, due probably to the liberation of proliferation factors having promoted an endogenous recruitment. Indeed, these cells were able in vitro to secrete neurotrophic factors, such as BDNF, GDNF, LIF and some VEGF (data not shown), thus facilitating also the vascularization in these zones. To note that this last factor was not a main factor, as in histological terms, it did not seem that there was a massive neovascularization. One could suppose that in the case of Mk-JA, the secretion of factors was useful for the cell autostimulation to differentiate. The non-exposure to a peri-inflammatory environment probably facilitated rather the neuronal differentiation and the reintegration in the neuronal circuits, whereas a situation in a peri-inflammatory structure led rather to a tendency to try to cope with this peri-inflammatory phenomenon instead of the neuronal reconstruction.

It thus seems that these cells acted accordingly to the needs of the timing and the place where they were reimplanted. In this sense, it has been shown previously (Brunet et al., 2005) that these same cells reimplanted in the contralesional hemisphere were no longer detectable, at least three months after reimplantation, as if their help was not needed. Another example of this type appeared in a monkey submitted to the same autologous cell therapy in an MPTP parkinsonian model in whom the needle penetration provoked a minor haemorrhage far

(12mm) above the reimplantation site: in the histological analyses, a migration of a part of the reimplanted PKH cells around the haemorrhagic phenomenon was observed. We do not know exactly what attracted the cells in terms of chemo-attractivity, but we could suppose that SDF1 (a chemo-attracting factor that is quite wide-spread among the stem cells) probably played a role. In this sense, it would be interesting to demonstrate if there is liberation of SDF1 in the ibotenic acid model, which could explain the migration phenomena. SDF1 is known to be liberated late after the lesional phenomenon (Hattori et al., 2003), contrarily to interleukines-1 or TGFβ, which are molecules that will rather prevent the migration in order to avoid anarchy to take place (for review, see Ebadi et al., 1997). In terms of secreted factors, there is also the possible participation of EGF, which is able to facilitate the proliferation (for review, see Moyse et al., 2008), which could explain the presence of MIB1 positive cells in Mk-JO. Thus, the peri-inflammatory context would promote the maintaining at a progenitor/stem cell level, up to facilitate the division of some cells, supposed to be slow, as it was the case in vitro. We can thus imagine that in an acute pathological context, there is a recruitment of the DCX cells, with perhaps a stimulation of their proliferation capacity, and that in primates, DCX and/or Nestin and DCX-GFAP cells participate to an endogenous phenomenon of greater plasticity, which could explain why primates' brain repair is more efficient than in the rodents.

Therefore, overall, these adult brain cells used as autologous cell therapy showed abilities to migrate and to act in repairing a lesioned brain area, and thus in enhancing the functional recovery, in a way that seemed adapted to the environment in which they were reimplanted, in the present case in an acute peri-inflammatory or in a post-recovery motor context. Along this line, these same monkey (Chlorocebus aethiops) adult brain cells reimplanted in the right caudate nucleus of minor parkinsonian monkeys migrated in the whole reimplanted striatum and in the contralesional striatum through the corpus callosum, and had a neurotrophic effect on endogenous TH neurons by secreting BDNF and GDNF (Brunet et al., 2009). Thus, these cells seem able to play a therapeutic role in repairing brain damage, according to the needs of the reimplantation context without external interventions inducing specific characteristics. Future studies would be necessary to investigate further their mechanisms as well as their clinical impact.

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3.3 DOES THE RIGHT DORSOLATERAL PREFRONTAL CORTICAL BIOPSY HAVE AN EFFECT ON THE PERFORMANCE OF THE MONKEYS?

3.3.1 INTRODUCTION

The prefrontal cortex (PFC) is the anterior part of the frontal lobe of the brain, situated rostral to premotor regions. This is one of the brain's regions having undergone the strongest expansion during the course of the primate's evolution until hominids. PFC is involved in different cognitive functions (among which language, working memory, reasoning, and more generally executive functions). Although the neural system responsible for working memory (online maintenance of items for immediate use) is known to include a large number of brain regions, there is evidence from neurophysiological and lesional studies in monkeys that PFC is a critical component (Fuster, 1990; Goldman-Rakic, 1990). Brain-imaging studies, using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (e.g. Courtney et al., 1998; Courtney et al., 1997; D'Esposito et al., 1995; Fiez et al., 1996; Jonides et al., 1993; McCarthy et al., 1994; Owen et al., 1996a,b,c; Petrides et al., 1993; Ungerleider et al., 1998) have also implicated the human prefrontal cortex in working memory by mediating the temporal association of sensory stimulus and behavioural response (Funahashi et al., 1989; Fuster, 2000a,b,c).

The PFC projects back to all areas from which it receives sensory input as well as to structures with motor functions, including the premotor cortex, the superior colliculus, and the basal ganglia, though not directly to the primary motor cortex; premotor areas receive inputs from the PFC and, in turn, project to the primary motor cortex and to the spinal cord (Bates and Goldman-Rakic, 1993; Dum and Strick, 1991, 2005; Fang et al., 2006; Kurata, 1991; Leichnetz, 1986; Lu et al., 1994; Matelli et al., 1986; Rizzolatti and Luppino, 2001; Sakagami and Watanabe, 2007). PFC is divided into different main zones: the orbitofrontal cortex (OFC), the dorsolateral prefrontal cortex (dlPFC), the ventromedial prefrontal cortex (vmPFC), the ventrolateral prefrontal cortex (vlPFC), the anterior cingulate cortex (ACC). A number of authors (e.g. Fulton, 1950; Fuster, 1990; Goldman-Rakic, 1987a,b; Petrides et al., 2002) have argued for a functional dissociation of frontal lobe regions. Indeed, studies in non-human primates (Wilson et al., 1993) and functional imaging data on healthy subjects (McCarthy et al., 1994; Petrides et al., 1993) showed that distinct regions of PFC are implicated in the realisation of a task, according to the type of treatment of information to carry out.

In the present study, which is part of a more comprehensive lesional work (see Brunet et al., 2005), a unilateral biopsy of cortical tissue was performed surgically on the right dlPFC of intact adult monkeys. Regarding a number of studies (Barraclough et al., 2004; Fuster, 2000a,b,c; Goldman-Rakic 1995a,b; Miller and Cohen, 2001; Wise et al., 1996), dlPFC is considered as the integrative center of high order cognitive processing in the brain and as playing a central role in the executive control of behaviour. The dlPFC is known as one of the major cortical areas responsible for maintaining, manipulating and integrating information in working memory. In addition to initial evidence derived from cell recording (Fuster, 1973; Goldman-Rakic et al., 1992), such a role has been supported by data from fMRI (Belger et al., 1998; D'Esposito et al., 1995; Jonides et al., 1993; Prabhakaran et al., 2000; Ricciardi et al., 2006).

Different lesional studies (Bachevalier and Mishkin, 1986; Goldman and Rosvold, 1970; Goldman et al., 1970a,b; Goldman et al., 1971; Goldman-Rakic, 1987; Levy and Goldman-Rakic, 1999; Levy and Goldman-Rakic, 2000; Mishkin, 1954; Mishkin and Manning, 1978; Mishkin and Pribram, 1954; Mishkin and Pribram, 1955; Mishkin and Pribram, 1956; Passingham, 1975; Petrides, 1991; Petrides, 1995; Petrides, 2000a,b; Pribram and Mishkin, 1956; Stamm, 1973; Stamm and Weber-Levine, 1971) have shown that damage to the dlPFC, especially in the depths of the principal sulcus including the areas 46, 9/46, and 8Ad, causes profound impairments in spatial working memory, and that inferior prefrontal areas seem to play an important role in nonspatial processing. Two early studies (Mishkin and Manning, 1978; Passingham, 1975) showed that lesions of the vlPFC in non-human primates interfere with the processing of nonspatial information, including color and form.

Mishkin et al. (1982) identified an occipito-temporo-prefrontal pathway for object vision and an occipito-parieto-prefrontal pathway for spatial vision with the [2-14C]deoxyglucose method, building on the theory of dorsal and ventral streams to adaptative behaviour (Goodale and Milner, 1992). Iba and Sawaguchi (2002) demonstrated that a subset of neurons in dlPFC show a visuospatial mnemonic process that is related to the selection of visual spatial target, and Funahashi (2006), reviewing various studies, showed that neurons in the dlPFC respond during the cue, delay, and response periods of spatial working memory tasks. Mohr et al. (2006) and Mohr and Linden (2005) extended the ventral-dorsal dissociation of visual processing for color and spatial information to manipulation processes.

In their review, Constantinidis and Procyk (2004) discussed a number of studies supporting the notion that dlPFC exerts complementary function to posterior parietal cortex, as it receives projections from sensory associative and limbic areas, and performs a

reconstruction of the external environment for being able to plan movements and give answer to this environment (Shadlen and Newsome, 2001). Goldman-Rakic and collaborators (Scalaidhe et al., 1997; Wilson et al., 1993) have shown that, in monkeys, the dlPFC areas that are reciprocally connected with parietal visual areas exhibit sustained delay activity that is primarily related to spatial information. Thus, neurons capable of holding specific visuospatial coordinates on line have been observed predominantly within the dlPFC, which receives a direct and robust projection from the posterior parietal cortex (Goldman-Rakic, 1999). In particular, areas 8a and the caudal part of area 46 receive a dense projection from parietal areas 7a and 7ip (Cavada and Goldman-Rakic, 1989a,b). These areas are, in turn, connected to a number of cortical and subcortical regions thought to make up the spatial working memory circuit (Selemon and Goldman-Rakic, 1988). The corresponding parietal and prefrontal areas exhibit comparable properties and it was proposed by Goldman-Rakic and collaborators that prefrontal neurons exhibit the same type of location and motion selectivity as that described for posterior parietal neurons (Shadlen and Newsome, 2001).

Functional similarities between the two regions include memory-related activity. Indeed, neuronal discharges in lateral intra-parietal area (LIP) and 7a persist after the offset of transient visual stimuli, and are linked to their spatial locations (Andersen et al., 1987; Chafee and Goldman-Rakic, 1998; Constantinidis and Steinmetz, 1996; Gnadt and Andersen, 1988; Quintana and Fuster, 1992). Posterior parietal areas and dlPFC are coactive during some working memory tasks (Friedman and Goldman-Rakic, 1994), and neuronal responses in the two regions are virtually undistinguishable, at least during the execution of an occulomotor delayed response (ODR) task (Chafee and Goldman-Rakic, 1998). Similar percentages of neurons display persistent activity with the same time courses in the prefrontal and parietal cortices (Quintana and Fuster, 1992).

In contrast, Goldman-Rakic and collaborators (Scalaidhe et al., 1997; Scalaidhe et al., 1999; Wilson et al., 1993), based on electrophysiological data in vlPFC including areas 12 lateral, 12 orbital and 45, reported neuronal responses that were selectively active in relation to information about pattern, colour, object and face. Injection of tracers into vlPFC resulted in retrograde labelling of neurons in the inferotemporal cortex, which is itself involved in object and face processing (Bates and Goldman-Rakic, 1993; Scalaidhe et al., 1997; Scalaidhe et al., 1999). Support for vlPFC involvement in object, pattern and face processing was obtained in neuroimaging studies in humans (Allison et al., 1999; Courtney et al., 1996; Courtney et al., 1997; Haxby et al., 1996; McCarthy et al., 1996; Puce et al., 1999). A difference between dlPFC and vlPFC has also been shown anatomically. Barbas (1988)

reported a connection between ventral PFC and ventral visual areas implicated in pattern recognition and discrimination, distinct from a connexion between the mediodorsal PFC and territories in the medial and dorsolateral occipital and parietal areas associated with visuospatial functions. This dissociation was confirmed by several connectional studies (Barbas and Mesulam, 1981; Barbas and Pandya, 1989; Chavis and Pandya, 1976).

Goldman-Rakic and colleagues dissected the afferent/efferent dlPFC network and postulated that these connections are the anatomical support of spatial working memory (Cavada and Goldman-Rakic, 1989a,b; Selemon and Goldman-Rakic, 1988). Regarding the relatively greater proportion of projections from the dorsal stream to the dlPFC and, similarly, the greater proportion of projections from the ventral stream to the vlPFC, Goldman-Rakic (1988) proposed that working memory is mediated by the sustained activity of neurons in parallel, distributed cortical networks. The same author also illustrated how interconnected dlPFC and parietal areas shared common connections with the ACC, thereby underlying a link between working memory and motivation. On this basis, Goldman-Rakic and collaborators developed the domain-specific model, proposing that the lateral PFC is segregated according to the processing of spatial and nonspatial attributes of information (Levy and Goldman-Rakic, 2000). The dlPFC is engaged in online maintenance of spatial memoranda, while the vlPFC supports nonspatial (e.g. face, objects) memoranda. In essence, the segregation of the dorsal "where" and ventral "what" visual pathways in posterior extrastriate, parietal, and temporal cortices are proposed to be conserved in the lateral PFC. In line with this model, different studies have shown that auditory (Bushara et al., 1999; Rama et al., 2004; Romanski and Goldman-Rakic, 2002; Romanski et al., 1999) and somatosensory (Hagen et al., 2002; Romo et al., 1999) stimuli evoke activity in the PFC in a domain-specific manner.

Another major model proposing a functional specialization for the dorsal and ventral lateral PFC is the process-specific model, developed by Petrides. It proposes another functional topography. The dorsal and ventral lateral prefrontal cortices are suggested to perform qualitatively different operations, with regard to the level of information processing (Petrides, 2000a,b; Petrides et al., 2002), and/or the rule or manner in which information is processed (Miller and Cohen, 2001; Passingham et al., 2000; Rushworth et al., 1997). The process-specific model proposes a hierarchy in which the vlPFC is engaged in processes such as the active encoding and retrieval of information, whereas the mid-dorsolateral PFC is involved in higher order executive control functions like the monitoring and manipulation of stored information. For example, Petrides (1994) showed that lesions sparing the principal

sulcus but including dorsal area 9 do not affect performance on spatial delayed-response tasks, but do impair performance on both spatial and non-spatial self-ordered tasks that require, in addition to maintenance, monitoring and manipulation of information in working memory. Other studies have led to different conclusions or have highlighted other parameters.

In a PET study, Kojima et al. (2007) found a segregation of working memory based on domain, but between the posterior parietal-premotor areas and the dorsolateral prefrontal-hippocampus areas, for spatial and non-spatial working memory tasks respectively. However, these authors reported muscimol microinjection into dlPFC impairing the performance of both spatial and non-spatial working-memory tasks. Ninokura et al. (2003) found that cells responding selectively to the physical properties (color and shape) of objects were localized in vlPFC and cells responding selectively to the numerical position (rank order) of objects were localized in dlPFC. Other studies (Baker et al., 1996; McCarthy et al., 1996) have contrasted activation during object and spatial working memory tasks. Both studies reported greater activation in the right PFC during the spatial task, and one (Baker et al., 1996) reported greater activation in the left PFC during the object task. Thus, these studies suggest that domain specificity for spatial and object working memory is primarily a hemispheric laterality effect, rather than a dorsal-ventral distinction.

Working memory function is essential for maintaining a focus on goal hierarchies, monitoring the status of competing options, storing affective information relevant to attributes and assessments of options, and predicting future outcomes and probabilities of meeting goals. Consequently, the PFC plays a key role in decision making. In PFC, visual information is integrated with reward expectation and prior knowledge, each PFC region making distinct contributions to decision making.

Krawczyk (2002) proposed a division of PFC for decision making into three primary regions: first, the OFC and vmPFC, which are most relevant to deciding based on reward values and contribute affective information regarding decision attributes and options (Bechara et al., 1997; Berns et al., 2001; Breiter et al., 2001; Damasio, 1995; Elliott et al., 1999, 2000; O'Doherty et al, 2003; Rogers et al., 1999; Rolls, 2000); second, the dlPFC, playing a critical role in making decisions that call for the consideration of multiple sources of information, and may recruit separable areas when making well defined versus poorly defined decision (Baker et al., 1996; D'Esposito et al., 1995; Duncan and Owen, 2000; Fletcher et al., 1997; Goel and Dolan, 2000; Goldberg et al., 1994; Goldman-Rakic, 1992; Holyoak and Kroger, 1995; Prabhakaran et al., 1997, 2000; Shimamura, 2000; Waltz et al., 2000); third, the anterior and ventral cingulate cortex, which is especially relevant in sorting among conflicting options, as

well as signalling outcome-relevant information (Botvinick et al., 1999; Carter et al., 1998; North and O'Carroll, 2001).

Lee et al. (2007) suggested a major implication of OFC and ACC in encoding and updating the utilities associated with different sensory stimuli and alternative actions, respectively, as well as in decision-making in a social context, and a major implication of lateral PFC in maintaining the state representation necessary to identify optimal actions in a given environment. Barraclough et al. (2004) and Seo et al. (2007), in studies on monkeys performing a competitive and a matching game respectively, showed the implication of dlPFC in decision making. Especially, Barraclough et al. (2004) showed that neurons in dlPFC encoded the animals' past decisions and payoffs, as well as the conjunction between the two, providing signals necessary to update the estimates of expected rewards. The authors thus proposed PFC as playing a key role in optimizing decision making strategies.

Ichihara-Takeda and Funahashi (2007) showed that OFC plays a role in monitoring the proximity of the reward trial and detecting reward delivery, whereas the dlPFC plays a role in performing cognitive operations and integrating cognitive and motivational information. Their results also indicate that spatial information and the monkey's motivational state independently affect neuronal activity in both areas. Watanabe et al. (2005) showed a relation between preferred reward, better task performance and enhanced working-memory related neuronal activity in the lateral PFC, suggesting an important role of lateral PFC in the integration of cognitive and motivational operations to obtain a reward more effectively (Sakagami and Watanabe, 2007).

An fMRI study by Heekeren et al. (2006) suggested that human dlPFC integrates sensory information over time and converts such information into a categorical decision about motion direction, as mentioned above concerning spatial working memory. There is also evidence that dlPFC is involved in deciding under uncertain circumstances that have no objectively correct answer, in comparing between exemplars or attributes, and in competing options for decision making (Fletcher et al., 1997; Goel and Dolan, 2000; Goldberg et al., 1994; Holyoak and Kroger, 1995).

The dlPFC is also involved, with parietal cortex, in the neural processing of similarity comparisons leading to decision making, as shown by different works using cognitive tasks, fMRI, PET or electrophysiology (Bjork et al., 1998; Elliott and Dolan, 1998; Goel and Dolan, 2000; Goldberg et al., 1994; Mellers et al., 1998; Podell et al., 1995). Other studies based on PET, fMRI or neuropsychological investigation on the Raven's progressive matrices test (Baker et al., 1996; Duncan and Owen, 2000; Holyoak and Kroger, 1995; Prabhakaran et al.,

1997, 2000; Waltz et al., 2000) demonstrated dlPFC activity in a range of tasks having relational processing and integration as a common element.

Wallis (2007), reviewing anatomical, neuropsychological and neurophysiological studies, suggested a key role of OFC in processing reward, lateral PFC in using this processing to plan and organize behaviour towards obtaining the outcome, and medial PFC in evaluating the overall action in terms of its success and the effort that was required. Moreover, in a larger view, Opris and Bruce (2005) also pointed out different works having highlighted some of the cognitive interactions involving decision making, such as prefrontal and dorsal visual extrastriate cortices (Kang et al., 2006), the PFC and basal ganglia (Opris and Bruce, 2005; Pasupathy and Miller, 2005), the frontal eye fields (FEF) and superior colliculus (SC) (Sommer and Wurtz, 2001), and the LIP and SC (Pare and Wurtz, 2001).

All these different aspects of working memory have not been yet studied on the kind of tasks we used in our experiment, but regarding this non-exhaustive amount of studies, showing a clear implication of dlPFC in different cognitive functions, especially spatial working memory and decision making, we could expect a possible effect of the right dlPFC biopsy on monkeys performance when executing fine manual dexterity tasks. Indeed, these motor tasks consist in boards which contain vertical and horizontal wells, static in a task and rotating in the other, in which little food pellets are placed that the monkeys have to retrieve by using precision grip. The wells are always set in the same configuration, which of course varies when the board rotates, the tasks being thus repetitive along the training sessions, leading the monkeys to possibly retrieve the pellets from the wells in a spatial sequential order. In this context, we hypothesized that the right dlPFC biopsy would have no effect on the motor control *per se*, but may affect the sequential order of pellets retrieval.

3.3.2 METHODS

3.3.2.1 Subjects and Behavioural Tasks

For the present study, data were collected in a group of 5 male long-tailed macaques (*Macaca Fascicularis*), weighting between 3 and 6 kg and ranging from 2.5 to 5.5 years old at the time of initiation of motor training sessions. All the behavioural and surgical procedures were approved by the local ethical committee, in accordance with the Guidelines for the Care and Use of Laboratory Animals and approved by Swiss veterinary authorities.

These monkeys were trained as described in the “General Material and Methods”. For the present investigation, the performances at the modified Brinkman board task and the

rotating Brinkman board task were analyzed. For each monkey, the motor performances for the left and the right hands were analyzed individually. Analyses were conducted on 11-15 recorded sessions pre-biopsy and/or cortical chamber implantation (see "Surgical procedures") as well as 11-15 recorded sessions post-biopsy and/or cortical chamber implantation. The behavioural sessions were analyzed with a video recorder allowing frame by frame analysis, with a resolution of 25 frames per second.

The following parameters, already described in the "General Methods" chapter, were analyzed: i) The number of pellets successfully grasped in 30 seconds (score) was counted; this parameter was analyzed only for the modified Brinkman board task; ii) The "contact time", which is the time the monkey took to retrieve each food pellet from the well. For the modified Brinkman board task, this parameter was analyzed on the 5 first and 5 last vertical and horizontal visited wells (20 wells in total). For the rotating Brinkman board task, this parameter was analyzed on all wells (n=32). To note here that the analyzes of the contact time in the rotating Brinkman board task differed slightly in terms of temporal resolution between the two biopsied monkeys and the three control monkeys, as the performances of the two biopsied monkeys were analyzed in a first type of protocol allowing a resolution of 0.25s, whereas for the three other monkeys, a new protocol was established, which allowed a resolution of 0.04s. Nevertheless, as the post-surgery performance of each monkey was compared to his own pre-surgery performance, this reduced the possibility of erroneous conclusions, although it has to be taken into account. Beyond that, no other parameter was concerned by this difference of protocol. iii) Another analyzed parameter was the sequence of prehension, which is the order in which the animal took the food pellet. For the modified Brikman board task, the cumulated distance to visit the wells was quantified as well, allowing to determine the cumulated distance needed to retrieve all the pellets. Concretely, if the monkey took a pellet adjacent to the previous one, the cumulated distance would be shorter than if he took a more distant one in a more random order. In the same line, the sequence of prehension according to the orientation of the wells (vertical and horizontal) was also observed. For the rotating Brinkman board task, different parameters were noticed: absolute well orientation (vertical or horizontal), ring position (4 in total), sector (8 in total) when entering the fingers in the well, sector when exiting the fingers from the well, thus relative well orientation (4 in total) when entering and exiting from the well. For both tasks, the errors, mainly the loss of food pellet, were noticed, as well as the position of the wrist to grasp the horizontal slots (Radial Abduction-Internal / Cubital Adduction-External), the first finger

introduced in the horizontal wells (thumb or index), and if the monkey retreived two pellets at the same time before bringing them to the mouth.

These data were analyzed and coded on an analysis protocol sheet and a database was then created on Excel. Graphics, panels and statistical analyses were performed using the softwares Excel, and SigmaPlot/SigmaStat. Some graphics were also produced with an home-made program in Matlab.

3.3.2.2 Surgical Procedures

All surgical procedures (ethical and veterinarian authorizations, preparation, sterile conditions, anaesthesia and medication) were the same as described in the "General Methods" chapter. The surgery took place once the monkey reached a behavioural plateau. The procedures concerning the cortical chamber implantation can be found in the "General Method" chapter. On the surgery day when the chamber was implanted, two monkeys (Mk-JO and Mk-AV) were subjected to a right dlPFC biopsy which was performed as follows. A square osseous sector of 8mm x 8mm was opened above the right dlPFC and the dura mater was incised. Then, a little piece of cortical tissue was extracted using a surgical blade (no 11, Paragon®). The osseous sector was then replaced on the brain and sewed at two sides to the skull. The muscle and the skin were then sutured. As the three other monkeys (Mk-JA -also biopsied, but later on after M1 lesion-, MK-VA and MK-SL) were not subjected to this prefrontal cortical biopsy, but were implanted with a chronic recording chamber on the left hemisphere, they were considered as control subjects for the purpose of this study.

3.3.2.3 Histology of the prefrontal biopsy

The procedures of necropsy and histology were already described in the "General Methods" chapter. Analyzes and reconstructions of the biopsy in the frontal plane were performed on Nissl-stained slices with Mercator®. Three-dimensional mapping and volume quantifications were performed on Map3D®.

3.3.3 RESULTS

3.3.3.1 Histology

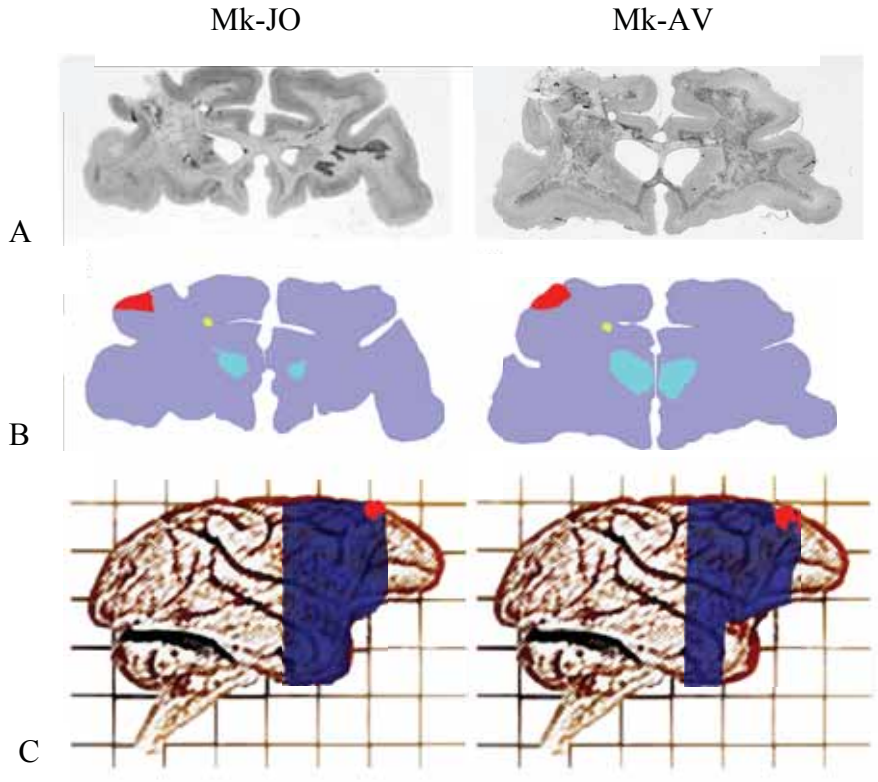


Figure 3.3.1: A. Nissl-stained slices in the frontal plane of the biopsied monkeys' brain, at the level of the center of the biopsy in the rostro-caudal axis. B. Reconstructions of the biopsy performed from Nissl-stained slices in order to define its size and location. The biopsy is represented in red, the ventricles are in turquoise, whereas the yellow circle corresponds to the pipette passing through the whole brain rostro-caudally and allowing to align the brain slices for 3D reconstruction, represented in C, superimposed on a standard macaque monkey brain illustration.

Both in Mk-JO and Mk-AV, the biopsy was situated in the areas 9 and 46 of the dorsolateral prefrontal cortex (Figure 3.3.1). Nevertheless, the quantification of the biopsy size revealed a larger biopsy in Mk-AV than in Mk-JO; indeed, the biopsy was 44mm^3 and 20.3mm^3 respectively in these two monkeys. This was due to the mechanical removal of the tissue perfomed without precise definition of the volume to extract, but with the objective to remove little tissue, but enough for the cultures. Afterwards, as it was noted that fewer tissue would be sufficient for the cultures, the biopsies performed in the next monkeys, namely Mk-JA after the M1 lesion and Mk-WI were very smaller and reached a volume of 8.8mm^3 and 10.3mm^3 respectively.

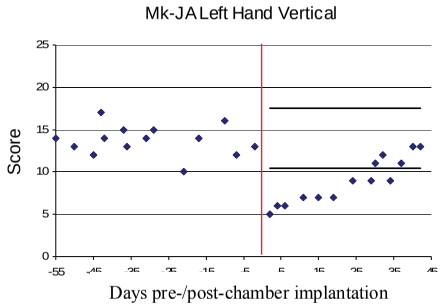
3.3.3.2 Behaviour: Motricity

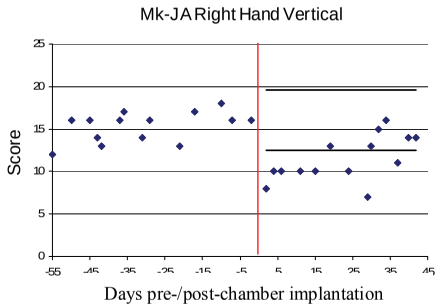
3.3.3.2.1 Modified Brinkman board task

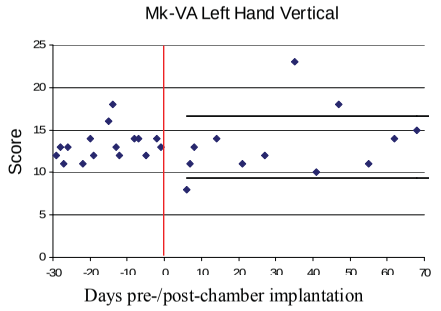
3.3.3.2.1.1 Score

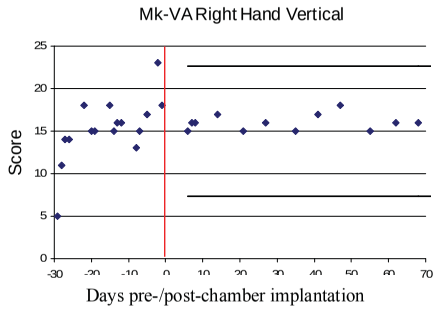
Control monkeys

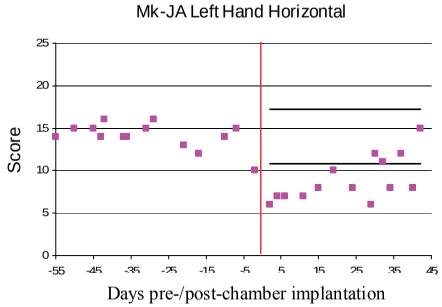
Mk-JA Left Hand | Mk-JA Right Hand | Mk-VA Left Hand | Mk-VA Right Hand

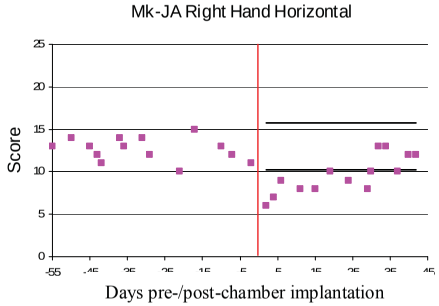


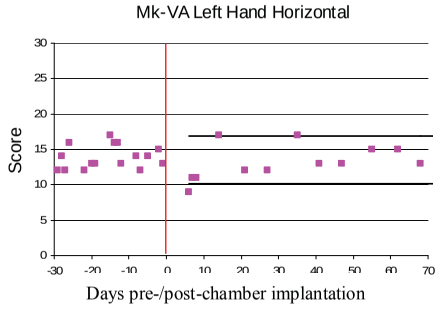


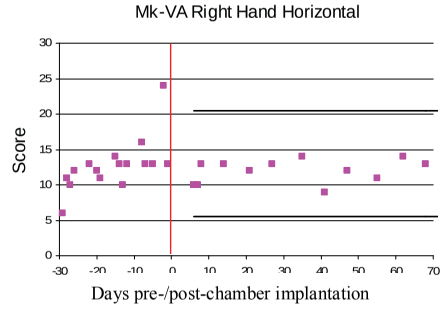


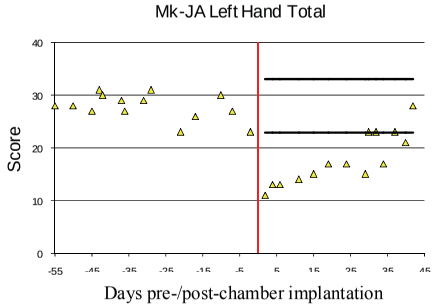


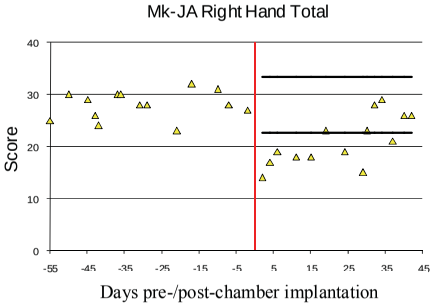


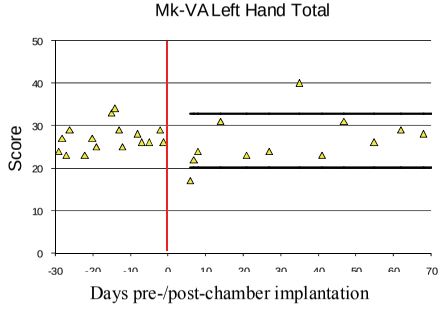


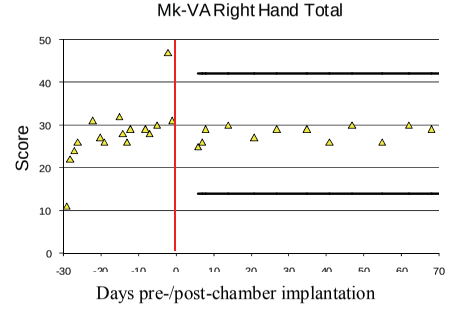


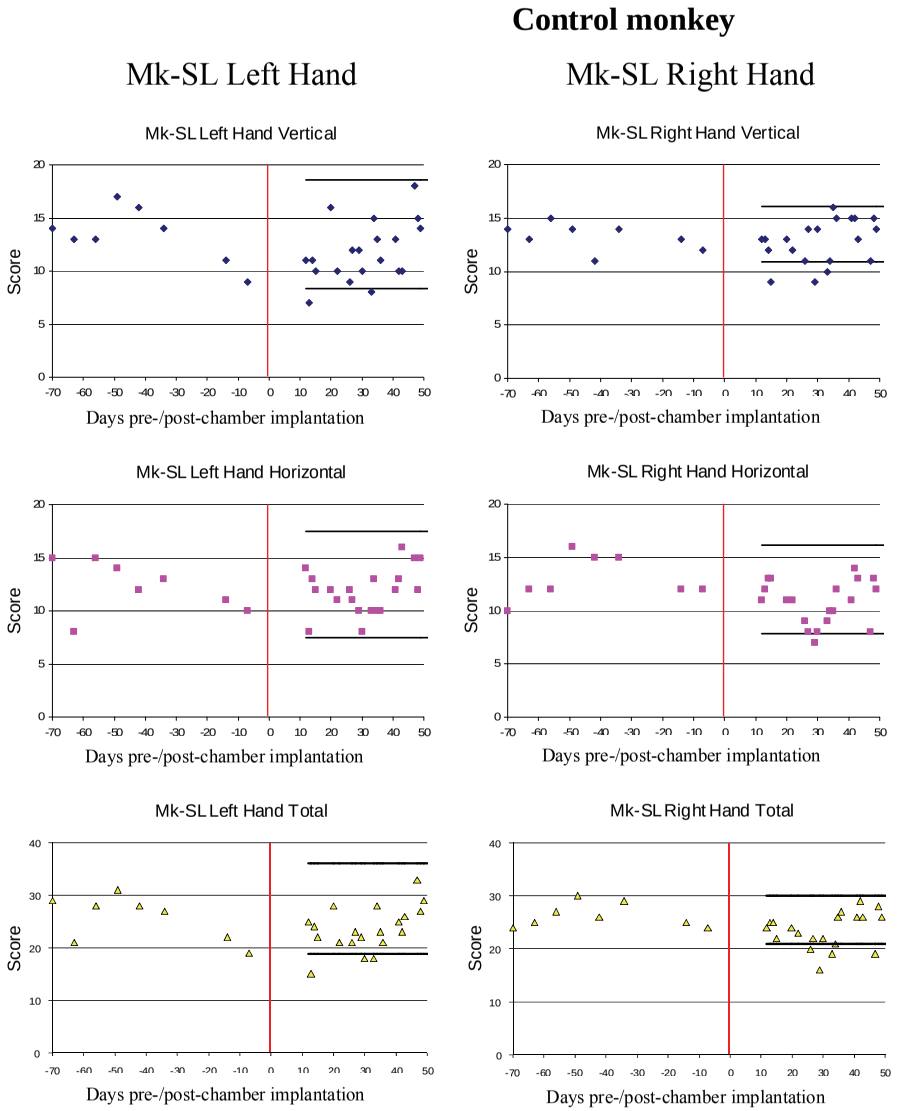












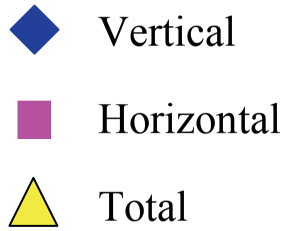
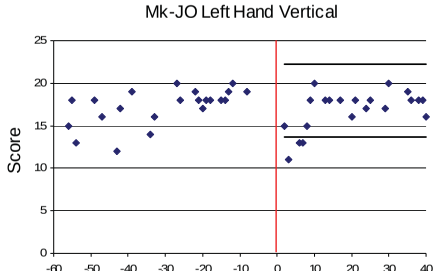
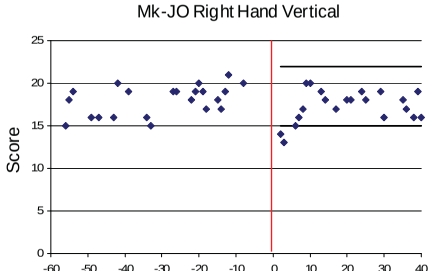


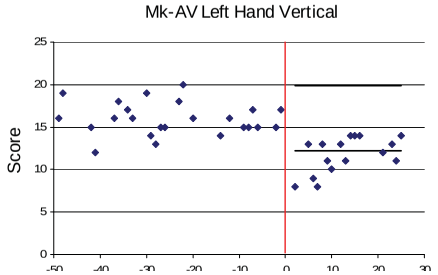
Figure 3.3.2 : Number of pellets retrieved in 30 seconds (score; y axis) for the vertical (blue), horizontal (pink) and total (yellow) wells along sessions pre- and post-surgery (x axis) in the control (chamber implant uniquely; n=3) and biopsied (chamber implant and dlPFC biopsy; n=2) monkeys on the modified Brinkman board task, for both hands. The post-surgery performance was compared to the pre-sugery one, given by its median value plus or minus two standard deviations. The red vertical line indicates the surgery. Post-surgery, the two black lines indicate the superior and inferior limits (mean plus and minus two standard deviations respectively) of the pre-surgery values.

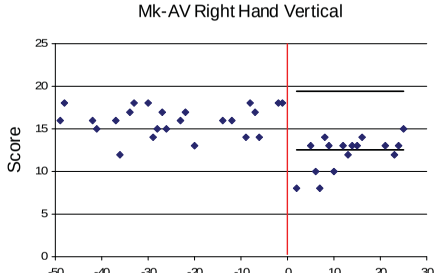
Biopsied monkeys

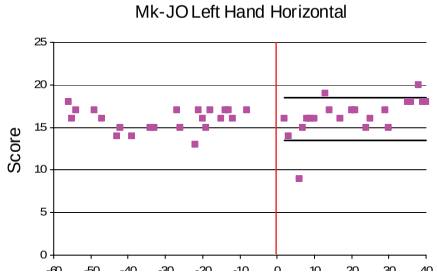
Mk-JO Left Hand | Mk-JO Right Hand | Mk-AV Left Hand | Mk-AV Right Hand

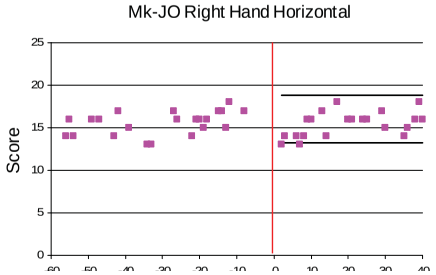


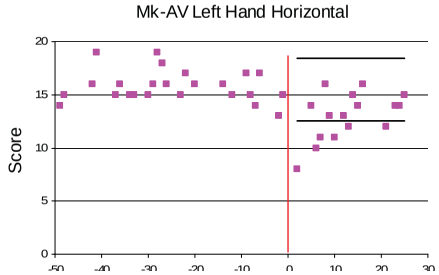


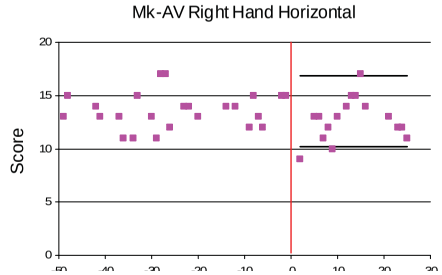


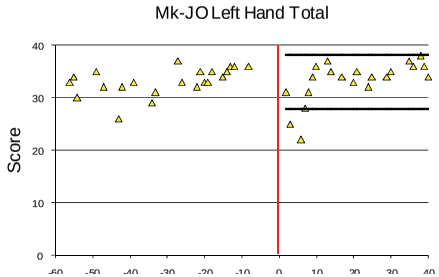


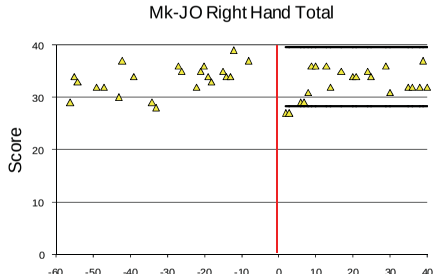


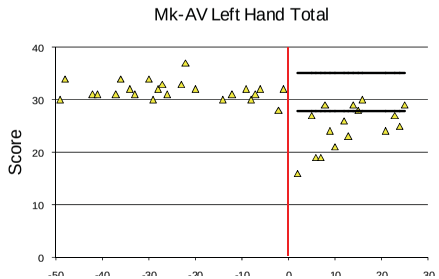


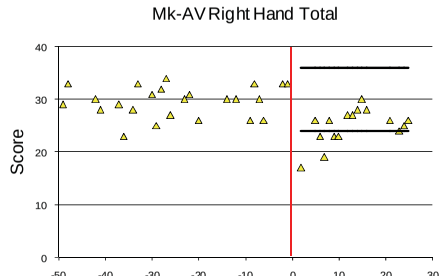












On the score parameter (number of pellets retrieved in 30 seconds) of the modified Brinkman board task, the post-surgery performance was compared to the pre-surgery one by observing the distribution of the scores for the vertical, the horizontal and the total wells with regard to the pre-surgery scores. Data situated outside the mean plus or minus two standard deviations area were considered as deviant consequently to the surgery (Fig. 3.3.2).

In the control monkeys (chamber implantation only), a decrease of performance was observed after the implantation of the chamber, especially in Mk-JA in his left and right hands for the vertical, horizontal and total wells during about 35-40 days. In the two other control monkeys, Mk-VA and Mk-SL, the training sessions did not begin immediately after the surgery, leading thus to a lack of data during the 6 and 12 first days after the surgery, respectively. Nevertheless, in Mk-VA, a very transitory decrease of the score was found in his left hand, as the score at the first session was below two standard deviations for all the wells orientations and the total wells cumulated, whereas no effect appeared in his right hand. In Mk-SL, such a transitory decrease occurred in both hands for the vertical and total wells cumulated, as these scores were repeatedly below two standard deviations during about 33 days. To note that as in Mk-VA and Mk-SL the first days after the surgery are lacking, a possible stronger acute effect of chamber implantation was impossible to assess.

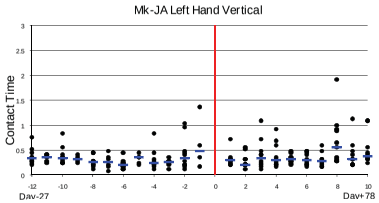
In the biopsied monkeys, an effect of the surgery (chamber implant and dlPFC biopsy) on behavioural score was also observed. Mk-JO showed an acute effect on the score during about 7 days, for the vertical and total total wells particularly in his right hand and for all the wells in his left hand. A decrease of the score occurred in Mk-AV, during about 24 days in his left hand for all the wells and during about 13 days in his right hand for the vertical and the total wells, particularly.

Overall, a decrease of the motor performance in terms of number of pellets taken in 30 seconds was observed after surgery in all monkeys, though to a lesser extent in Mk-VA and Mk-SL, probably due to the lack of data immediately following the surgery. Therefore, as an effect was present in both groups of monkeys, it could not be attributed to the dlPFC biopsy specifically, but may rather reflect a general drop in performance following any type of heavy surgery implicating deep anaesthesia.

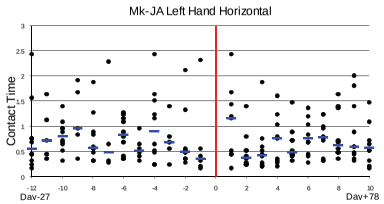
3.3.3.2.1.2 Contact Time

Control monkeys

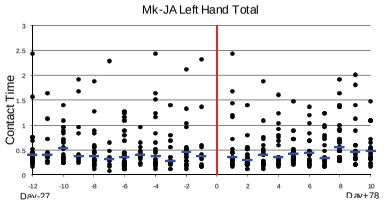
Mk-JA Left Hand



Sessions pre-/post-chamber implantation

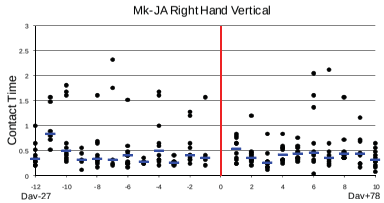


Sessions pre-/post-chamber implantation

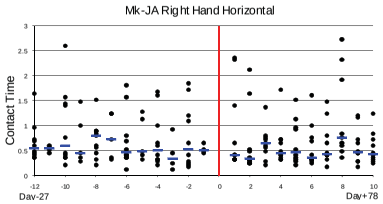


Sessions pre-/post-chamber implantation

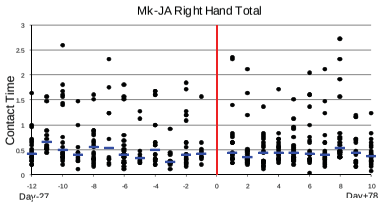
Mk-JA Right Hand



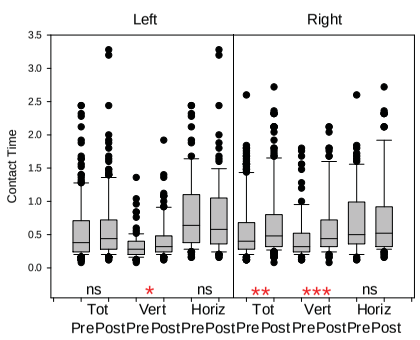
Sessions pre-/post-chamber implantation



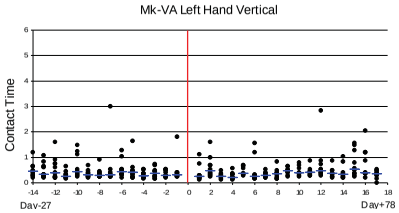
Sessions pre-/post-chamber implantation



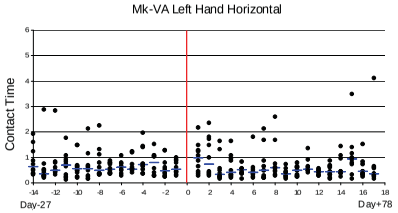
Sessions pre-/post-chamber implantation



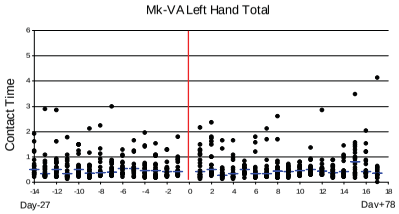
Mk-VA Left Hand



Sessions pre-/post-chamber implantation

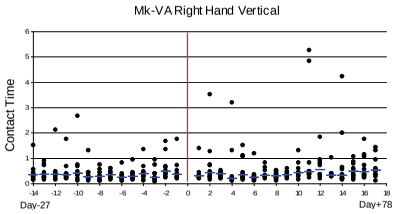


Sessions pre-/post-chamber implantation

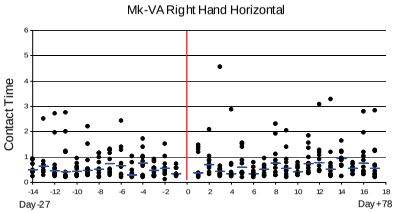


Sessions pre-/post-chamber implantation

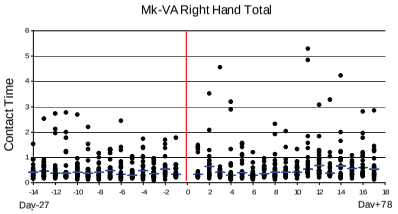
Mk-VA Right Hand



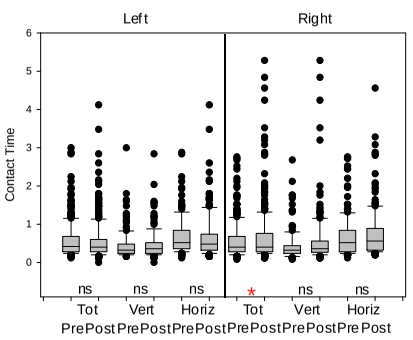
Sessions pre-/post-chamber implantation



Sessions pre-/post-chamber implantation



Sessions pre-/post-chamber implantation



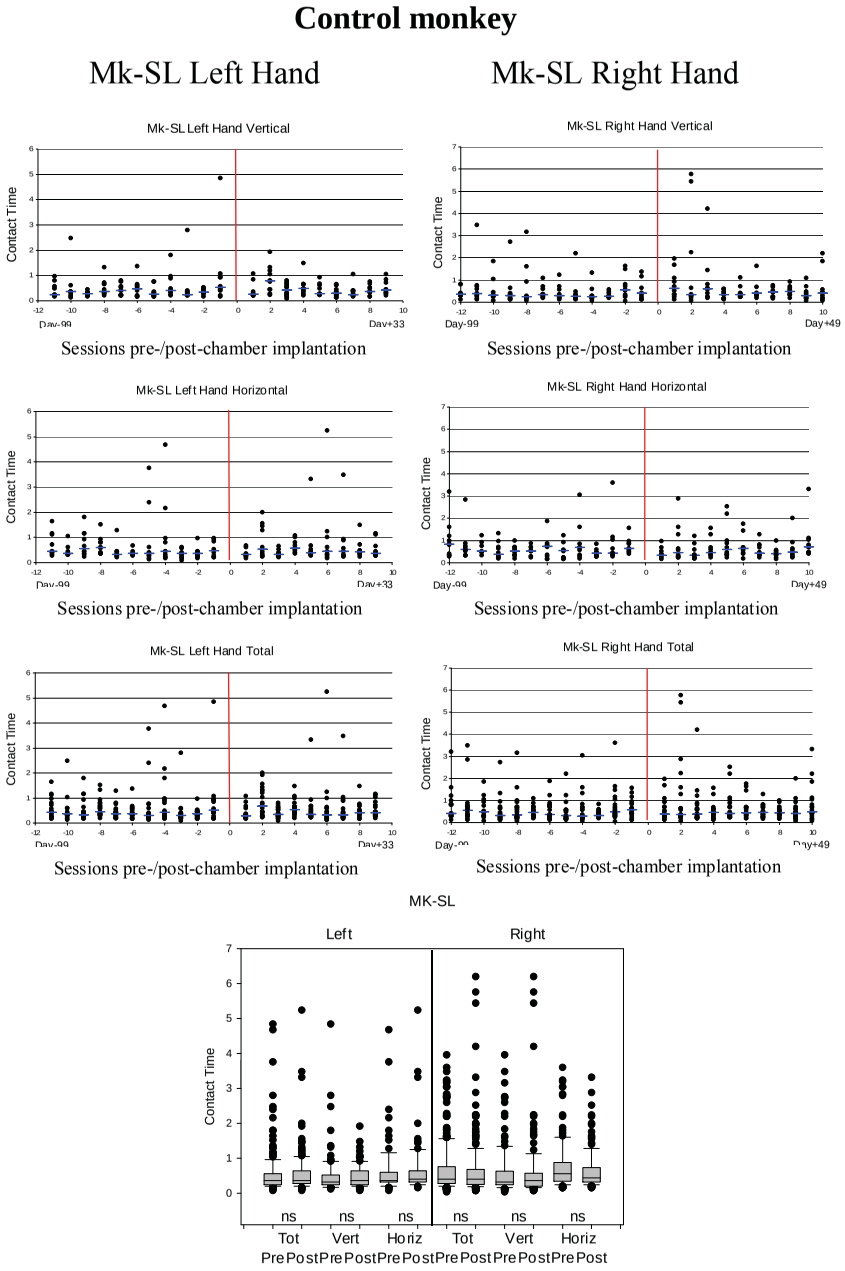
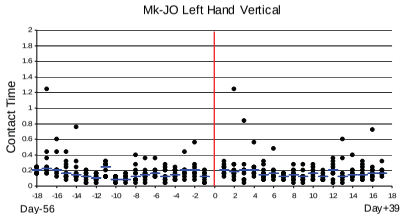


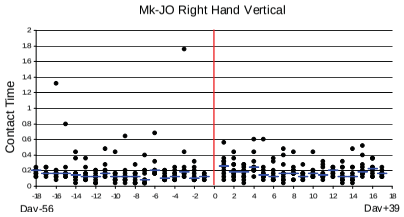
Fig. 3.3.3 : Graphics with XY axes: Contact time (y axis) to retrieve a pellet out of the well, for the vertical, horizontal and total wells along training sessions pre- and post-surgery (x axis) in the control (chamber implant uniquely; n=3) and biopsied (chamber implant and dlPFC biopsy; n=2) monkeys on the modified Brinkman board task, for both hands. The red vertical line indicates the surgery. Statistical comparisons were performed between pre- and post-surgery contact times distributions, represented in the boxplots.

Biopsied monkeys

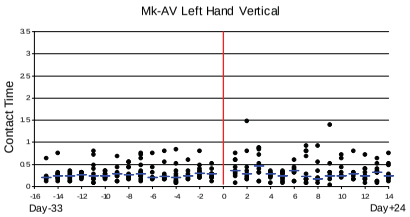
Mk-JO Left Hand | Mk-JO Right Hand | Mk-AV Left Hand | Mk-AV Right Hand



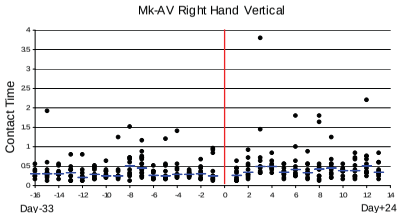
Sessions pre-/post-biopsy/chamber implantation



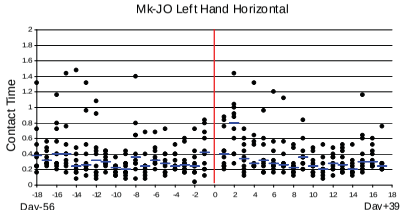
Sessions pre-/post-biopsy/chamber implantation



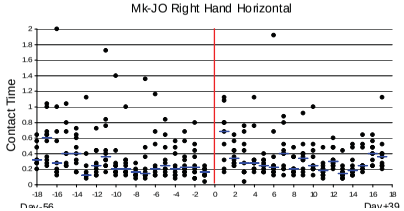
Sessions pre-/post-biopsy/chamber implantation



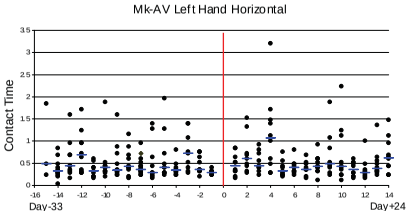
Sessions pre-/post-biopsy/chamber implantation



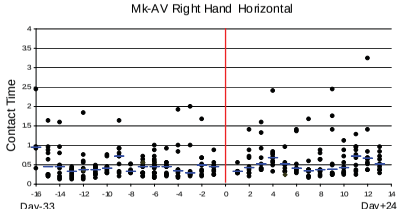
Sessions pre-/post-biopsy/chamber implantation



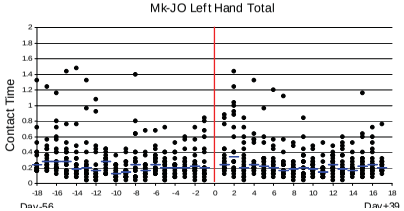
Sessions pre-/post-biopsy/chamber implantation



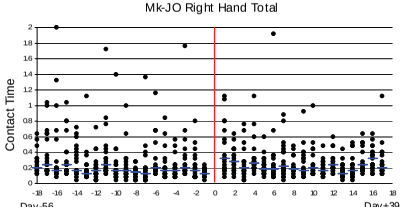
Sessions pre-/post-biopsy/chamber implantation



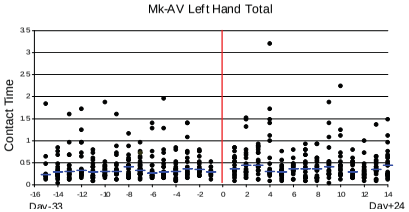
Sessions pre-/post-biopsy/chamber implantation



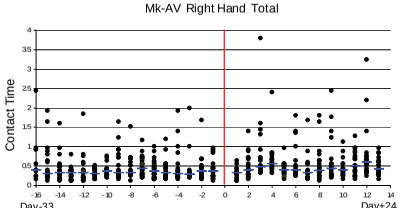
Sessions pre-/post-biopsy/chamber implantation



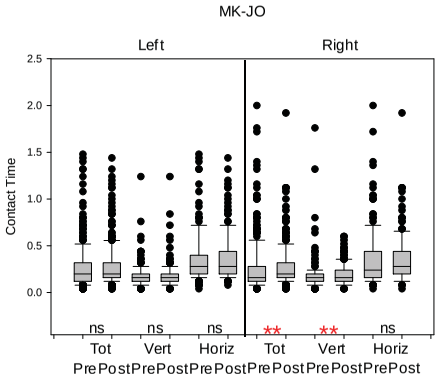
Sessions pre-/post-biopsy/chamber implantation

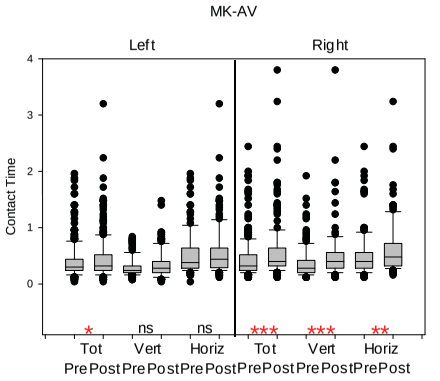


Sessions pre-/post-biopsy/chamber implantation



Sessions pre-/post-biopsy/chamber implantation





When looking at the contact time on the modified Brinkman board task (Fig. 3.3.3), as for the score, little effect was observed in the two control monkeys Mk-VA and Mk-SL, probably due again to the lack of data on the days immediately following the surgery. Thus, the only significant decrease of motor performance, expressed by an increase of the contact time, was found in Mk-VA in his right hand for the total wells. In contrast, in Mk-JA with training sessions following immediately the surgery, there was an increase of contact time in his right (vertical and total wells) and left (vertical wells) hands.

A similar increase of contact time was observed in the biopsied monkeys, particularly in their right hands (vertical and total wells for Mk-JO and all wells for Mk-AV). Mk-AV showed also such an increase in his left hand for the total wells.

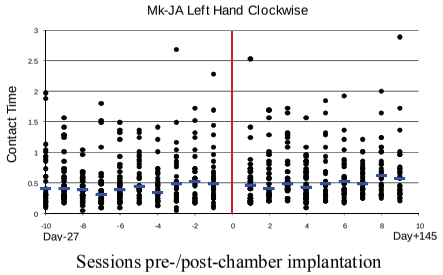
Again, as days of training are lacking immediately after the surgery in Mk-VA and Mk-SL, it was not possible to assess the acute phase following the surgery. Nevertheless, as for the score, an effect of the surgery appeared in both control and biopsied monkeys, which could therefore not be attributed to the dlPFC biopsy specifically. To not that the observed effect concerned mainly the right hand, suggesting that it would be related to the chamber implantation. Thus, these contact time data are in line with the observations on the score data, which led to attribute these effects to a general decrease of performance consequent to the surgery and chamber implantation above M1.

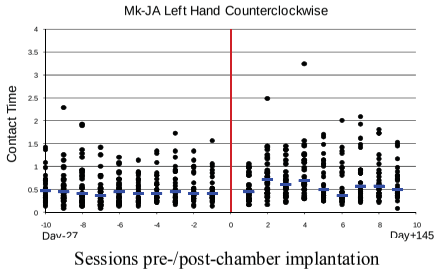
3.3.3.2.2 Rotating Brinkman board task

3.3.3.2.2.1 Contact Time

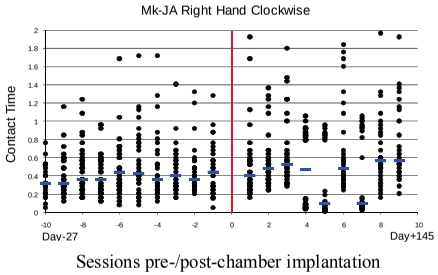
Control monkeys

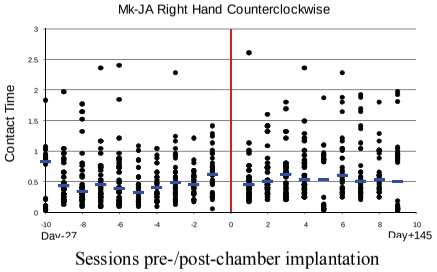
Mk-JA Left Hand



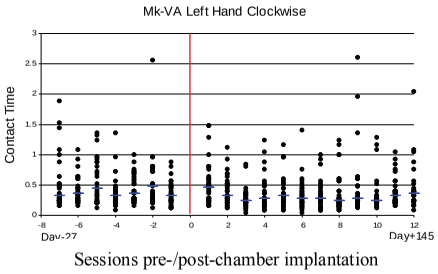


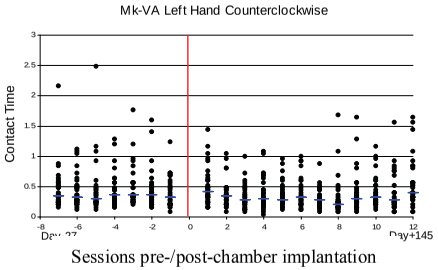
Mk-JA Right Hand



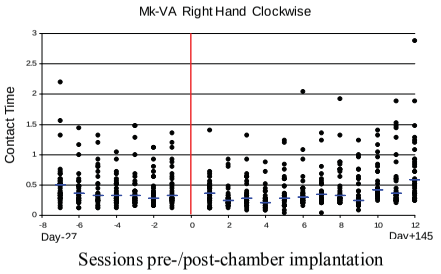


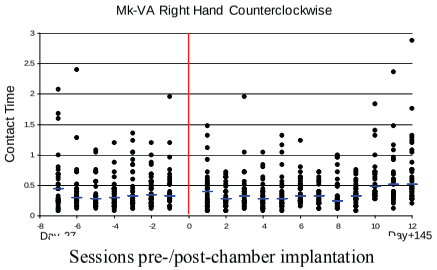
Mk-VA Left Hand

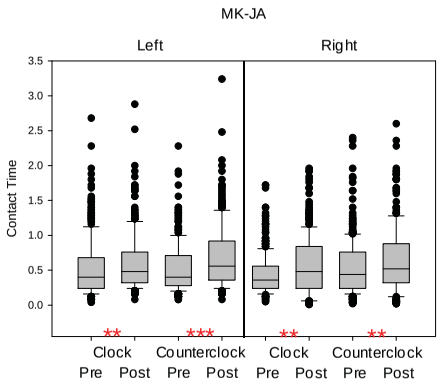


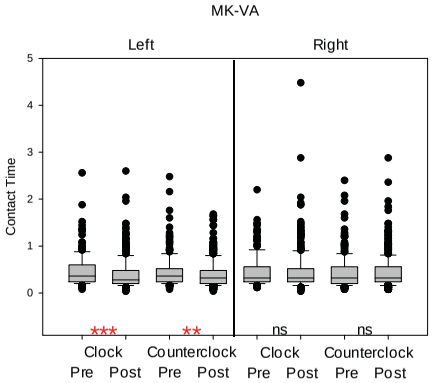


Mk-VA Right Hand









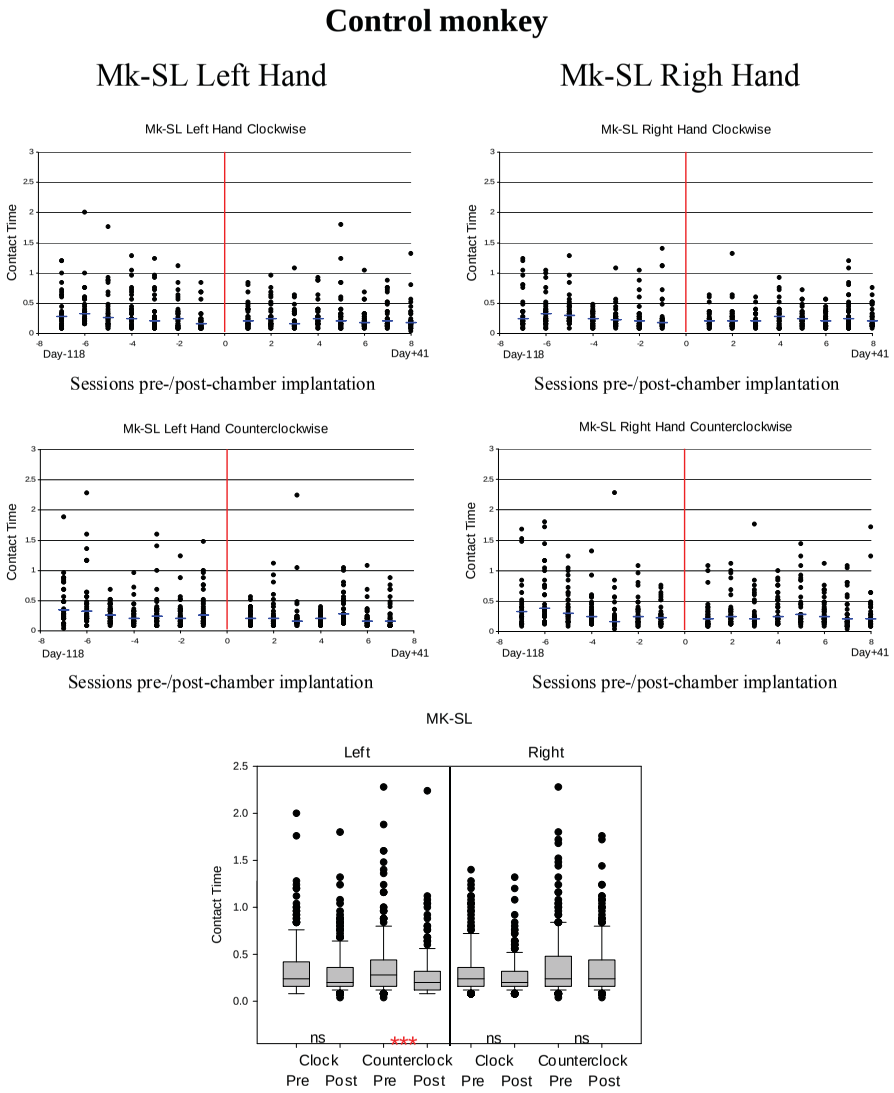
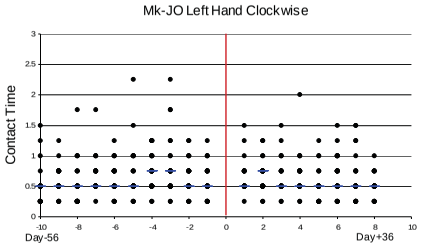


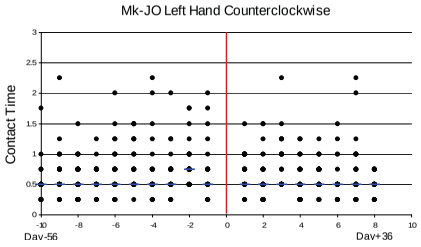
Fig. 3.3.4 : Graphics with XY axes: Contact time (y axis) to retrieve a pellet out of the well, for the clockwise and contraclockwise orientations along training sessions pre- and post-surgery (x axis) in the control (chamber implant uniquely; n=3) and biopsied (chamber implant and dlPFC biopsy; n=2) monkeys on the rotating Brinkman board task, for both hands. The red vertical line indicates the surgery. Statistical comparisons were performed between pre- and post-surgery contact times distributions, represented in the boxplots.

Biopsied monkeys

Mk-JO Left Hand

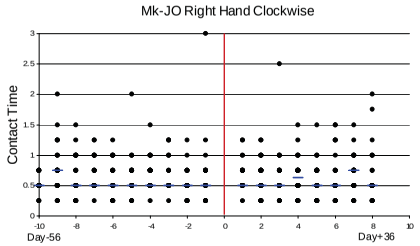


Sessions pre-/post-biopsy/chamber implantation

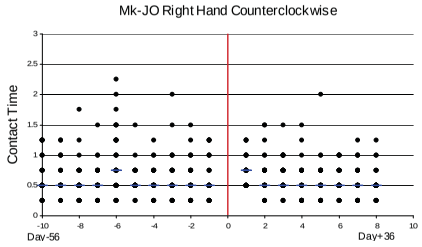


Sessions pre-/post-biopsy/chamber implantation

Mk-JO Right Hand

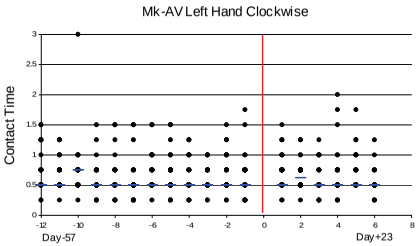


Sessions pre-/post-biopsy/chamber implantation

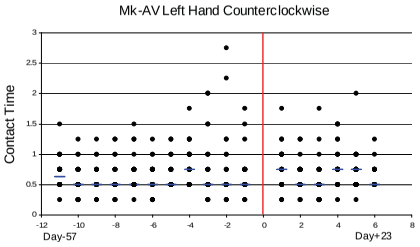


Sessions pre-/post-biopsy/chamber implantation

Mk-AV Left Hand

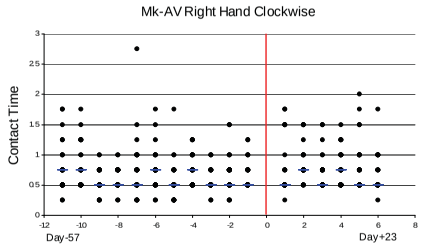


Sessions pre-/post-biopsy/chamber implantation

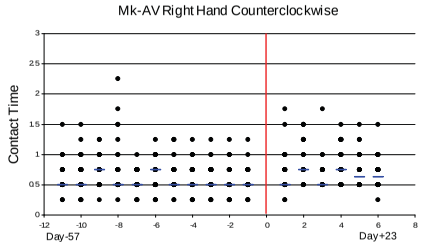


Sessions pre-/post-biopsy/chamber implantation

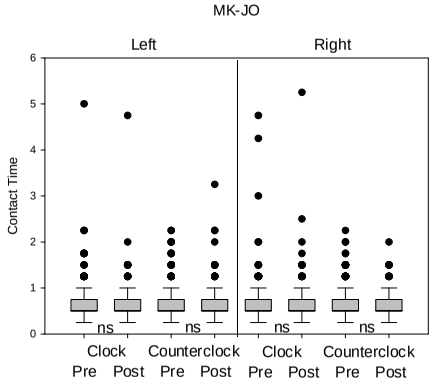
Mk-AV Right Hand

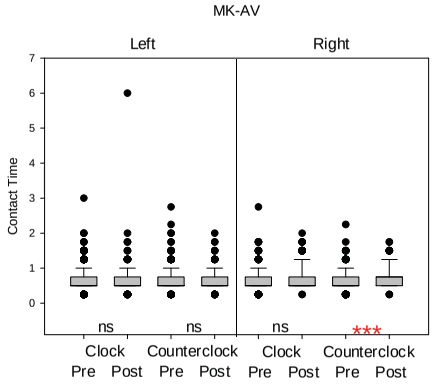


Sessions pre-/post-biopsy/chamber implantation



Sessions pre-/post-biopsy/chamber implantation





On the contact time at the rotating Brinkman board task (Fig. 3.3.4), effects in terms of increase and of decrease of contact time were observed in the control monkeys. In Mk-JA an increase effect was present in both hands and in each rotation orientation (clockwise and contraclockwise), whereas the two other control monkeys had a decrease effect only on their left hands, in each rotation orientation in Mk-VA and in the contraclockwise rotation orientation in Mk-SL. To note that for this task, there was the same lack of training sessions immediately after the surgery as for the modified Brinkman board task. In the biopsied monkeys, little effect was found, namely uniquely an increase of contact time in Mk-AV on his right hand when the board rotated contraclockwise.

One cannot exclude that this lack of effect in the biopsied monkeys could be due to the less high temporal resolution of contact time analysis (see "Methods"), therefore less discriminant. Nevertheless, as observed on the standard Brinkman board task, as post-surgery effects on the motor performance occurred in the control monkeys too, there was no specific effect of the biopsy in terms of contact time in the rotating Brinkman board task.

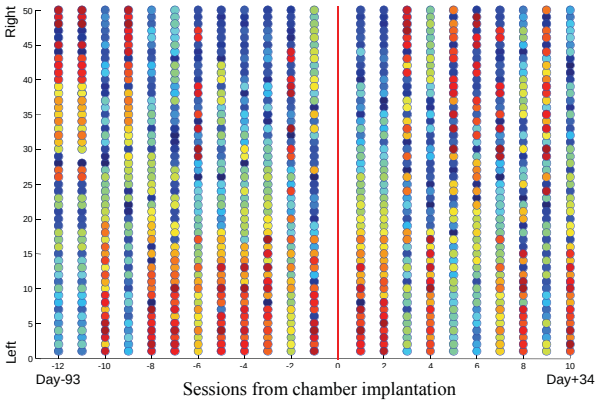
3.3.3.3 Behaviour: Strategy

3.3.3.3.1 Modified Brinkman board task

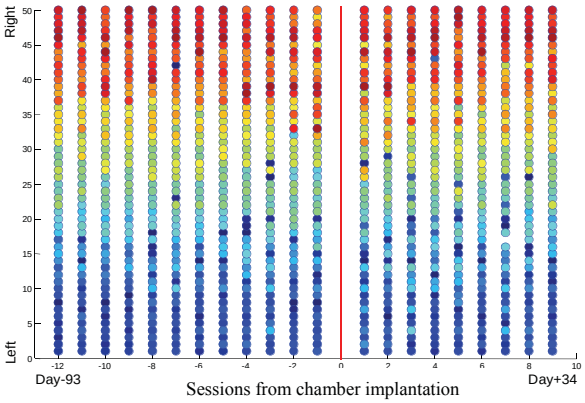
3.3.3.3.1.1 Picking Sequence on the left-right axis

Control monkeys

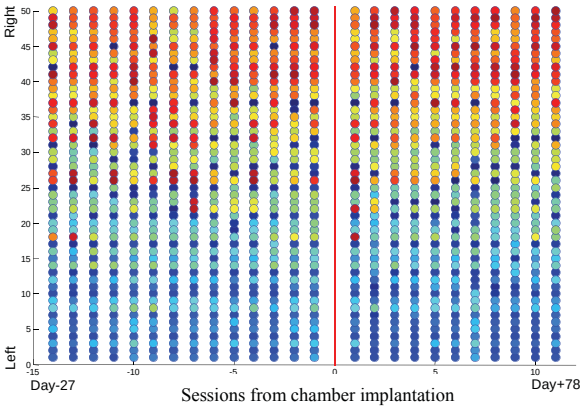
MK-JA Left Hand



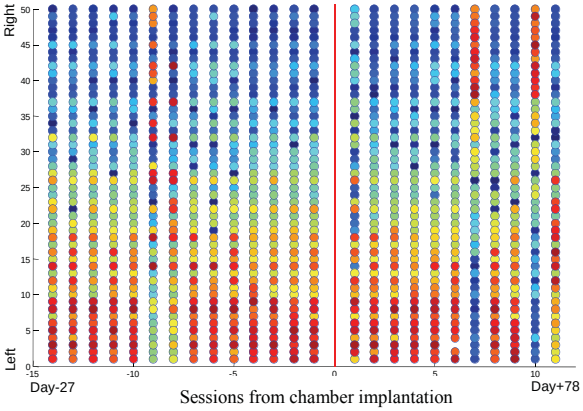
Mk-JA Right Hand



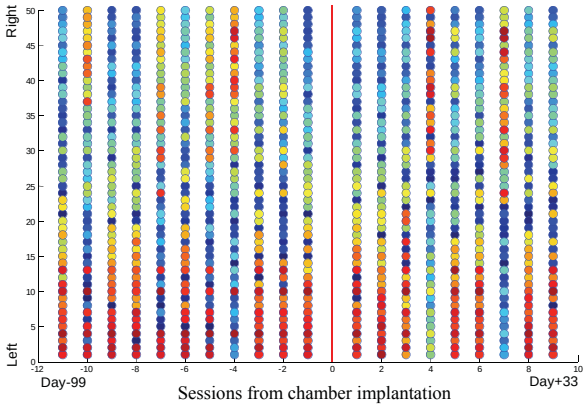
Mk-VA Left Hand



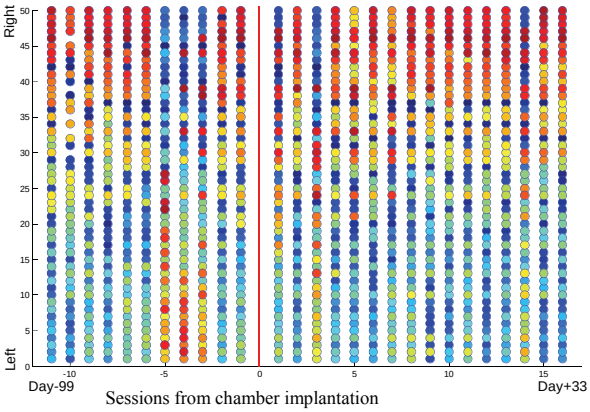
Mk-VA Right Hand



Mk-SL Left Hand



Mk-SL Right Hand



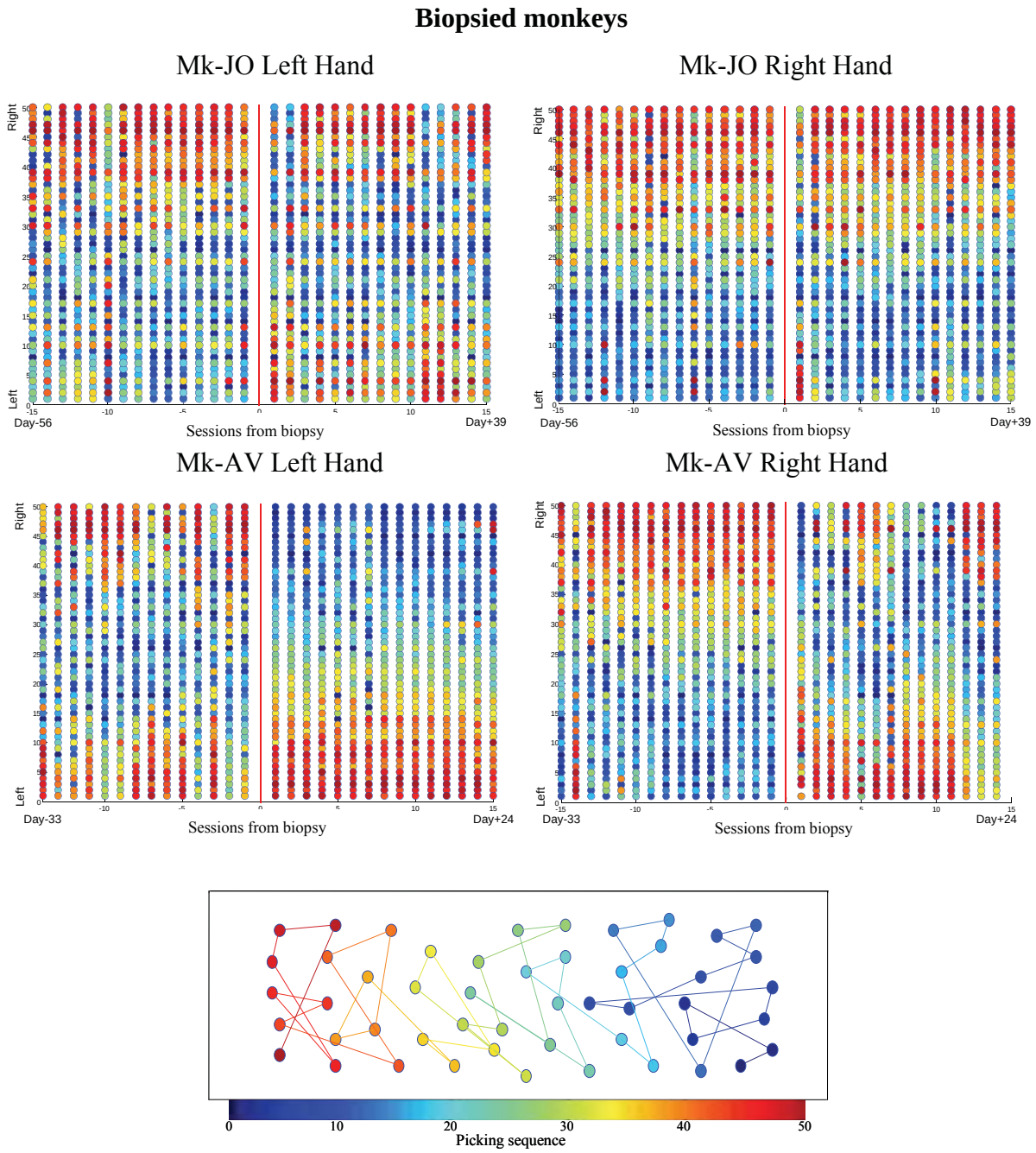


Fig. 3.3.5; Picking sequence on the left-right axis in the modified Brinkman board task for both hands of the three control and the two biopsied monkeys. X axis represents training sessions, one column corresponding thus to one training session. Y axis represents the 50 wells of the board, ordered according to their position on the left-right axis, independently of their position on the up-down axis. Colors indicate the picking sequence, ranging from 1 (blue) to 50 (red). Red vertical lines represent the surgery. The board at the bottom of the figure represents the modified Brinkman board with its 50 wells, coloured and linked according to the prehension sequence of a given session. In the present example, the monkey began to pick the pellets in the right side of the board, and then progressively moved towards the left side (Mk-AV, left hand post-lesion).

When looking at the picking sequence on the left-right axis at the modified Brinkman board task (Fig. 3.3.5), the data indicated that, before the surgery, all monkeys performed the task with a given strategy. Indeed, the pellets were not retrieved randomly along the left-right axis; the strategy of prehension differed across the monkeys.

After the surgery, no change of prehension strategy was observed in the control monkeys. Mk-JA retrieved the pellets after the surgery with his left hand sometimes from the left to the right and sometimes from the right to the left, as before the surgery. In the same way, he took the pellets with his right hand from the left to the right side of the board before and after the surgery. This left to right prehension order was also used by Mk-VA with his left hand, whereas with his right hand, he retrieved the pellets from right to left, with exceptionally a less organized or a left to right sequence, both before and after the surgery for both hands. For his part, Mk-SL showed sometimes a left to right, sometimes a right to left sequence, which was exceptionally less organized, with his left hand, both before and after the surgery. With his right hand, the picking sequence went from left to right, exceptionally from right to left or in a less organized way, before and after the surgery.

On the contrary, post-surgery changes occurred in the biopsied monkeys. Indeed, before the surgery, Mk-JO took the pellets from left to right mainly with his left hand, with sometimes a picking sequence from the center to the extremities or less organized. After the surgery, he retrieved the pellets in a sequence going from the center to the left and then to the right side mainly, and sometimes from right to left or in a less organized way. With his right hand, the pre-surgery sequence went mainly from left to right. Post-surgery, only the two first sessions differed, with a sequence going from the center to the right and then to the left side. Before the surgery, Mk-AV began to take the pellets out of the wells with his left hand from the center to the extremities, sometimes to the left, sometimes to the right side, whereas after the surgery, a change occurred toward a right to left picking sequence. In the same way, with his right hand, the pre-surgery picking sequence went from left to right, whereas a different strategy was used after the surgery, namely a right to left or center to extremities picking sequence.

Overall, a clear change of prehension strategy in terms of picking sequence on the left-right axis at the modified Brinkman board task occurred in the biopsied monkeys, though to a lesser extent for the right hand of Mk-JO, whereas no such change was observed in the control monkeys. Therefore, this change of pehension strategy may well be a consequence specific to the dlPFC biopsy.

3.3.3.3.1.2 Cumulated Distance: all wells

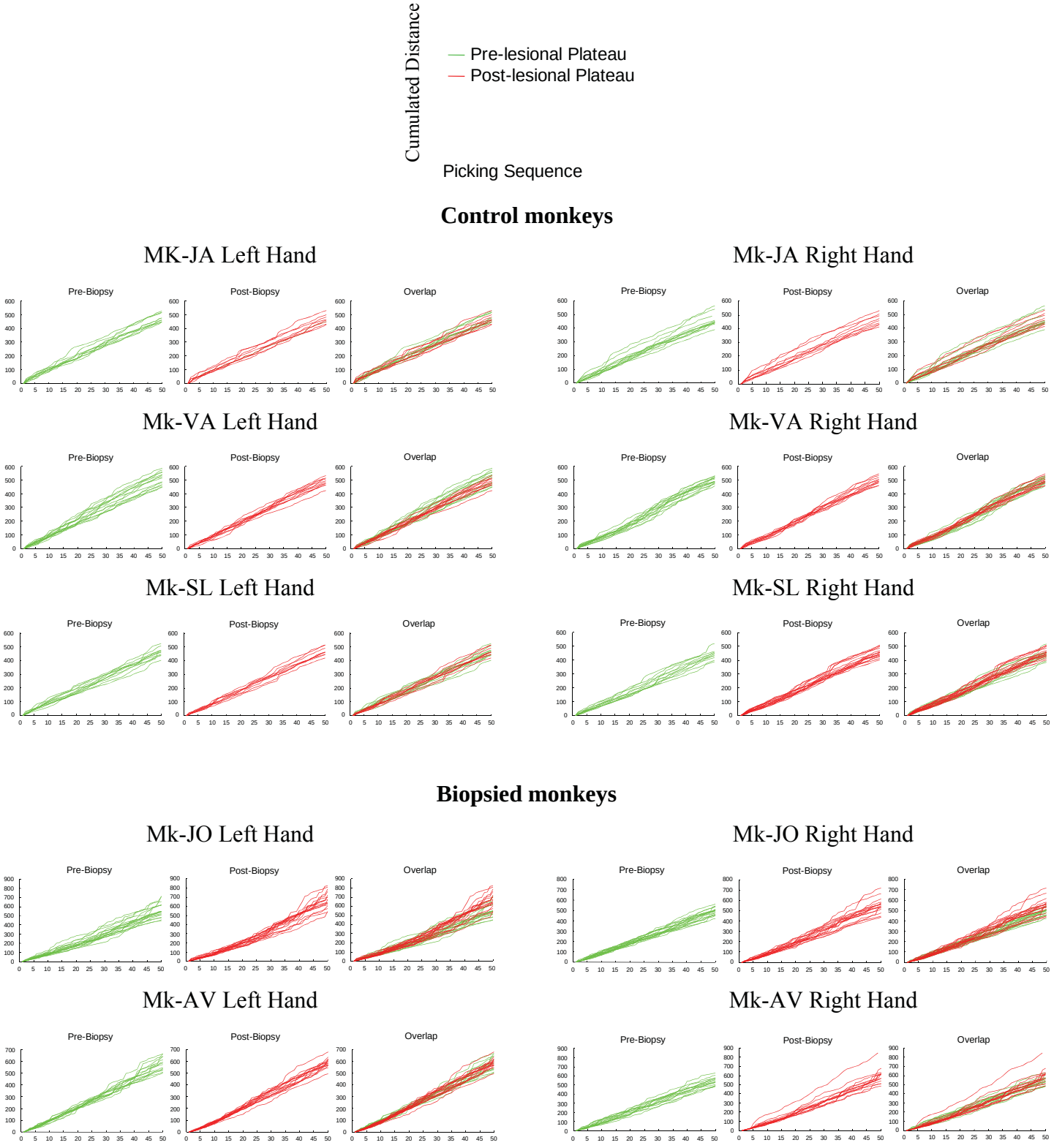


Fig. 3.3.6 : Cumulated distance to perform the modified Brinkman board task, represented for the three control monkeys and the two biopsied monkeys for their both hands. X axis represents the picking sequence, whereas on the Y axis appears the cumulative distance (mm) when grasping the pellets along the performance of the task. For each hand, three graphics are presented and correspond to the pre-surgery sessions (green), to the post-surgery sessions (red) and to the overlap.

On the cumulated distance covered to perform the modified Brinkman board task (Fig. 3.3.6), no significant differences appeared in the control monkeys, showing a similar distance pre- and post-surgery.

On the contrary, in the biopsied monkeys, a significant increase of cumulated distance was observed after the surgery, both on the left ($p=***$) and right ($p=**$) hands in Mk-JO and on the right hand ($p=*$) uniquely in Mk-AV.

These changes may be due to the biopsy, as they occurred only in the biopsied monkeys. They seem to be linked to the changes observed in the picking sequence, which was less efficient in terms of cumulated distance after the surgery.

3.3.3.3.1.3 Picking sequence of the vertical and the horizontal wells

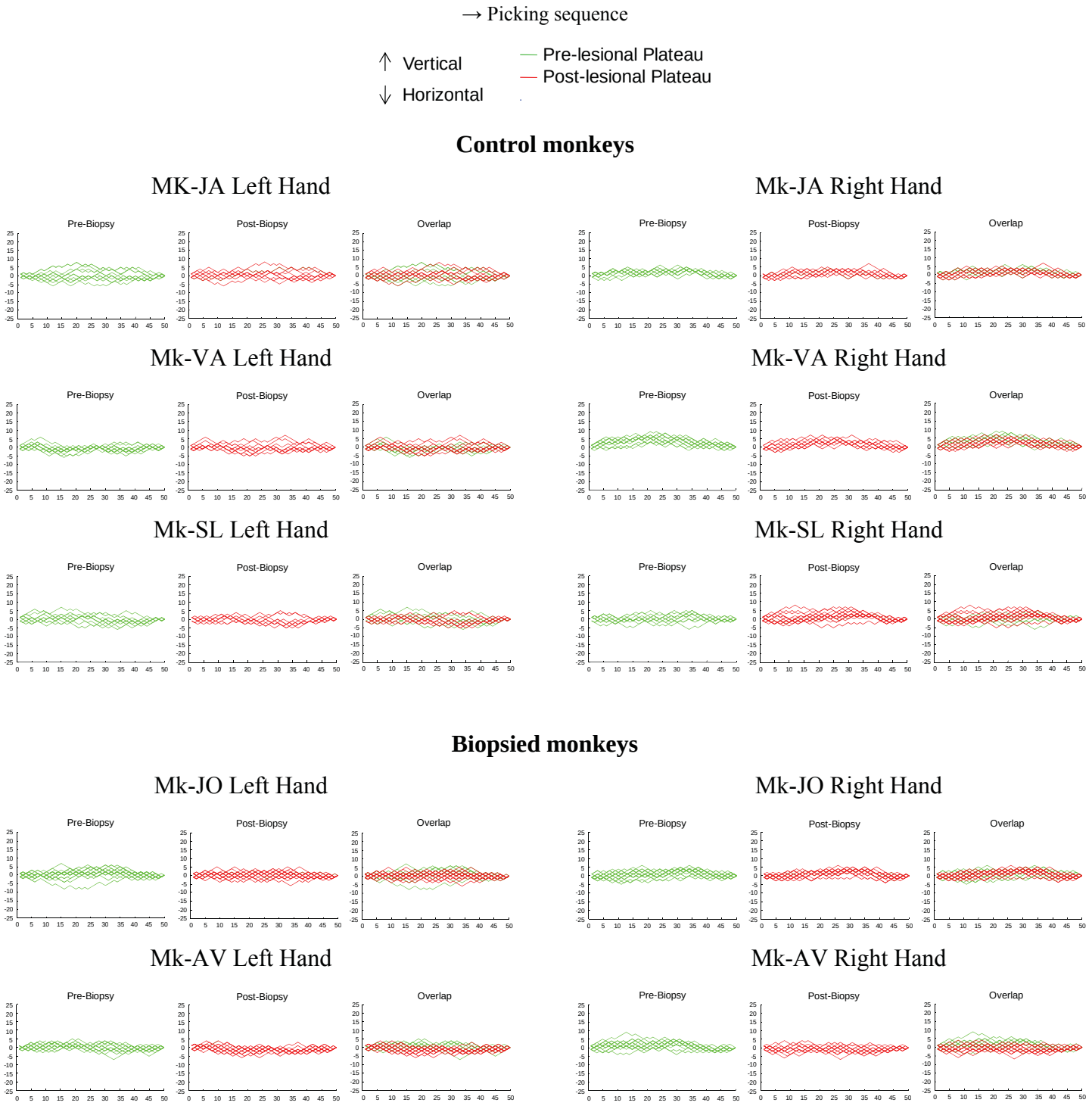


Fig. 3.3.7 : Picking sequence of the vertical and the horizontal wells represented for the left and the right hand of the control (n=3) and the biopsied (n=2) monkeys. X axis represents the picking sequence, whereas on the Y axis, the grasp of a vertical or an horizontal pellet leads to a rise or a fall of 1 unit, respectively. For each hand, three graphics are presented and correspond to the pre-surgery sessions (green), to the post-surgery sessions (red) and to the overlap.

When looking at the differences between pre- and post-surgery on the sequence of prehension in terms of vertical and horizontal wells (Fig. 3.3.7), no change appeared after the surgery, both in the control and in the biopsied monkeys, which retrieved the pellets as much in the vertical wells as in the horizontal wells.

Thus, the surgery, either the chamber implant or the biopsy did not affect the prehension in the vertical and horizontal wells of the monkeys. To note that a change occurred when the monkeys were M1 lesioned (see chapter "Does the motor cortex lesion affect the prehension's strategy of the monkeys?"), leading to link this way of grasping pellets preferentially in the vertical or horizontal wells to the motor capacity itself.

3.3.3.3.1.4 Preferential wrist orientation: horizontal wells

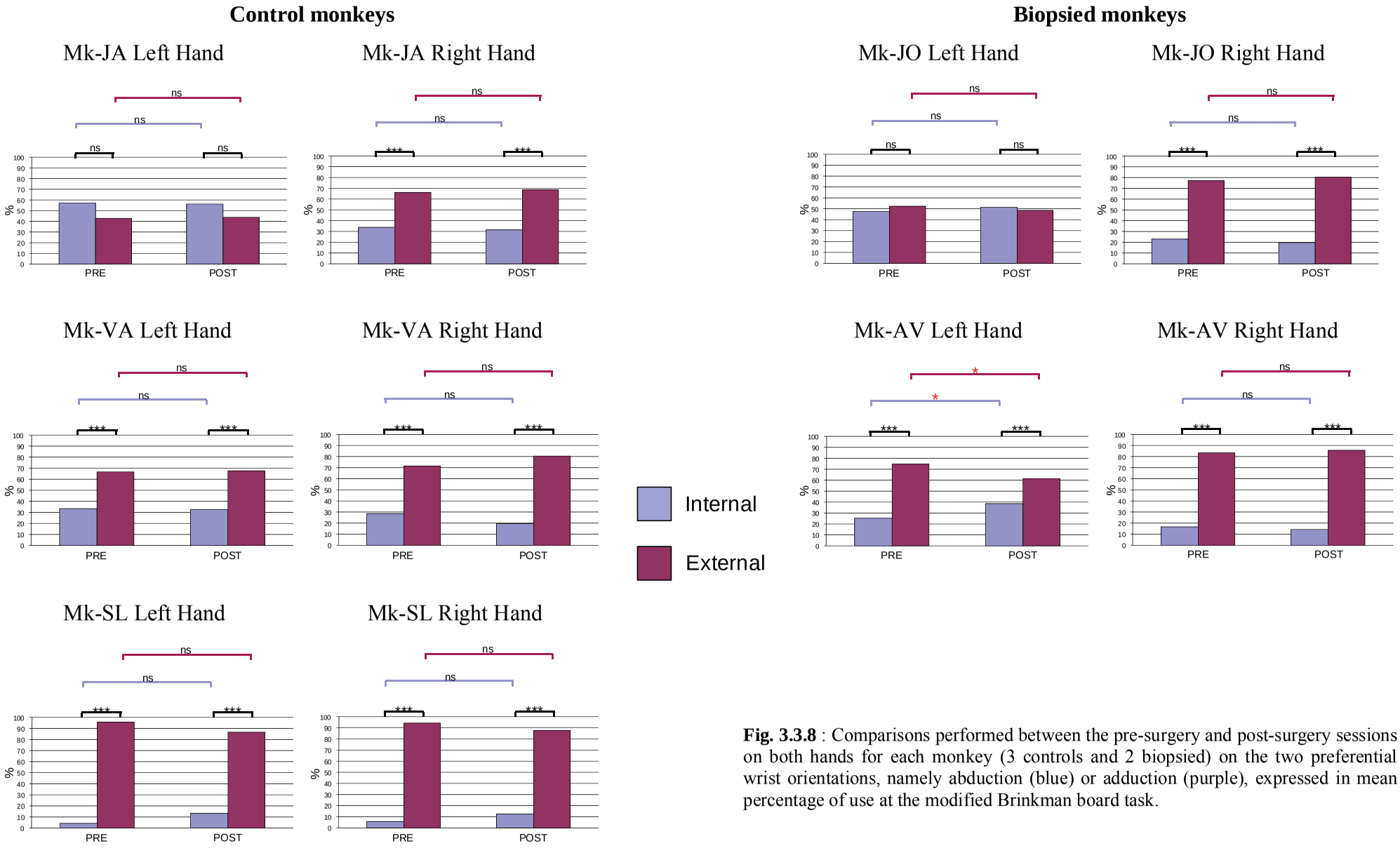


Fig. 3.3.8 : Comparisons performed between the pre-surgery and post-surgery sessions on both hands for each monkey (3 controls and 2 biopsied) on the two preferential wrist orientations, namely abduction (blue) or adduction (purple), expressed in mean percentage of use at the modified Brinkman board task.

3.3.3.3.1.5 Preferential finger: horizontal wells

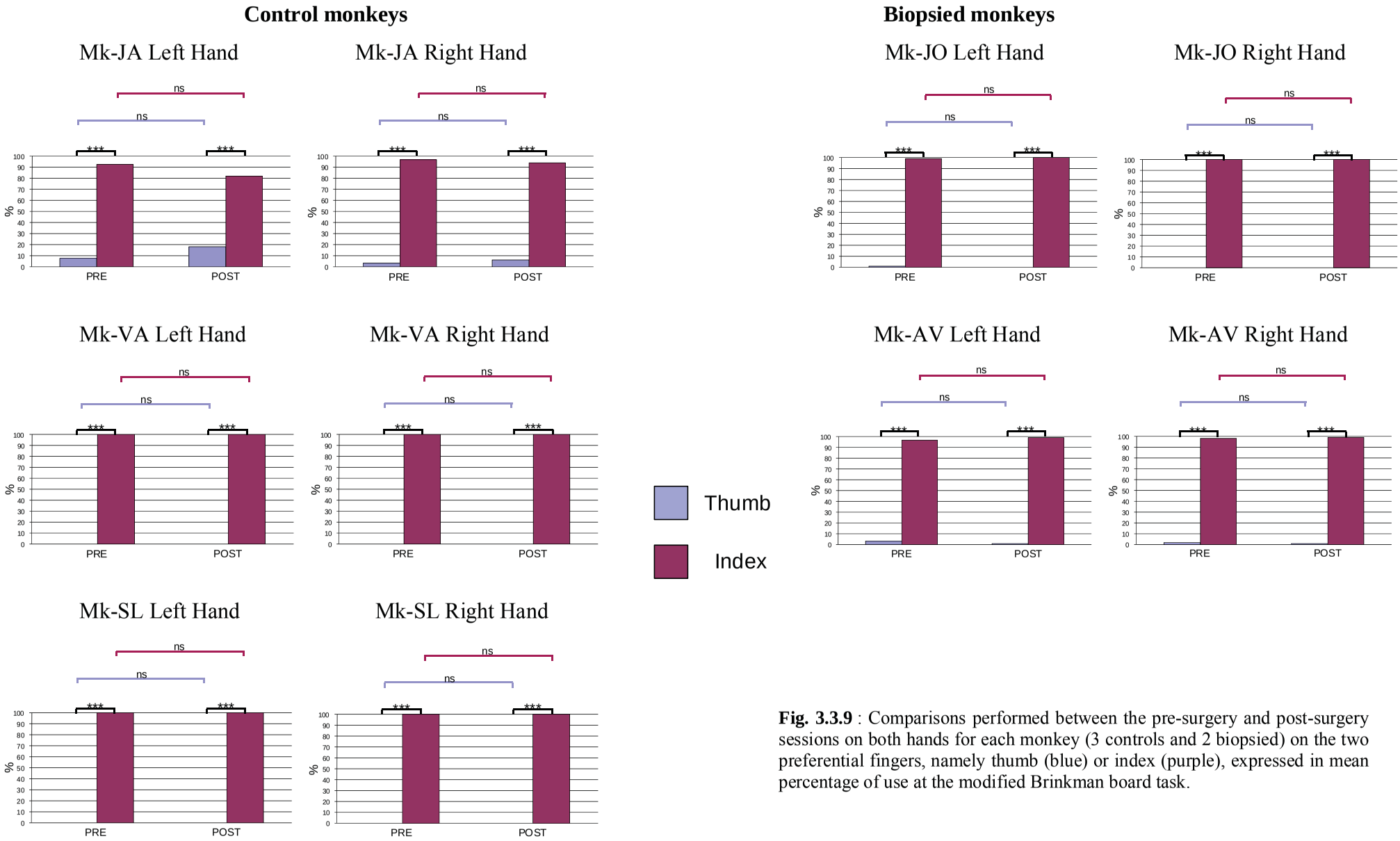


Fig. 3.3.9 : Comparisons performed between the pre-surgery and post-surgery sessions on both hands for each monkey (3 controls and 2 biopsied) on the two preferential fingers, namely thumb (blue) or index (purple), expressed in mean percentage of use at the modified Brinkman board task.

3.3.3.3.1.6 Errors

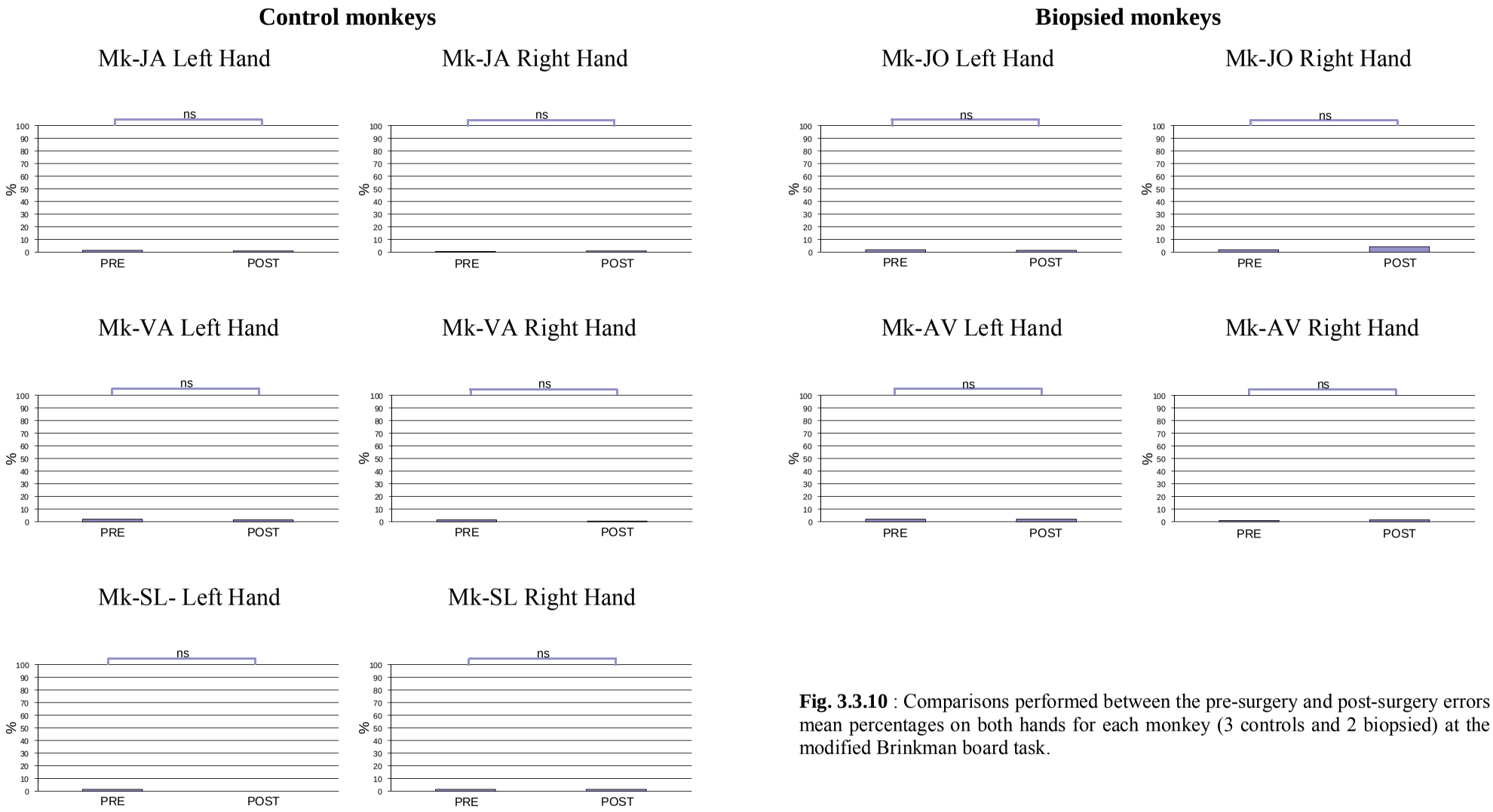


Fig. 3.3.10 : Comparisons performed between the pre-surgery and post-surgery errors mean percentages on both hands for each monkey (3 controls and 2 biopsied) at the modified Brinkman board task.

3.3.3.3.1.7 Retrieval of 2 pellets at the same time

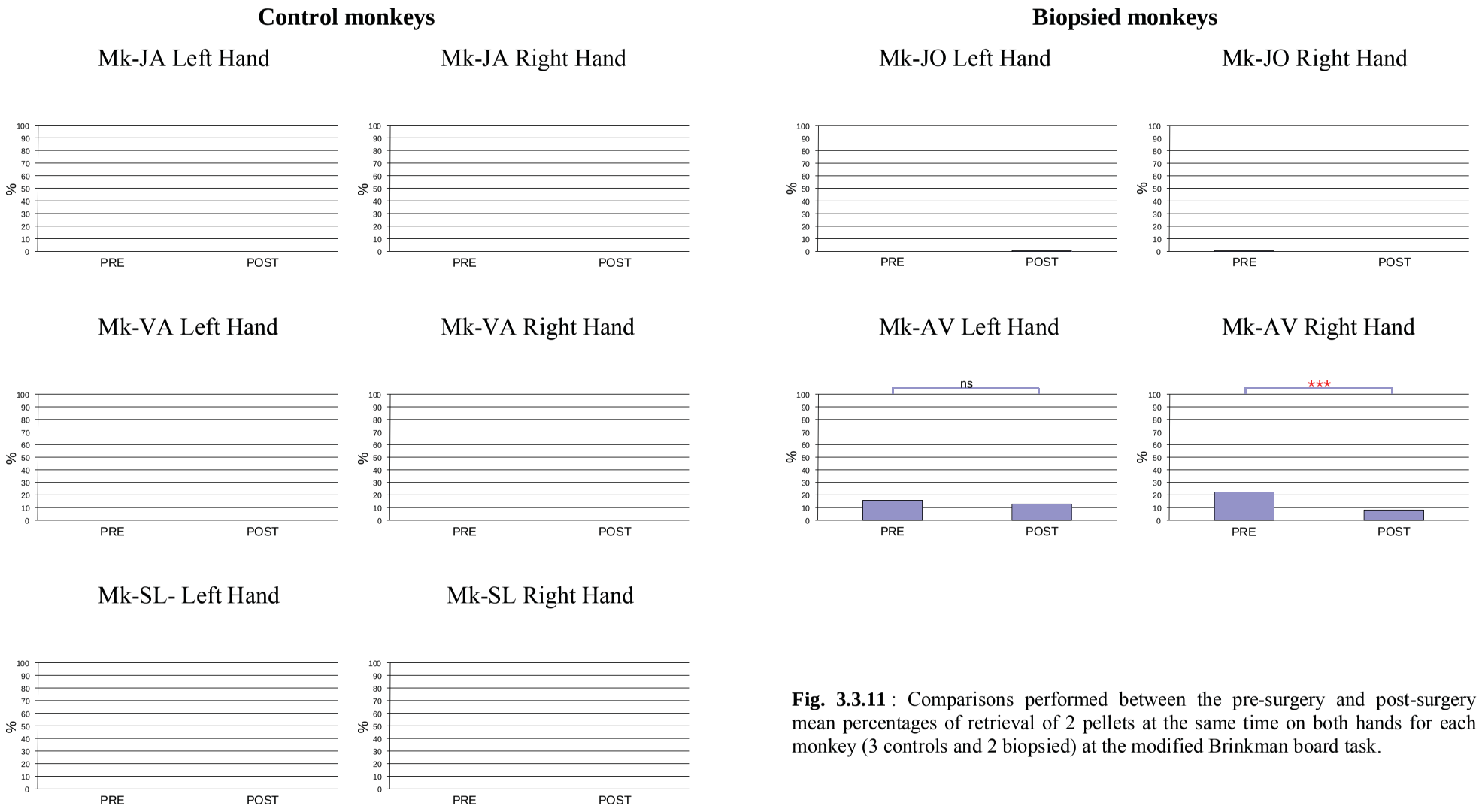


Fig. 3.3.11 : Comparisons performed between the pre-surgery and post-surgery mean percentages of retrieval of 2 pellets at the same time on both hands for each monkey (3 controls and 2 biopsied) at the modified Brinkman board task.

Pre-surgery, the preferential wrist orientation was mainly the adduction, except in Mk-JA and Mk-JO for their left hand with an equal use of adduction and abduction (Fig. 3.3.8). Post-surgery, the unique difference was found in the biopsied monkey Mk-AV on his left hand, with an increase of the abduction use.
It was delicate to attribute this unique difference to the biopsy, as it was not massive in the biopsied monkeys; nevertheless, as Mk-AV had the largest biopsy (Fig. 3.3.1), one cannot totally exclude this possibility.

The preferential finger used to enter in the well before the surgery was the index for all the monkeys, without change after the surgery (Fig. 3.3.9). The thumb was poorly used, in the control monkey Mk-JA and the biopsied monkey Mk-AV. This parameter was therefore not much informative.

In the same way, the errors committed to perform the task were not informative, as very few errors were committed by the monkeys, both before and after the surgery (Fig. 3.3.10).

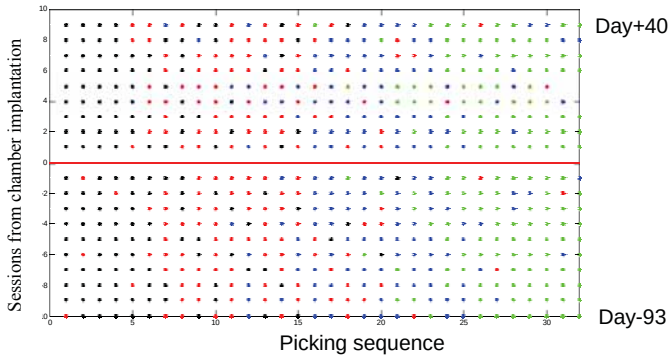
The only monkey using the grasp of two pellets at the same time was Mk-AV, who used less this strategy after the surgery with his right hand (Fig. 3.3.11). As for the preferential wrist orientation, one cannot exclude that this difference could be a consequence of the dlPFC biopsy, but as this parameter was absent in the other monkeys, no comparison with the control monkeys could be done, rendering any conclusion delicate. Anyway, in Mk-AV, the change on the grasp of two pellets at the same time was not massive; therefore, it did not seem that this parameter was very pertinent here.

3.3.3.3.2 Rotating Brinkman board task

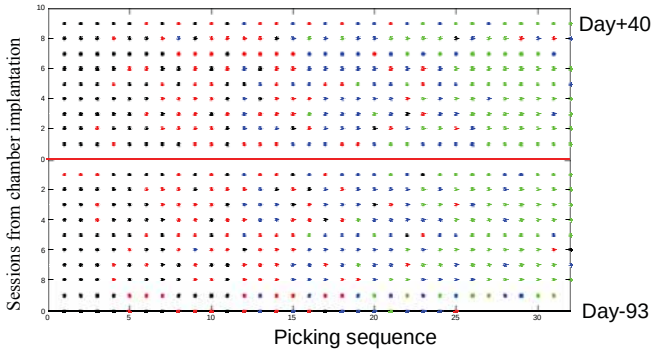
3.3.3.3.2.1 Picking sequence on the four rings

Control monkeys

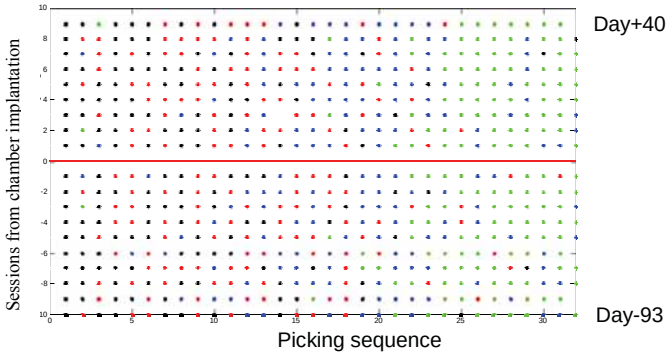
Mk-JA Left Hand Clockwise



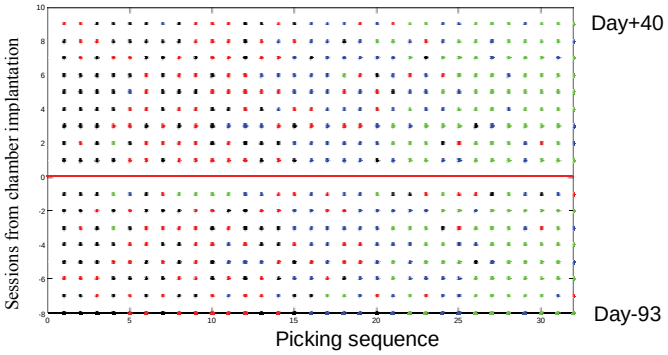
Mk-JA Right Hand Clockwise



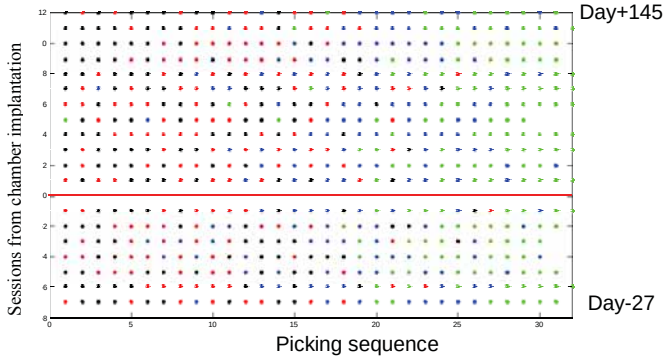
Mk-JA Left Hand Contraclockwise



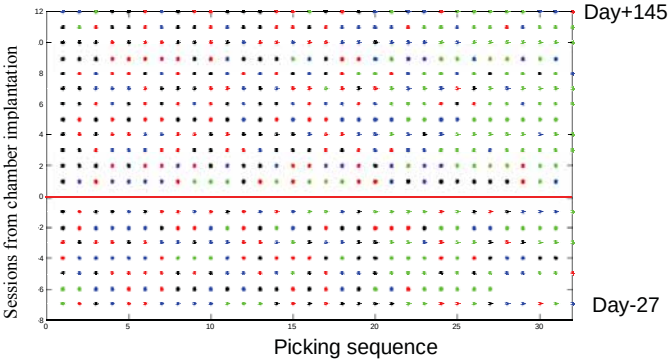
Mk-JA Right Hand Contraclockwise



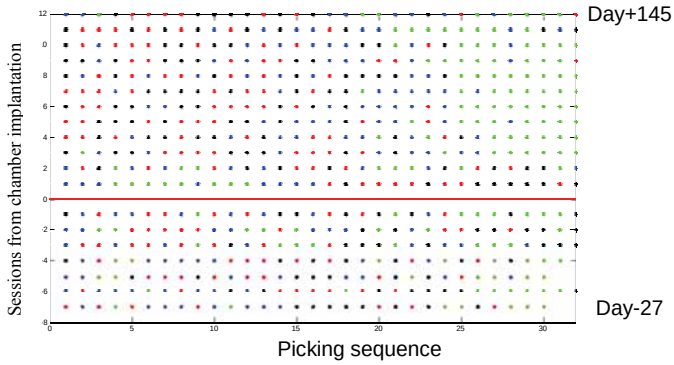
Mk-VA Left Hand Clockwise



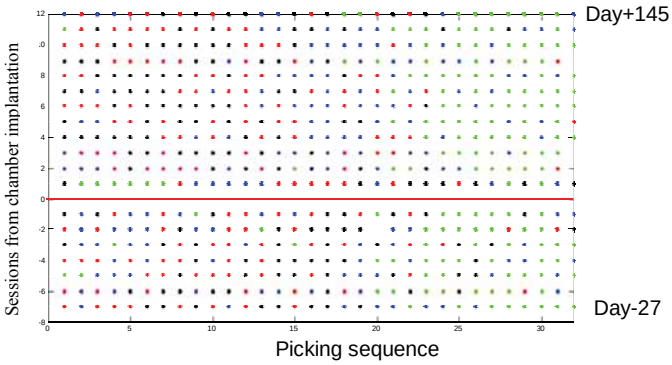
Mk-VA Right Hand Clockwise



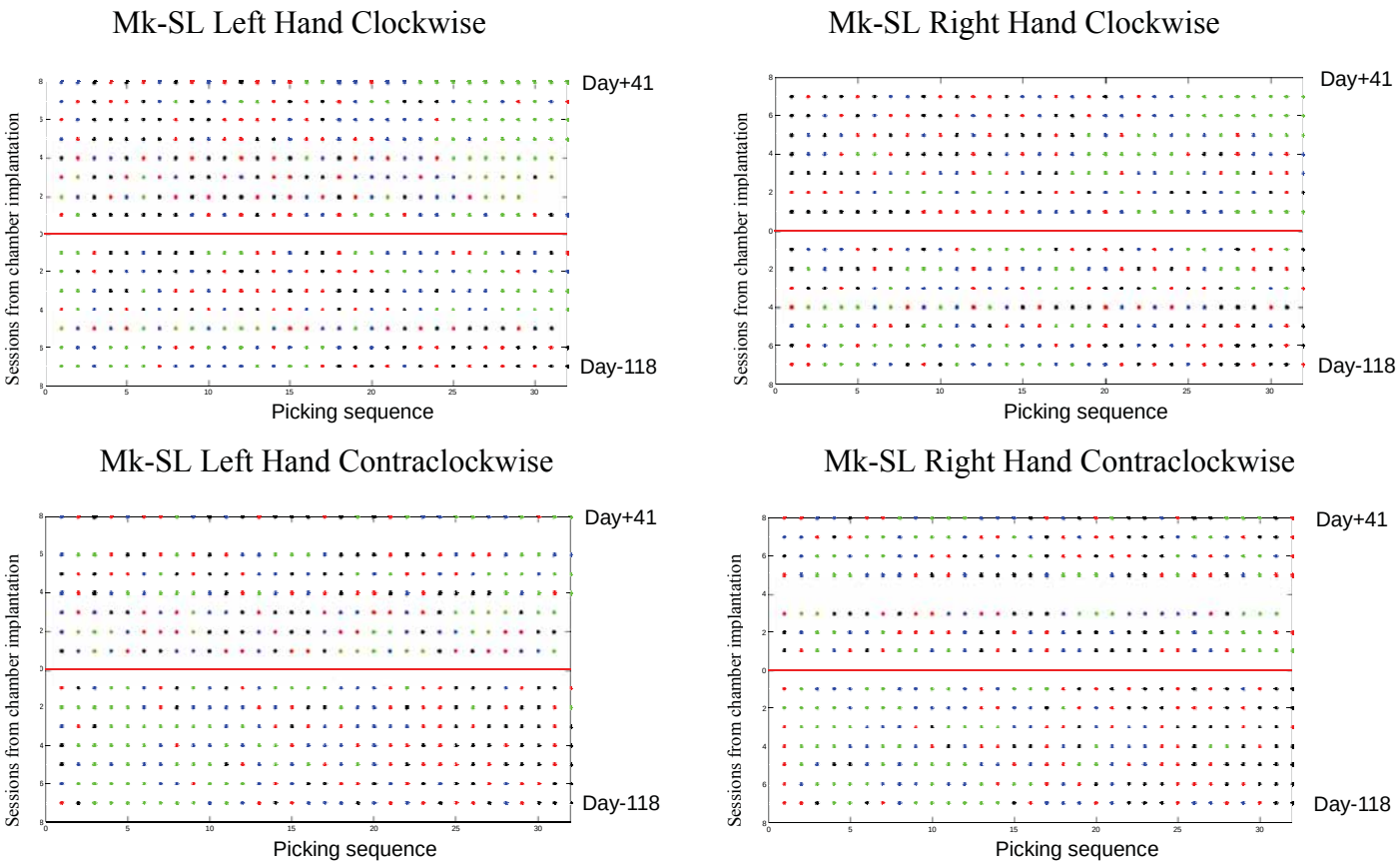
Mk-VA Left Hand Contraclockwise



Mk-VA Right Hand Contraclockwise



Control monkey



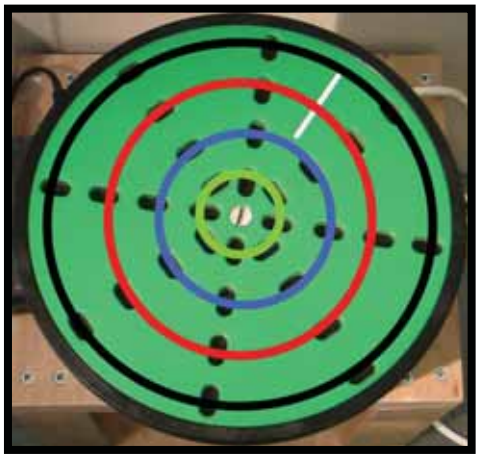
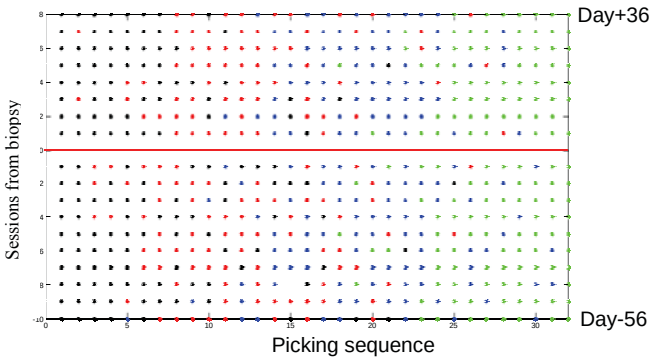


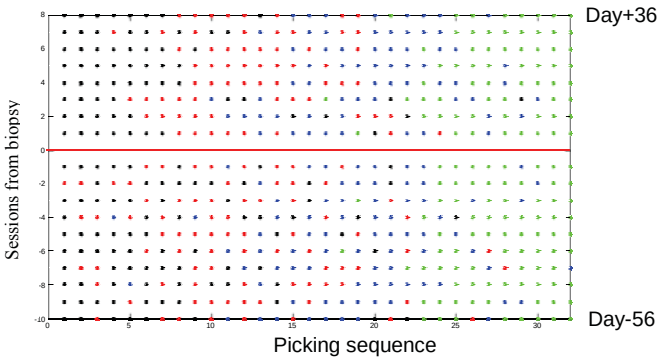
Figure 3.3.12: Picking sequence on the four rings of the Rotating Brinkman board task for both hands of each monkey (3 controls and 2 biopsied). X axis represents the picking sequence, each retrieval being coloured according to the corresponding ring (black for the first, red for the second, blue for the third and green for the fourth). Y axis indicates the training sessions, one line corresponding thus to one training session. Red horizontal lines represent the surgery.

Biopsied monkeys

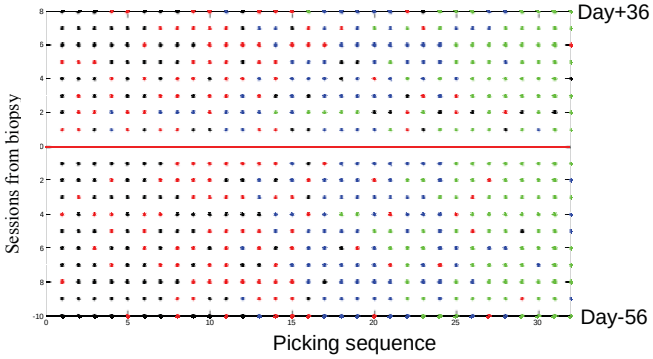
Mk-JO Left Hand Clockwise



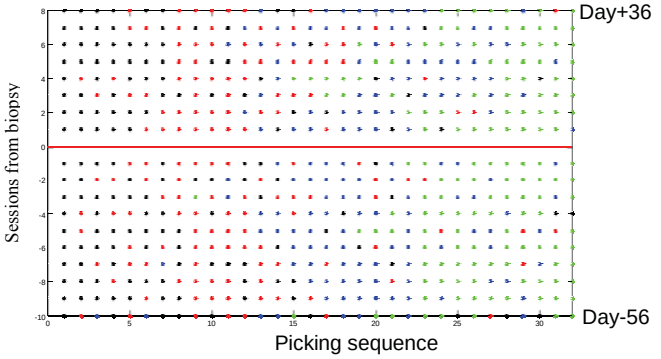
Mk-JO Right Hand Clockwise



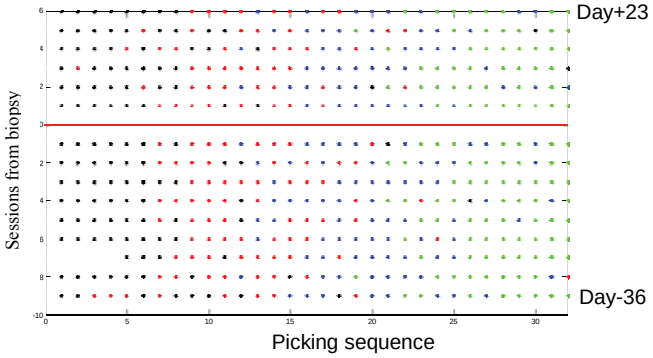
Mk-JO Left Hand Contraclockwise



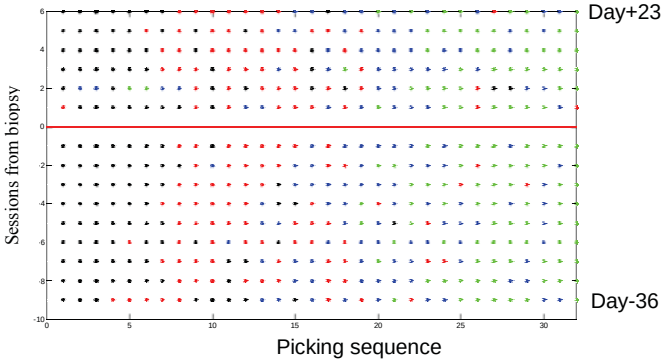
Mk-JO Right Hand Contraclockwise



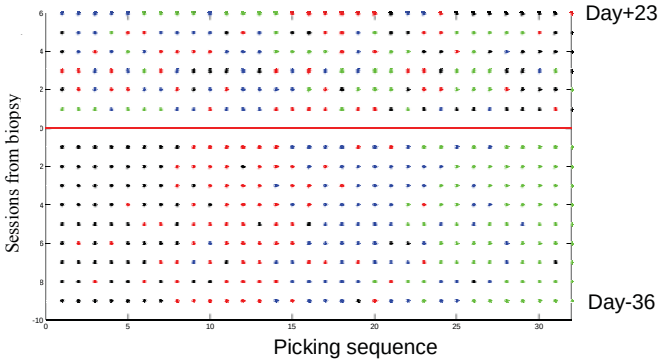
Mk-AV Left Hand Clockwise



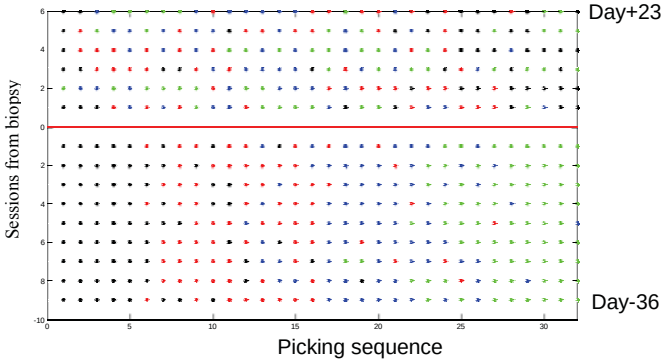
Mk-AV Right Hand Clockwise



Mk-AV Left Hand Contraclockwise



Mk-AV Right Hand Contraclockwise



When looking at the prehension sequence on the four rings before the surgery (Fig. 3.3.12), a general trend appeared, consisting in taking the pellets from the most external (either 1 or 1 and 2 mixed) to the most internal (4) rings, meaning that the monkey retrieved the pellets that were the closest to him, except Mk-SL, showing a quite randomly distributed prehension sequence, even with pellets in ring 4 at the beginning. Furthermore, in Mk-VA, the rings were more interpenetrating, even with a trend to retrieve the pellets from the internal to the external rings when the board rotated contraclockwise.

Post-surgery, no major change was observed in the control monkeys. Indeed, the prehension sequence in Mk-JA went from the most external to the most internal rings for both hands and each rotation orientation, pre- and post-surgery. In Mk-VA, pre-surgery, the sequence went from the rings 1, 2 and 3 mixed to the ring 4, with sometimes pellets in ring 4 retreived at the beginning particularly when the board rotated contraclockwise. After the surgery, no change occurred, except perhaps an effect at the first session after the surgery with his right hand in both rotation orientations and his left hand when the board rotated contraclockwise, as the pellets in the ring 4 were grasped earlier. Mk-SL showed a randomly distributed picking sequence when the board rotated clockwise with his left and right hand, both pre-and post-surgery. Before the surgery, when the board rotated contraclockwise, the sequence went rather from the internal to the external rings, but not in a systematic way. After the surgery, the grasp of pellets in the ring 4 seemed more randomly distributed.

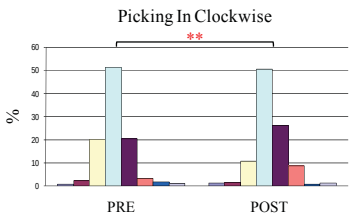
In the biopsied monkeys, no major effect was found in Mk-JO, whose prehension sequence went from the most external to the most internal rings for both hands, before and after the surgery. A little effect appeared in his left hand when the board rotated contraclockwise on the two first sessions following the surgery, with more pellets in ring 1 retrieved at the end and the pellets in the rings 3 and 4 taken earlier. Before the surgery, Mk-AV showed a very systematic picking sequence, grasping pellets in the ring 1 first, then in the ring 2, followed by the ring 3, to end finally in the ring 4. This strategy was used for both hands and both rotation orientations. After the surgery, no effect was found when the board rotated clockwise, whereas a massive change occurred in both hands when the board rotated contraclockwise, with a picking sequence no more organized and randomly distributed.

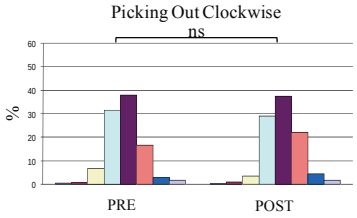
Thus, overall, a marked change occurred in Mk-AV for both hands and when the board rotated contraclockwise, probably consequently to the biopsy, as this radical change was observed uniquely in this monkey, who had the largest biopsy.

3.3.3.3.2.2 Prehension area

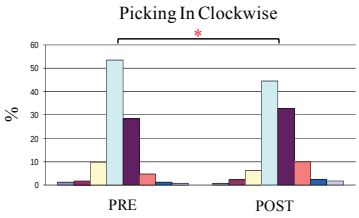
Control monkeys

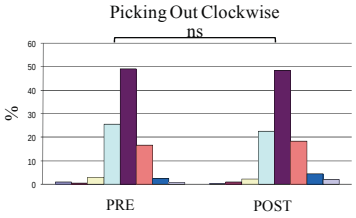
Mk-JA Left Hand Clockwise



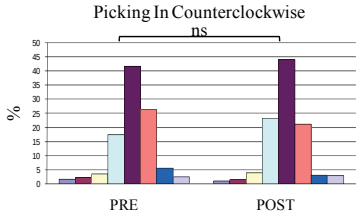


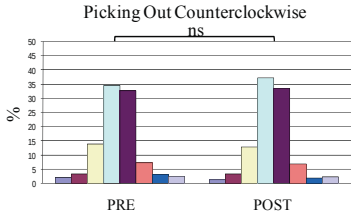
Mk-JA Right Hand Clockwise



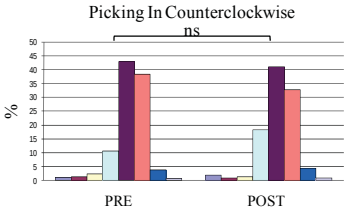


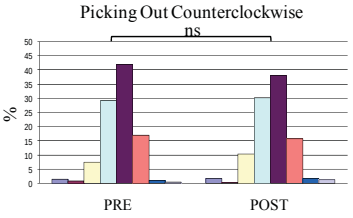
Mk-JA Left Hand Contraclockwise



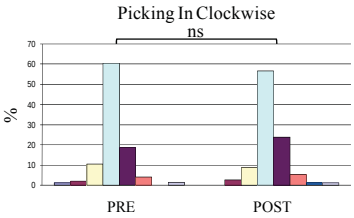


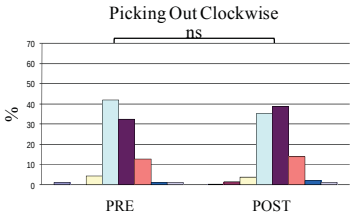
Mk-JA Right Hand Contraclockwise



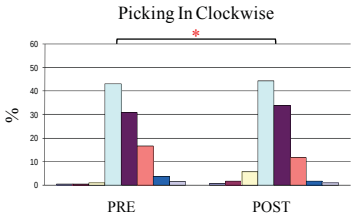


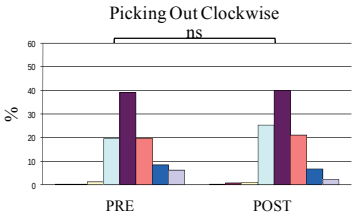
Mk-VA Left Hand Clockwise



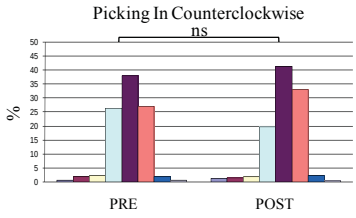


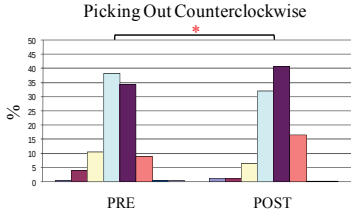
Mk-VA Right Hand Clockwise



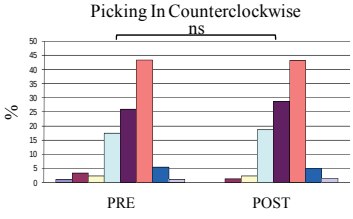


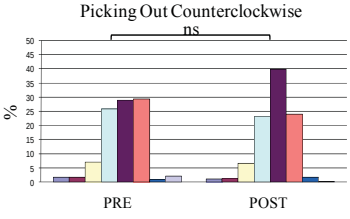
Mk-VA Left hand Contraclockwise



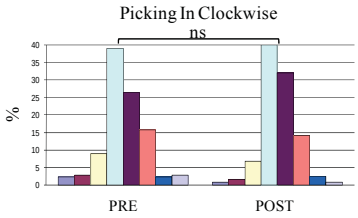


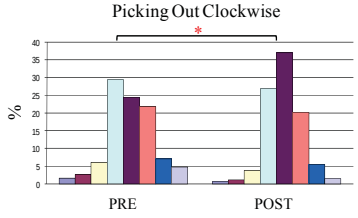
Mk-VA Right Hand Contraclockwise



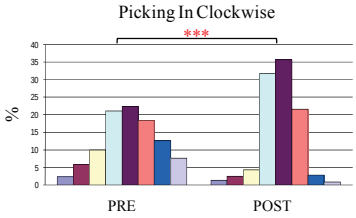


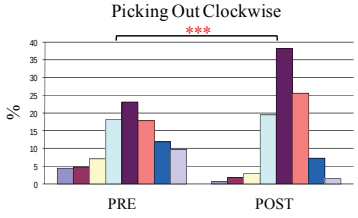
Mk-SL Left Hand Clockwise



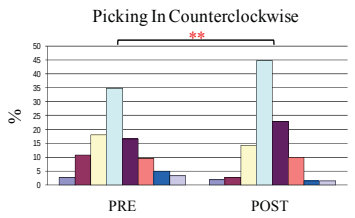


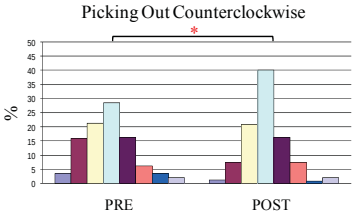
Mk-SL Right Hand Clockwise



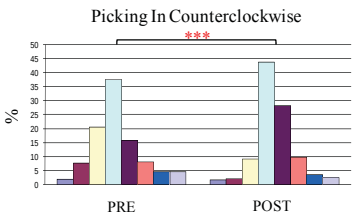


Mk-SL Left Hand Contraclockwise





Mk-SL Right Hand Contraclockwise



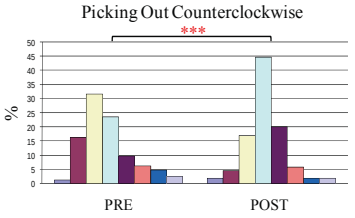


Figure 3.3.13: Comparisons of the distributions of the prehension areas in the rotating Brinkman board task performed for the left and right hands and for each rotation orientation between pre- and post-surgery for the three control and the two biopsied monkeys. "Picking In" and "Picking Out" correspond to the moments at which the monkey entered his finger in the well and took the pellet out of it, respectively. Coloured bars express the mean percentage of prehension areas used, each color representing a given sector, as shown on the coloured circle at the bottom of the figure, where the position of the monkey placed in front of the board is illustrated.

When considering the prehension area (Fig. 3.3.13), it appeared that the monkeys grasped the pellets mainly in the nearest areas and rarely in the areas situated farther, such as areas 1, 2 and 8. Furthermore, differences across monkeys were observed, as well as between the clockwise and the contraclockwise directions. After the surgery, significant changes were observed both in the controls and in the biopsied monkeys.

Post-surgery, in the control monkeys, Mk-JA showed more often delayed entries when the board rotated clockwise with both hands, entering his finger less in the area 3 (and 4 with his right hand) and more in the areas 5-6. On the contrary, Mk-VA had more often earlier exits with his left hand when the board rotated contraclockwise, as he went out from the wells less in the areas 3-4 and more in the areas 5-6. The same was observed in his right hand when the board rotated clockwise, entering less in the areas 3-5 and more in the areas 6-7. Changes occurred also in Mk-SL. With his left hand, when the board rotated clockwise and to pick the pellets out of the wells, less exits occurred in the areas 2-3-4 whereas more exits were performed from the area 5, reflecting thus a reduction of the exit field to the area situated in front of him. When the board rotated contraclockwise, the picking in and out movements appeared earlier on the board, with fewer occurrences in the area 2 (and 3 for the picking in) and more occurrences in the area 4 (and 5 for the picking in). With his right hand, when the board rotated clockwise, a reduction of the prehension field took place, with fewer retrievals in the areas 1-2-3 and 7-8 and more retrievals in the areas 5-6 (and 4 for the picking in). When the board rotated contraclockwise, the picking in and out movements, as for his left hand, appeared earlier on the board, with fewer occurrences in the areas 2-3 (and 8 for the picking in) and more occurrences in the areas 4-5.

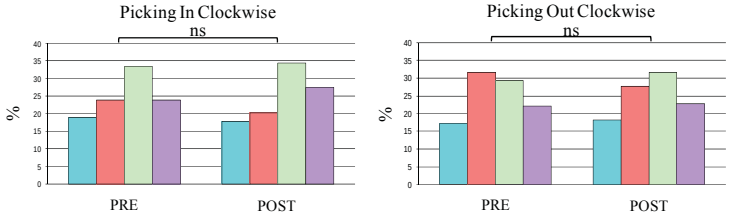
In the biopsied monkeys, after the surgery, some changes were also observed. Indeed, Mk-JO entered his finger earlier on the board with his right hand when the board rotated clockwise, namely less in the area 5 and more in the area 4. When the board rotated contraclockwise, the entries and exits appeared later, with fewer occurrences in the area 6 (and 5 for the picking out) and more occurrences in the area 4 (and 3 for the picking out). For his part, Mk-AV showed changes only when the board rotated contraclockwise. With his left hand, the picking in and out movements occurred later, entering and exiting his finger less in the areas 5-6 and more in the area 4 (and 3 for the picking out). With his right hand, the prehension field was larger, for both enter and exit movements, as there were fewer occurrences in the area 5 and more occurrences in the areas 4 and 6 (and 3 for the picking out).

Thus, some differences were observed between pre- and post-surgery in the area of prehension of the pellets at the rotating Brinkman board task, both in the control and in the biopsied monkeys. Therefore, these changes could not be attributed to the biopsy specifically.

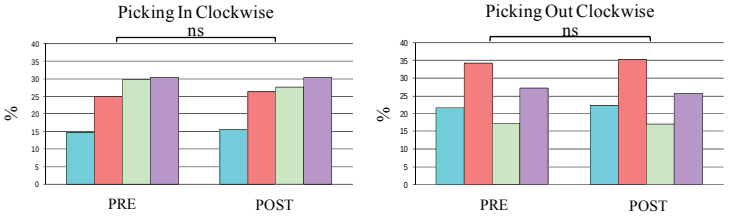
3.3.3.3.2.3 Prehension orientation

Control monkey

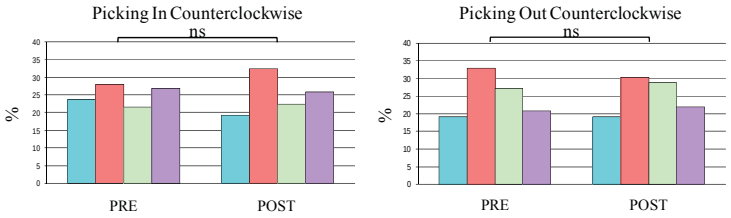
Mk-JA Left Hand Clockwise



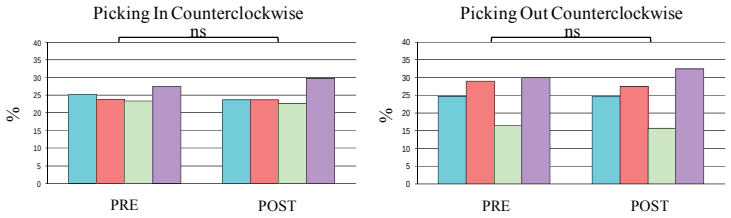
Mk-JA Right Hand Clockwise



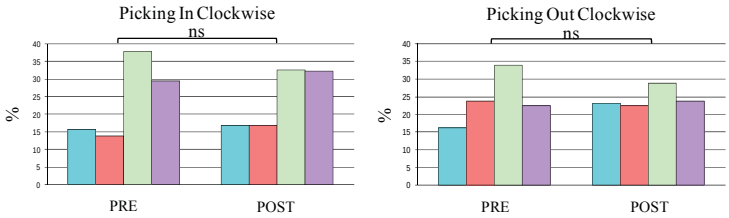
Mk-JA Left Hand Contraclockwise



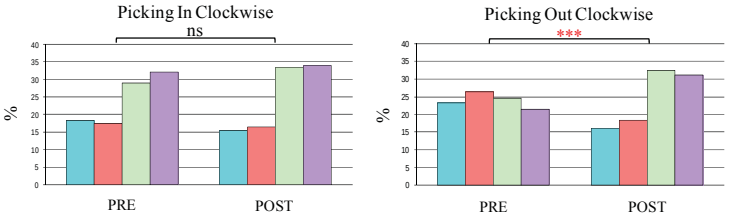
Mk-JA Right Hand Contraclockwise



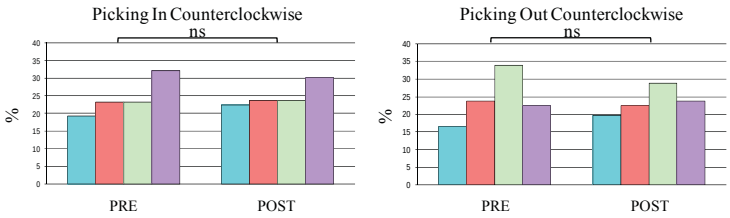
Mk-VA Left Hand Clockwise



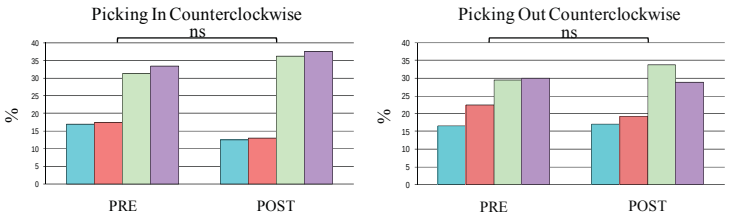
Mk-VA Right Hand Clockwise



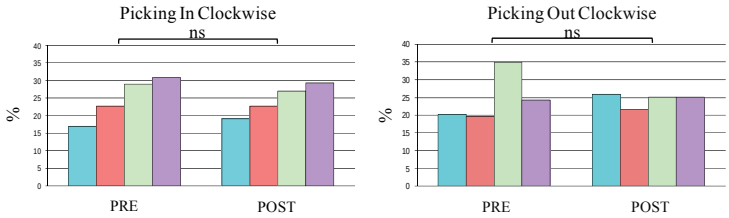
Mk-VA Left hand Contraclockwise



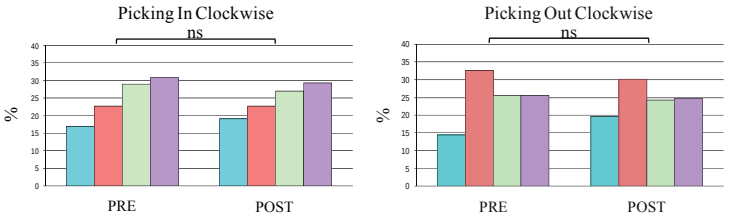
Mk-VA Right Hand Contraclockwise



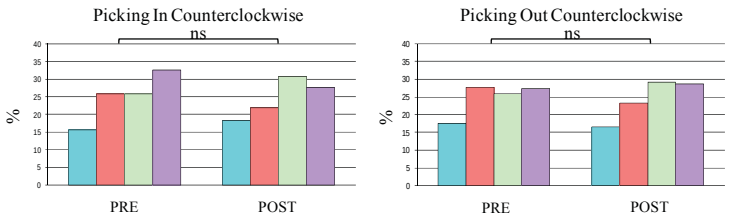
Mk-SL Left Hand Clockwise



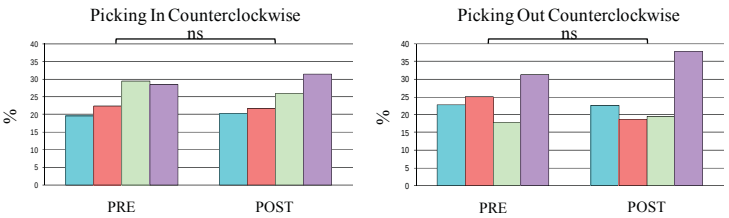
Mk-SL Right Hand Clockwise



Mk-SL Left Hand Contraclockwise



Mk-SL Right Hand Contraclockwise



Biopsied monkeys

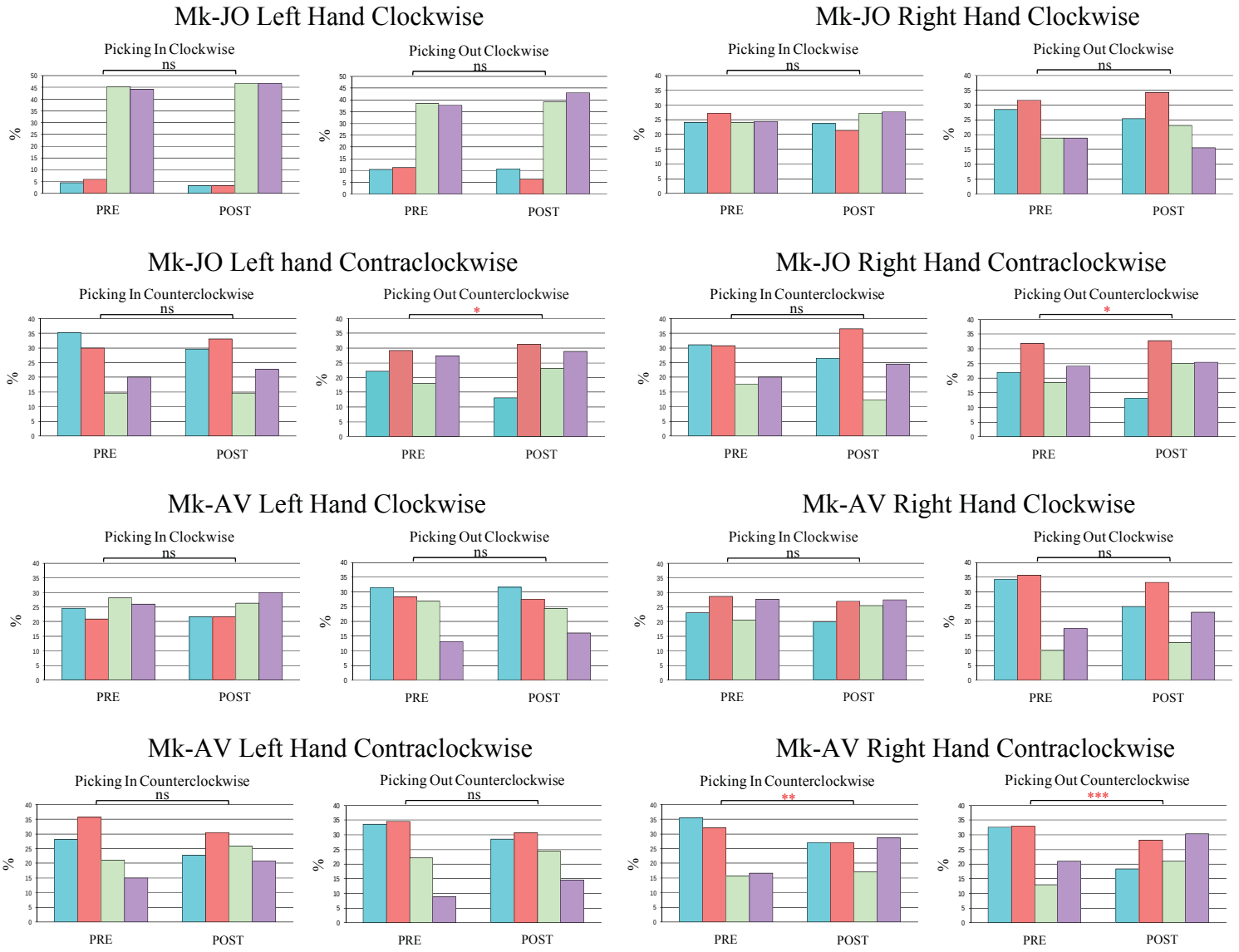


Figure 3.3.14: Comparisons of the distributions of the prehension orientations in the Rotating Brinkman board task performed for the contralesional hand between pre-surgery and post-surgery for all the monkeys. "Picking In" and "Picking Out" correspond to the moments at which the monkey entered his finger in the well and took the pellet out of it, respectively. Coloured bars express the mean percentage of prehension orientations used, each color representing a given orientation, as shown on the four coloured ovals at the center of the figure.

To the previous eight areas of prehension corresponded four different prehension orientations (Fig. 3.3.14). As for the areas, before surgery, there were differences in the orientations across monkeys and between rotation orientations. Some significant changes were observed after the surgery both in one control and in the two biopsied monkeys.

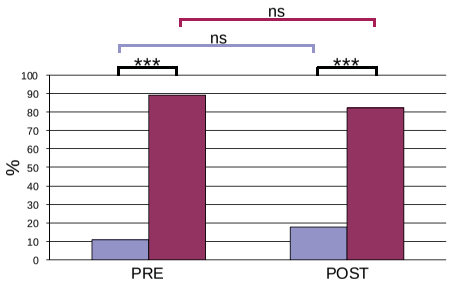
Indeed, in Mk-VA, when the board rotated clockwise and for the picking in the well, the orientations 1 and 2 decreased, whereas the orientations 3 and 4 increased. In the contraclockwise rotation, when exiting from the well, Mk-JO showed a decrease of the orientation 1 and an increase of the orientation 3 mainly, 2 and 4 lightly, both with his left and right hand. Finally, when the board rotated contraclockwise a decrease of the orientations 1 and 2, accompanied by an increase of the orientations 3 and 4 was observed in Mk-AV for the picking in and the picking out movements.

Although the observed changes occurred more and systematically in the contraclockwise rotation in the biopsied monkeys, caution is required to attribute them to the biopsy specifically, as one control monkey showed also a change to pick out the pellets with his right hand when the board rotated clockwise after the surgery. Furthermore, the changes seen in the biopsied monkeys were not massively distributed.

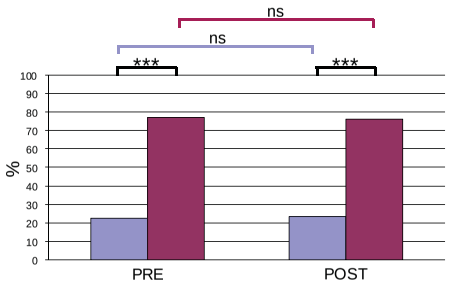
3.3.3.3.2.4 Preferential wrist orientation

Control monkeys

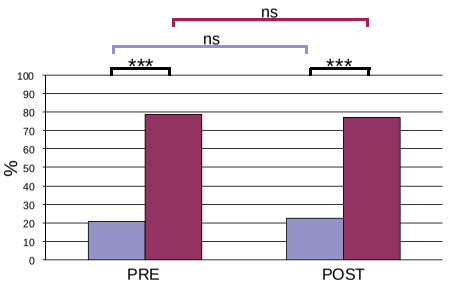
Mk-JA Left Hand Clockwise



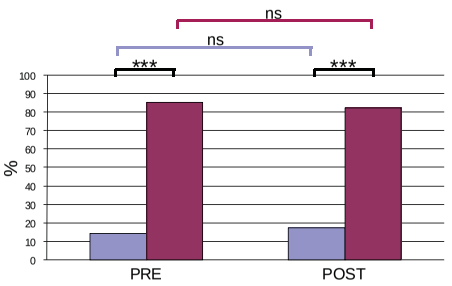
Mk-JA Left Hand Contraclockwise



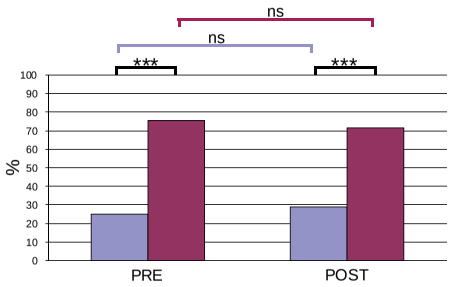
Mk-JA Right Hand Clockwise



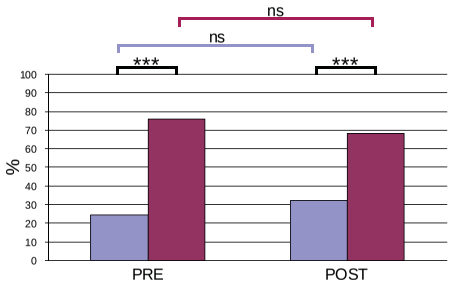
Mk-JA Right Hand Contraclockwise



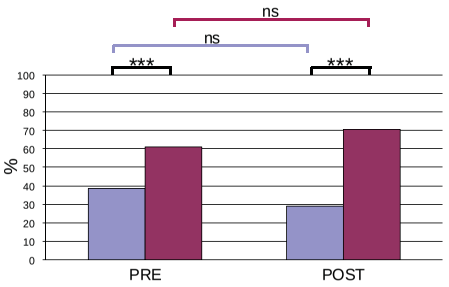
Mk-VA Left Hand Clockwise



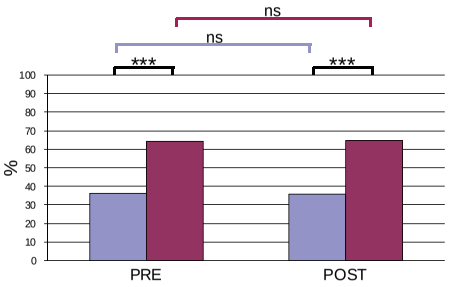
Mk-VA Left Hand Contraclockwise



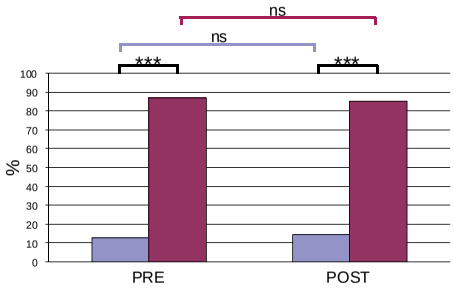
Mk-VA Right Hand Clockwise



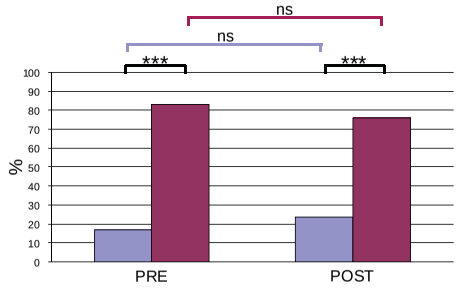
Mk-VA Right Hand Contraclockwise



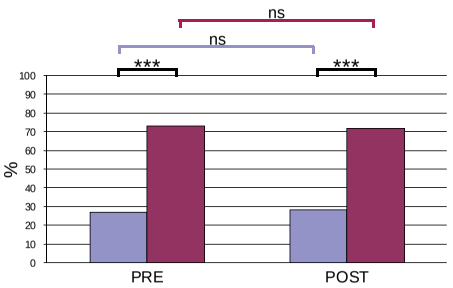
Mk-SL Left Hand Clockwise



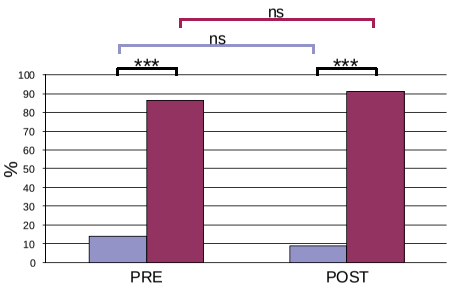
Mk-SL Left Hand Contraclockwise



Mk-SL Right Hand Clockwise



Mk-SL Right Hand Contraclockwise



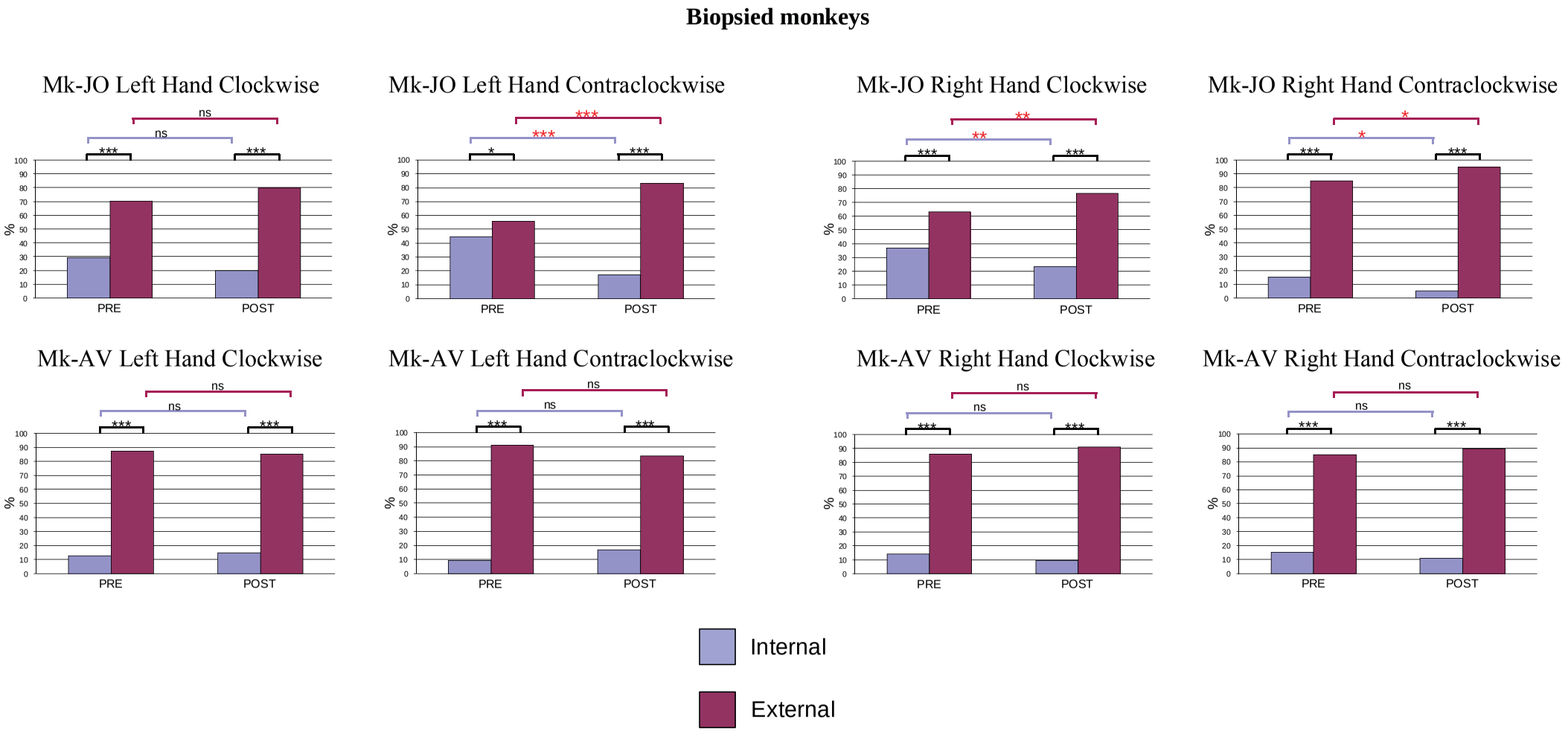
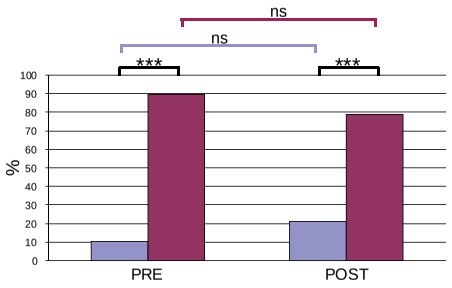


Figure 3.3.15 : Comparisons performed between the pre-surgery and post-surgery sessions on both hands for each monkey (3 controls and 2 biopsied) on the two preferential wrist orientations, namely abduction (blue) or adduction (purple), expressed in mean percentage of use at the rotating Brinkman board task.

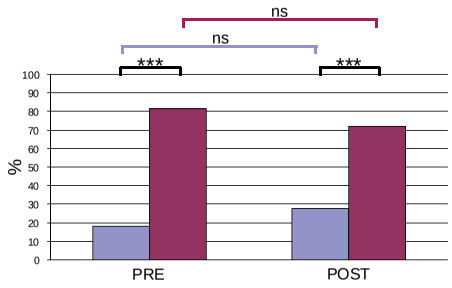
3.3.3.3.2.5 Preferential Finger

Control monkeys

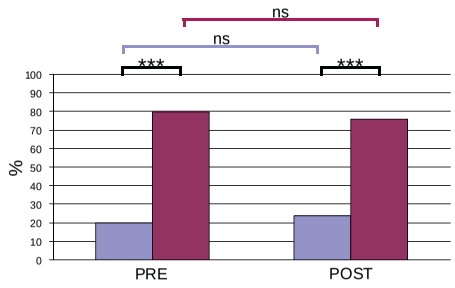
Mk-JA Left Hand Clockwise



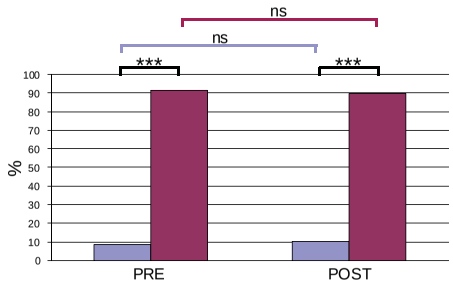
Mk-JA Left Hand Contraclockwise



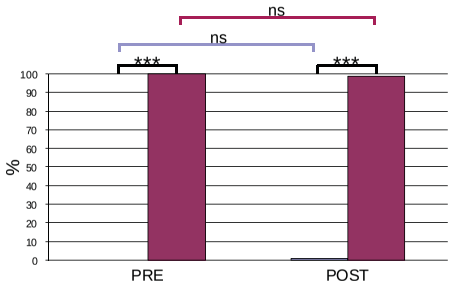
Mk-JA Right Hand Clockwise



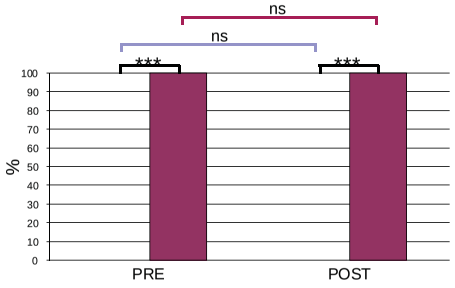
Mk-JA Right Hand Contraclockwise



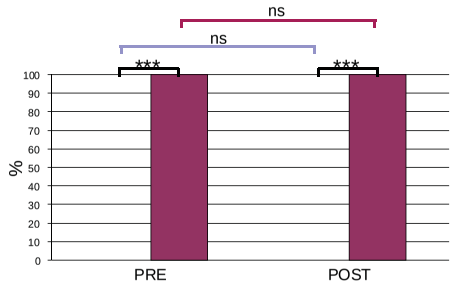
Mk-VA Left Hand Clockwise



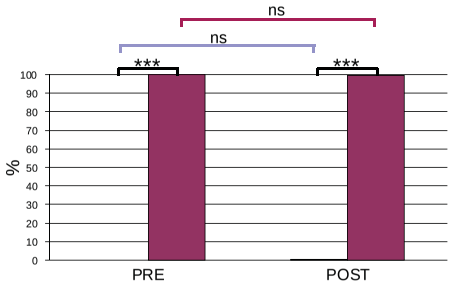
Mk-VA Left Hand Contraclockwise



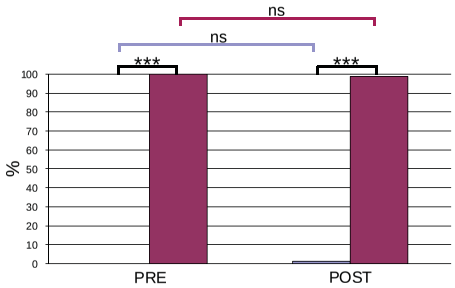
Mk-VA Right Hand Clockwise



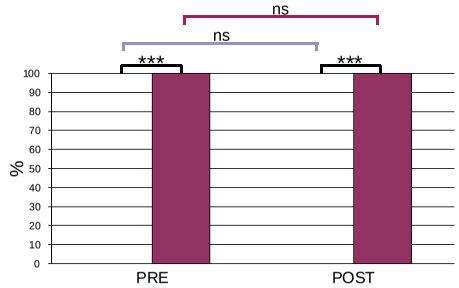
Mk-VA Right Hand Contraclockwise



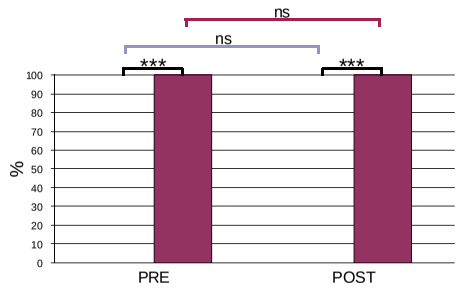
Mk-SL Left Hand Clockwise



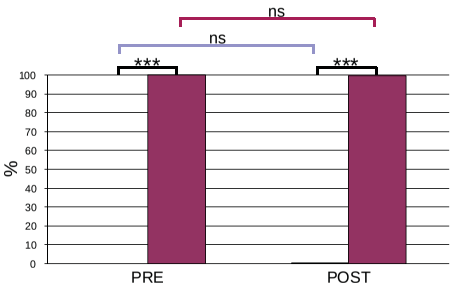
Mk-SL Left Hand Contraclockwise



Mk-SL Right Hand Clockwise



Mk-SL Right Hand Contraclockwise



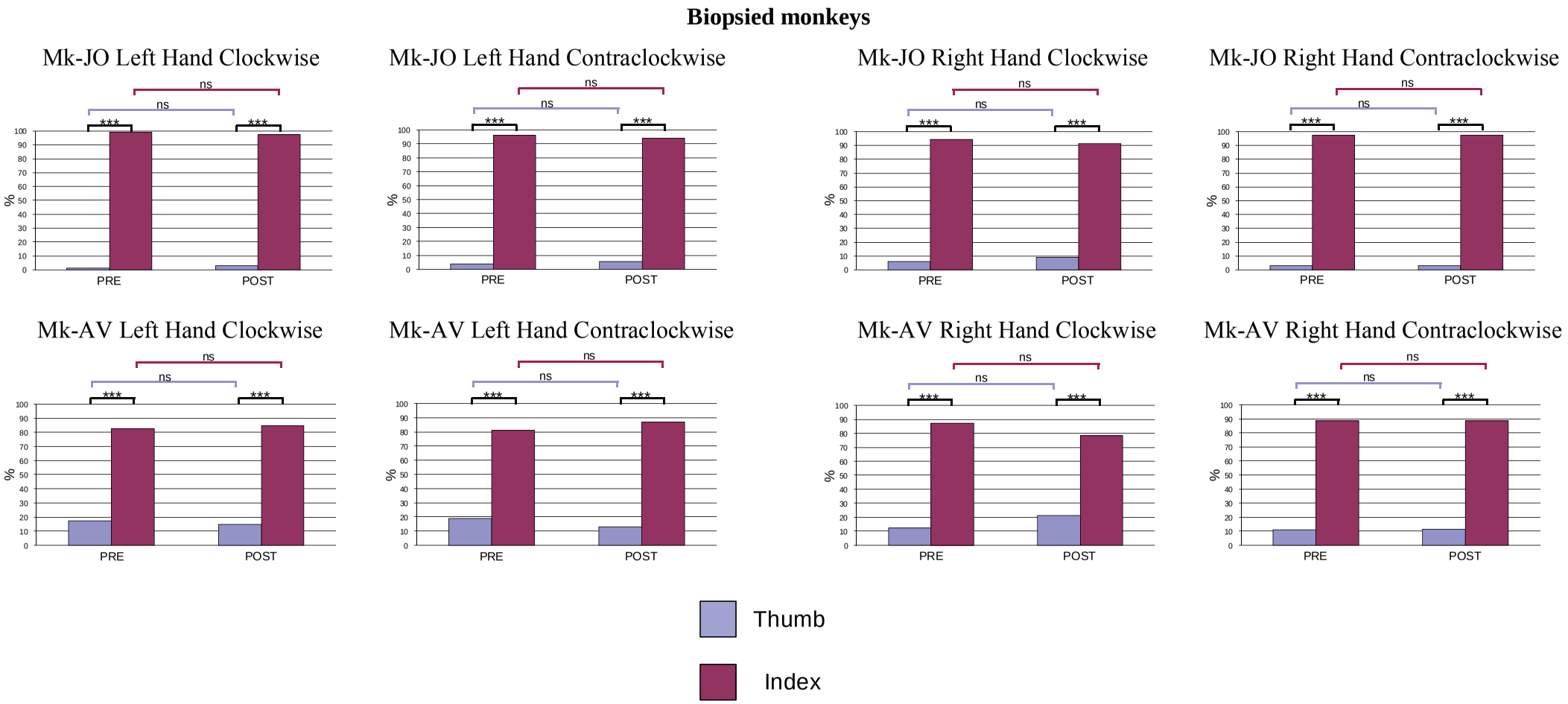
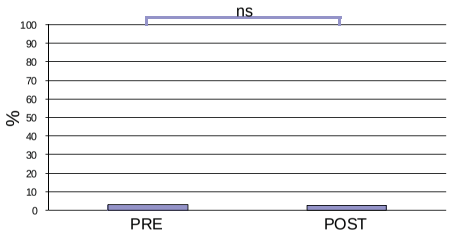


Figure 3.3.16 : Comparisons performed between the pre-surgery and post-surgery sessions on both hands for each monkey (3 controls and 2 biopsied) on the two preferential fingers used to enter in the well, namely thumb (blue) or index (purple), expressed in mean percentage of use at the rotating Brinkman board task.

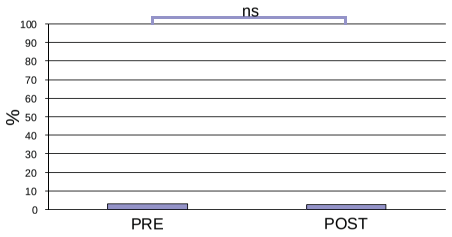
3.3.3.3.2.6 Errors

Control monkeys

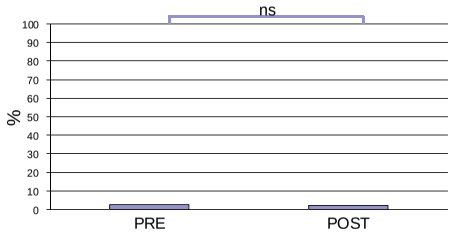
Mk-JA Left Hand Clockwise



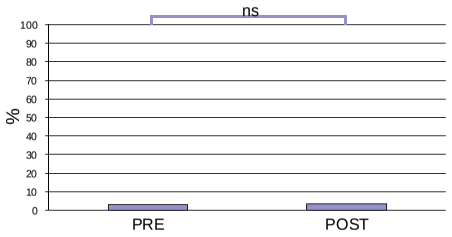
Mk-JA Left Hand Contraclockwise



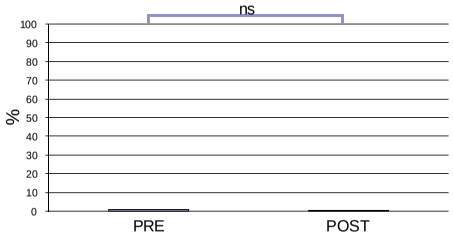
Mk-JA Right Hand Clockwise



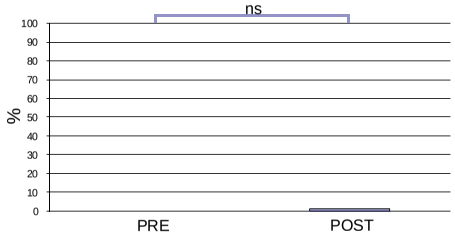
Mk-JA Right Hand Contraclockwise



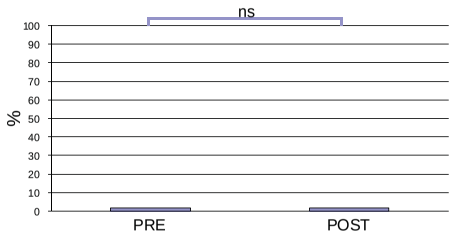
Mk-VA Left Hand Clockwise



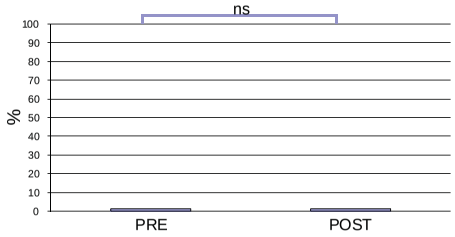
Mk-VA Left Hand Contraclockwise



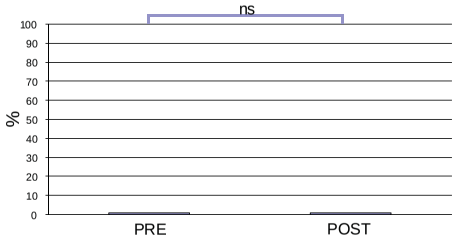
Mk-VA Right Hand Clockwise



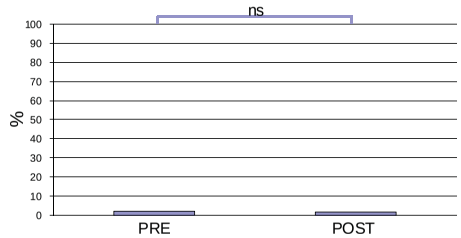
Mk-VA Right Hand Contraclockwise



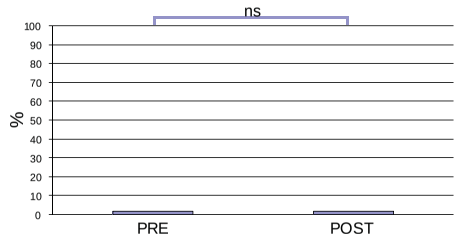
Mk-SL Left Hand Clockwise



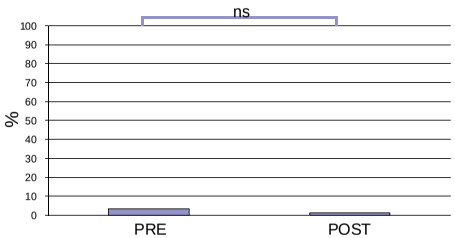
Mk-SL Left Hand Contraclockwise



Mk-SL Right Hand Clockwise



Mk-SL Right Hand Contraclockwise



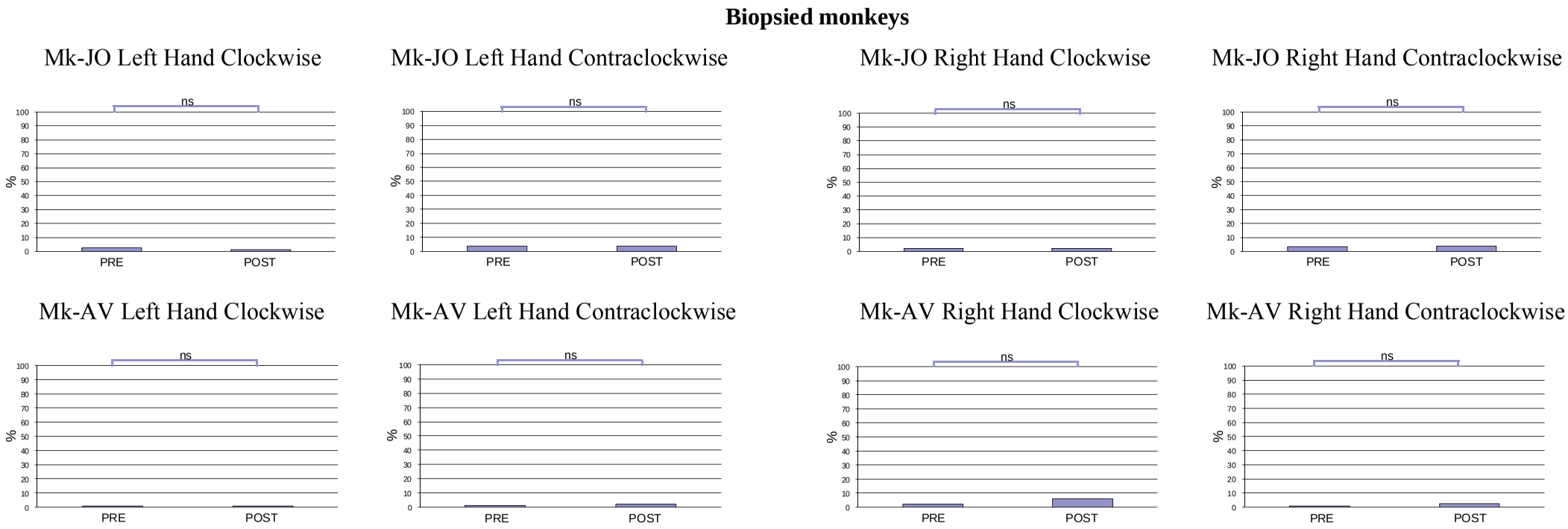
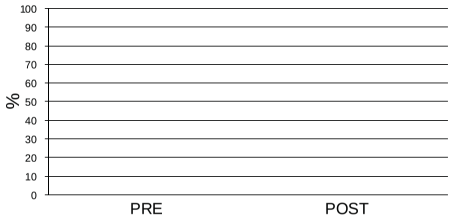


Figure 3.3.17 : Comparisons performed between the pre-surgery and post-surgery errors mean percentages on both hands for each monkey (3 controls and 2 biopsied) at the rotating Brinkman board task.

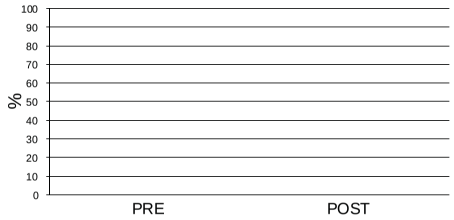
3.3.3.3.2.7 Retrieval of 2 pellets at the same time

Control monkeys

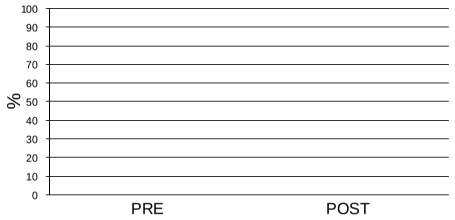
Mk-JA Left Hand Clockwise



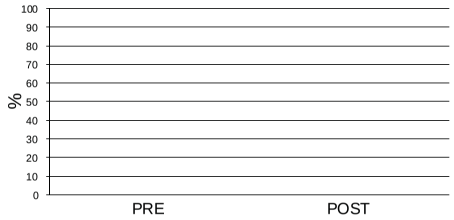
Mk-JA Left Hand Contraclockwise



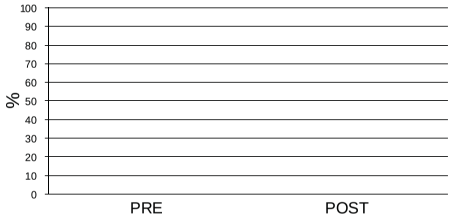
Mk-JA Right Hand Clockwise



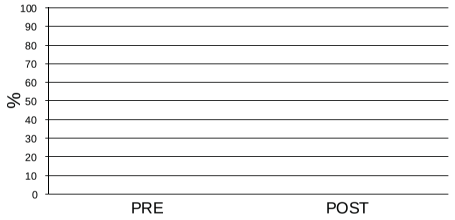
Mk-JA Right Hand Contraclockwise



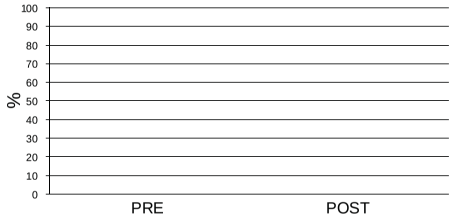
Mk-VA Left Hand Clockwise



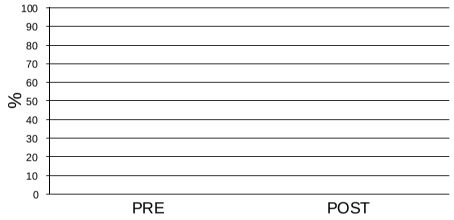
Mk-VA Left Hand Contraclockwise



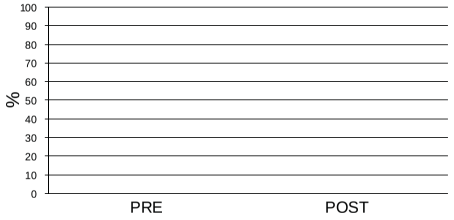
Mk-VA Right Hand Clockwise



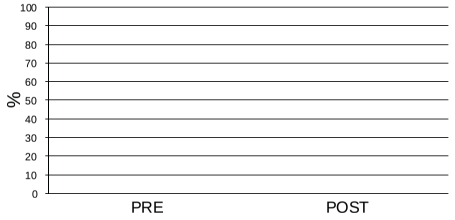
Mk-VA Right Hand Contraclockwise



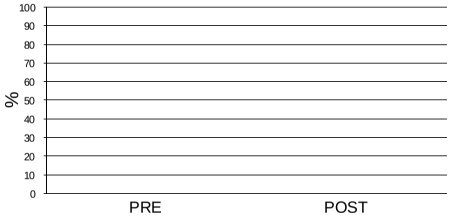
Mk-SL Left Hand Clockwise



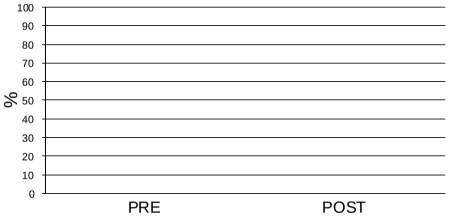
Mk-SL Left Hand Contraclockwise



Mk-SL Right Hand Clockwise



Mk-SL Right Hand Contraclockwise



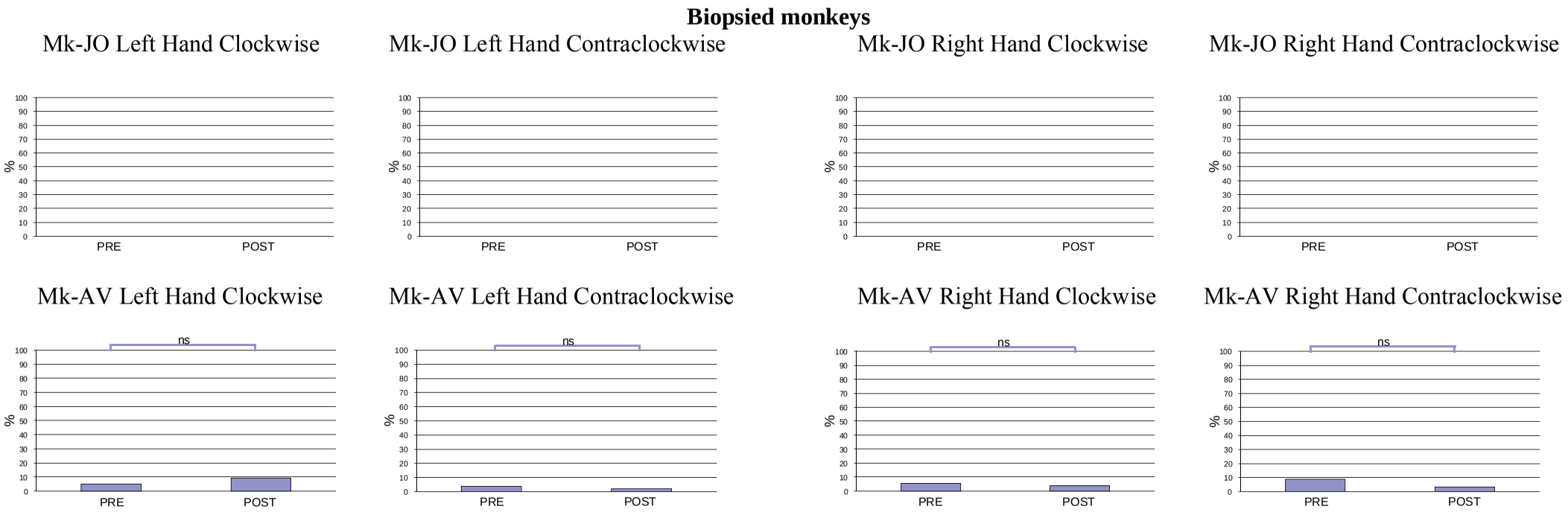


Figure 3.3.18 : Comparisons performed between the pre-surgery and post-surgery mean percentages of retrieval of 2 pellets at the same time on both hands for each monkey (3 controls and 2 biopsied) at the rotating Brinkman board task.

Before and after the surgery, the preferential wrist orientation was the adduction for all the monkeys (Fig. 3.3.15). When comparing the mean percentages of adduction and abduction orientations between the pre- and post-surgery sessions, the only significant changes appeared in Mk-JO, except for his left hand when the board rotated clockwise, toward less use of the abduction of the wrist to grasp the pellets. In the other monkeys, no significant change occurred.

As the observed difference between pre- and post-surgery sessions took place in a biopsied monkey, one cannot reject the possibility that it was a consequence of the dlPFC biopsy, although the absence of such effect in Mk-AV having the larger biopsy reduces this possibility.

All the monkeys showed a preference for the index to enter in the well both before and after the surgery (Fig. 3.3.16). The only monkeys using sometimes the thumb were Mk-JA (control), Mk-JO and Mk-AV (biopsied). For these three monkeys, no significant difference occurred after the surgery. Thus, as for the modified Brinkman board task, this parameter was not very informative for the rotating Brinkman board task.

Again, as for the modified Brinkman board task, the monkeys committed very few errors both before and after the surgery, rendering this parameter little informative (Fig. 3.3.17).

Mk-AV was the unique monkey using the strategy consisting in taking two pellets at the same time before bringing them to the mouth, as in the modified Brinkman board task (Fig. 3.3.18). After the surgery, no significant change was observed on this parameter. Here again, this parameter did not seem to be very informative.

	STRATEGY		CONTROL MONKEYS						BIOPSIED MONKEYS					
			Mk-JA Left Hand	Mk-JA Right Hand	Mk-VA Left Hand	Mk-VA Right Hand	Mk-SL Left Hand	Mk-SL Right Hand	Mk-IO Left Hand	Mk-IO Right Hand	Mk-AV Left Hand	Mk-AV Right Hand
ROTATING BRINKMAN BOARD	Picking sequence on the 4 rings	Clockwise	no	no	no	no	no	no	no	no	no	light
		Contraclockwise	no	no	light	no	no	no	light	no	yes	yes
	Prehension area	Picking In Clockwise	**	*	ns	*	ns	***	ns	*	ns	ns
		Picking Out Clockwise	ns	ns	ns	ns	*	***	ns	ns	ns	ns
		Picking In Contraclockwise	ns	ns	ns	ns	**	***	ns	***	***	*
		Picking Out Contraclockwise	ns	ns	*	ns	*	***	ns	***	**	***
	Prehension orientation	Picking In Clockwise	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		Picking Out Clockwise	ns	ns	ns	***	ns	ns	ns	ns	ns	**
		Picking In Contraclockwise	ns	ns	ns	ns	ns	ns	ns	ns	ns	***
		Picking Out Contraclockwise	ns	ns	ns	ns	ns	ns	*	*	ns	***
	Preferential wrist orientation	Clockwise	ns	ns	ns	ns	ns	ns	ns	**	ns	ns
		Contraclockwise	ns	ns	ns	ns	ns	ns	***	*	ns	ns
	Preferential finger	Clockwise	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		Contraclockwise	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Errors	Clockwise	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		Contraclockwise	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2 pellets	Clockwise	-	-	-	-	-	-	-	-	ns	ns
		Contraclockwise	-	-	-	-	-	-	-	-	ns	ns
STANDARD BRINKMAN BOARD	Picking sequence on the Left-Right axis		no	no	no	no	no	no	yes	2 sessions	yes	yes
	Cumulated distance		no	no	no	no	no	no	***	**	no	*
	Picking sequence on the vertical and horizontal wells		no	no	no	no	no	no	no	no	no	no
	Preferential wrist orientation		ns	ns	ns	ns	ns	ns	ns	ns	*	ns
	Preferential finger		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Errors		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2 pellets		-	-	-	-	-	-	-	-	ns	***

	MOTRICITY		CONTROL MONKEYS						BIOPSIED MONKEYS			
			Mk-JA Left Hand	Mk-JA Right Hand	Mk-VA Left Hand	Mk-VA Right Hand	Mk-SL Left Hand	Mk-SL Right Hand	Mk-IO Left Hand	Mk-IO Right Hand	Mk-AV Left Hand	Mk-AV Right Hand
ROTATING BRINKMAN BOARD	Contact Time	Clockwise	**	**	***	ns	ns	ns	ns	ns	ns	ns
		Contraclockwise	***	**	**	ns	***	ns	ns	ns	ns	***
STANDARD BRINKMAN BOARD	Contact Time	Vertical	*	***	ns	ns	ns	ns	ns	**	ns	***
		Horizontal	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
		Total	ns	**	ns	*	ns	ns	ns	**	*	***
	Score	Vertical	yes transitory	yes transitory	light acute phase	no	light acute phase	light transitory	acute phase	light acute phase	yes transitory	acute phase
		Horizontal	yes transitory	yes transitory	light acute phase	no	no	light transitory	acute phase	light acute phase	yes transitory	acute phase
		Total	yes transitory	yes transitory	light acute phase	no	light acute phase	light transitory	acute phase	light acute phase	yes transitory	acute phase

Table 3.3.1: Summary of the diverses analyzed parameters in the three control and two biopsied monkeys, for their left and right hand at the modified and rotating Brinkman board tasks. “no” is for no observed change; “ns” is for no significant change; “yes” and other explanations are related to the presence of a change; *, ** and *** mean statistical significances of $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

3.3.4 DISCUSSION

First of all, the present results demonstrate clear effects of the surgery with deep anaesthesia itself, indicating that these surgeries are not insignificant and may have effects, although transitory, on the behaviour. Therefore, it seems important to evaluate the monkeys continuously and to wait for a return to a behavioural plateau before going on with the experimental protocol.

Concerning the diverse changes observed (see Table 3.3.1), the differences that appeared after the surgery on the motor control itself were not specific to the dlPFC biopsy. Indeed, changes occurred both in the control and the biopsied monkeys on the two tasks. For the modified Brinkman board task, although two control monkeys (Mk-VA and Mk-SL) did not perform the task during 6-12 days after the surgery, data of the very acute phase thus lacking in these monkeys, a transient decrease of motor performance on the score parameter was found systematically, except in one control monkey (Mk-VA) whose performance with his right hand stayed constant. For the contact time, such a decrease of performance appeared both in control and biopsied monkeys, especially on their right hand, leading to suppose that the left chamber implantation could have played a role in these changes. Again, as immediate data in the two pre-cited monkeys were lacking, one cannot exclude that the quasi-absence of change in these monkeys was imputable to this lack. For the contact time at the rotating Brinkman board task, changes were observed in all the control monkeys on their left hand, with one exception when the board rotated clockwise (Mk-SL), and on the right hand of one of them (Mk-JA), whereas only one change in the biopsied monkeys occurred, namely in Mk-AV with his right hand when the board rotated contraclockwise. The quasi-absence of change in the biopsied monkeys could be explained by the lower temporal resolution of analysis in these monkeys, making the probability to find a difference lower than in the controls. Nevertheless, one can conclude that no specific effect of the dlPFC biopsy occured on this parameter.

On the strategy aspects, various changes that occurred were specific to the right dlPFC biopsy. Indeed, for the modified Brinkman board task, there was a clear change of the picking sequence on the left-right axis of the board for the two hands in the biopsied monkeys uniquely, with an importance more pronounced in Mk-AV, the monkey having had the largest biopsy ($44mm^3$, versus $20.3mm^3$ in Mk-JO). In the same line, the most drastic change that happened in the rotating Brinkman board task concerned Mk-AV for the picking sequence on the four rings, when the board rotated contraclockwise, and for his two hands. Associated to

the changes of picking sequence in the left-right axis of the modified Brinkman board task, the cumulated distance differed between before and after the surgery in the biopsied monkeys. The picking sequence changes led thus to less optimal cumulated distances, except in Mk-AV with his left hand. Indeed, to retrieve the pellets going from one extremitiy of the board to the other, going through the center, is more effective than to begin at the center to end at the extremities. One other parameter that seemed to affect only the biopsied monkeys was the preferential wrist orientation, with significant changes in Mk-JO for the rotating Brinkman board task and in Mk-AV on his left hand for the modified Brinkman board task.

The other observed parameters did not show any clear effect of the dlPFC biopsy, as either no change specific to the biopsy was shown, as seen in the prehension area and the prehension orientation, although a trend toward an effect on the prehension orientation to pick out the pellets when the board rotated contraclockwise could be attributed to the biopsied monkeys uniquely, or even no change at all occurred in any of the monkeys, either control or biopsied, as seen in the preferential finger and in the errors for both tasks, and in the picking sequence on the vertical and horizontal wells for the modified Brinkman board task. Concerning the retrieval of two pellets at the same time, only one monkey (biopsied, Mk-AV) used this strategy; in this monkey, a unique change appeared on this parameter on his right hand at the modified Brinkman board task. These parameters were thus less informative in this context.

Overall, in line with previous studies having demonstrated an implication of the dlPFC in the visuo-spatial working memory (e.g. Courtney et al., 1998; Funahashi et al., 1993; Smith and Jonides, 1999, Wilson et al., 1993), the present results showed changes consequent to the right dlPFC biopsy mainly in parameters implicating a treatment of the either static or rotation-moving visuo-spatial information, in relation to prehension movements and the planning of the optimal picking sequence to perform manual prehesion tasks, implicating thus decision making of which realization depends on dlPFC (e.g. Barraclough et al., 2004; Fletcher et al., 1997; Goel and Dolan, 2000; Goldberg et al., 1994; Heekeren et al., 2006; seo et al., 2007). Furthermore, these results are congruent with the model of Petrides suggesting that dlPFC is involved in higher order executive control functions, as well as with that proposing an hemispheric laterality effect with a greater PFC right activation during spatial tasks (Baker et al., 1996; McCarthy et al., 1996; Reuter-Lorenz et al., 2000), as in the present investigation, the dlPFC biopsy was performed in the right hemisphere.

To note that the here presented observed effects were transitory and of greater importance in the monkey subjected to the largest biopsy. For the continuation of the

experiments conducted in our laboratory in the context of brain cell autotransplantation in a primary motor cortex lesion model, a smaller tissue biopsy will be done in the order of 8-10mm^3, such as already performed in two monkeys with biopsy sizes of 8.8 mm^3 and 10.3mm^3 respectively. Along this line, future clinical trials should take these observations into account and try to minimalize as much as possible the size of the cortical biopsy preleved for autologous cell therapy. Furthermore, in future human patients, attention should be paid on eventual effects that were not evaluated here, such as on psychic state changes. From there, this therapeutical strategy seems very safe, as the observed effects were all transitory.

3.3.5 REFERENCES

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4 GENERAL DISCUSSION AND PERSPECTIVES

The past decade has witnessed important advances in stem cell biology with the validation of adult neurogenesis and the establishment of methods to isolate and propagate stem cell populations suitable for transplantation. In our investigations, a population of adult non-human primates' brain cells was extracted from the prefrontal cortex. This method was demonstrated here to be safe. Furthermore, the dlPFC biopsy had only a transitory effect, which did not affect the motor control itself, but the prehension's strategy aspects implicating a treatment of the visuo-spatial information in relation to prehension movements and the planning of the optimal picking sequence. As these effects were only transitory and of greater amplitude in the monkey having had the larger biopsy (Mk-AV), the present strategy seems safe, particularly if the size of the preleved cortical biopsy is reduced as much as possible. Nevertheless, in future clinical trials on human patients, a psychological assessment would be necessary to confirm the absence of persisting undesirable cognitive effects of the dlPFC biopsy.

The prefrontal cortical biopsied cells' population was isolated, cryopreserved and /or put into culture in a medium without any kind of purification and proliferation or differentiation induction, nor genetic engineering. In such an in vitro context, the cells created aggregates evoking a cell ecosystem mimicking what can be observed in vivo, such as in the stem cell niches, and were constituted by neural progenitor cells (DCX positive) and astrocytes (GFAP positive), of which partnership seems to insure the optimal survival. Furthermore, we showed by incorporation of BrdU that the functioning of these cells does not follow the stem cells classical view of exponential division, but rather divide in an asymetrical way, in which a dividing cell generates a quiescent progenitor cell and a new dividing cell.

In the present study, we demonstrated that once reimplanted in the donor injured brain - here in a motor cortex lesioned adult macaque brain-, these cells, similarly to post-mitotic neuroblasts, were able to migrate towards the lesioned area and to differentiate, expressing MAP2 and SMI-32. At a behavioural level, the cell transplantation led to a significant enhancement of functional recovery, as shown by the presence of a rebound of ~25% of supplemental functional recovery in the treated monkeys, which was absent in the control monkeys. This enhancement of functional recovery was time-locked to the cell transplantation (75-85 days), which took place at different time points with regard to the lesion in the two treated monkeys (15 days and more than 120 days).

The transplanted cells may first have acted by cell replacement, integrating the neural circuitry. Second, they may have delivered factors that could stimulate intrinsic repair from endogenous neural stem cells, as well as neurons, astrocytes and blood vessels. Of course, these two mechanisms are not exclusive. To note here that our approach with non-human primates, showing the same inter-individual variability as stroke patients (due to the size and the position of the lesion, as well as other parameters, such as the motivation, the enrichment of the environment and the social interactions) is in this sense close to clinical situations. Along this line, with the aim to mimic better stroke impairment, other types of lesion were considered, such as electrolytic capsular lesions and cortical lesions using endothelin (which constrict blood vessels). This last option may be used in future studies in our laboratory.

The same strategy of autologous adult brain progenitor cells transplantation in the context of Parkinson's disease in an MPTP monkey model led to a neuroprotective effect (Brunet et al., 2009b) and a significant enhancement of functional recovery as compared to control monkeys (Brunet et al., 2009a). It seems thus that these adult brain cells used as autologous therapy act in repairing a lesioned brain area accordingly to the needs of the environment in which they are reimplanted. They adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions, in the present case in an acute peri-inflammatory or in a post-recovery motor context, and without external interventions inducing specific characteristics. In this sense, the time point of cell transplantation after the lesion should take into account the inflammatory phenomenon, which leads to reactive gliosis and cell proliferation induction, to the detriment of the cells migration and differentiation, such as observed in one monkey (Mk-JO), potentially compromising optimal cell acting and even graft survival (Freed et al. 2001; Olanow et al. 2003). Furthermore, effective but safe cell doses will need to be established in primate models optimized to more accurately mimic the human condition. For example, neurotrophic factors protect cells from death at optimal doses but induce cell death at high doses (Friedman, 2000). In our case, the amount of 250'000 transplanted cells into the lesioned site in one monkey (Mk-JO), and 600'000 transplanted cells (300'000 into and 300'000 near the lesioned site) and 150'000 transplanted cells in the two reimplantation time points, respectively, in the other monkey (Mk-JA) was shown to be safe and efficient, indicating that a certain margin is acceptable. The survival of reimplanted cells reached 26-37%, and importantly, the reimplanted autologous brain cells did not form teratoma or tumor over time. Contrarily to the idea to purify the stem cell, the heterogeneity of neural precursor cells seems to be an important aspect of the promise of such cells. Their variable specialization and differentiation competence provides great promise for

application in clinic. The idea that 'one cell fits all' might turn out to be unnecessary (Sohur et al., 2006). Adult neural precursors with partial fate restriction may, at least in some cases, allow far more efficient production of desired cell types.

Further animal studies, especially on primates, are necessary to bring light on the molecular, vascular, glial, neuronal, behavioral, and environmental events that are important to the spontaneous behavioural recovery that is observed during the weeks after a stroke. The multiple signals that are responsible for endogenous precursor division, migration, differentiation, axon extension, circuit integration and survival will need to be elucidated in order for therapies to be developed efficiently. Circuit plasticity at the level of individual cells, neuronal or glial, adds a new layer of complexity to our understanding of how brain structure and function interact, as well as the complex interplay between neural precursors' potential and signals in their local microenvironment. Similar to the situation in intact CNS, approprate interactions among neurons, glial cells and vascular systems are crucial for CNS repair. Indeed, transplanted cells must interact with an extremely complex and intricate mature CNS environment in order to integrate into the brain. The success of clinical stem/progenitor cell-based strategies will depend on detailed understanding of stem/progenitor cell biology in the degenerating brain and detailed evaluation of their functional efficacy and safety in preclinical animal models. It is important to learn about the normal role of precursor cells and the normal function of neurogenesis in the neurogenic regions of the adult CNS (Sanai et al., 2004), in order to understand brain function in development, normal adulthood and disease states.

To note that enhancing neurogenesis has also been shown not to be beneficial with regard to the pathology of epilepsy (Scharfman, 2004; Scharfman and Hen, 2007). The inappropriate migration, differentiation, and integration of numerous new neurons in the hippocampal DG of animal models for temporal lobe epilepsy are likely to result in severe prolonged seizures (status epilepticus). Thus, elucidating the underlying mechanisms responsible for the migration of neuroblasts, the survival of newly generated neurons, and their functional maturation and subsequent inclusion in neural circuitry, in addition to the mechanisms underlying neurogenesis, is crucial.

For example, the roles of the DCX cells in the plasticity and in the neuropathological context have to be investigated; as shown in the present study, in the control monkeys, DCX positive cells were found to be recruited in the lesioned area, probably to enhance the capacity of plasticity of the lesioned target region. Although we already demonstrated that in vitro, the adult brain progenitor cells used in the present study were able to secrete BDNF, GDNF, LIF

and some VEGF, additional insight from animal experiments is also needed regarding the physiologically functional effects of transplanted NSC/NPC, such as the assessment of the ability of these cells to make synapses and to generate electric activity. Another investigation planned in our laboratory will be to measure the EMG responses of hand muscles following stimulation of M1, as well as of regions known to manage the function of M1 when damaged, such as PM, SMA, S1 or contralateral M1, both before and after an M1 lesion. The contralesional hand muscular responses elicited when stimulating these target regions will be compared between control and treated monkeys, in order to assess the possible influence of transplanted cells on plastic reorganization in areas of the motor cortex following cortical damage.

Another strategy that will be used to investigate in more detail the post-lesion cortical reorganization, with comparison between control and treated monkeys, will be the application of MRI and/or SSEP at regular interval time points on the behavioural recovery and plateau phases.

The present results need to be confirmed on a larger sample of animals, and behavioural analyses should be conducted on the hidden Brinkman board task, manual prehension task performed without visual feedback and allowing to measure somatosensory deficits, the Drawer task, measuring manual grip force, as well as the “Ballistic Arm Movements” (BAM) test, evaluating the monkey’s capacity to make rapid catching movements, and the "hindlimb grasp" test, allowing to assess whether hindlimb motor function was affected by the motor cortex lesion. The acceptance that the experimental protocol takes long time (transitory effect of the biopsy, time needed by the cells to migrate, differentiate and to become functional), which is a pre-requisite to assess the clinical impact of the transplanted cells, is crucial. In addition to the long duration of the process, regenerating long-distance projection neurons may require one or more adjunct therapies to modify local guidance cues. In this sense, further studies should assess the effectiveness of this autologous cell-based therapy applied in combination with other strategies (Rossi and Cattaneo, 2002). For example, helping the endogenous repair processes and the optimal acting of transplanted cells by blocking and inhibiting myelin associated inhibitors, such as Nogo by anti-Nogo-A antibody, will probably lead to a maximal benefit. Indeed, a multifaceted approach to treat human neurological diseases could better improve symptomology and overall quality of life. Further animal and human studies are required to determine the optimal prescription of restorative therapies alone or combined, such as cell-based approaches, small molecules, growth factors, electromagnetic stimulation, a range of devices and robots, and physiotherapy methods, including constraint-

induced movement therapy, in order to maintain an optimal activity and recruitment of neuronal circuits.

As the autologous cell therapy, which does not involve as complex ethical and/or immunosuppression concerns as in heterologous strategies, was demonstrated here to be safe and efficient, there is reasonable hope to successfully translate this approach, perhaps in combination with other therapy (ies) into clinics in a reasonably close future.

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5 SUPPLEMENTAL ANALYSES

The following part was accepted for publication in Journal of Neurophysiology.

5.1 EFFECTS OF UNILATERAL MOTOR CORTEX LESION ON IPSILESIONAL HAND'S REACH AND GRASP PERFORMANCE IN MONKEYS: RELATIONSHIP WITH RECOVERY IN THE CONTRALESIONAL HAND

5.1.1 INTRODUCTION

Following hemi-paralysis, for instance after a unilateral stroke affecting motor control, there is wide variability in the extent of recovery of motor control that depends on several parameters (e.g. the precise location and extent of the lesion, type and rapidity of intervention after the cerebral vascular accident, type of rehabilitative therapy; see e.g. van der Lee et al., 1999; Jorgensen et al., 1999a; Nudo et al., 2001; Zemke et al., 2003; Ward and Cohen, 2004; Masiero and Carraro, 2008; Oujamaa et al., 2009). A cursory evaluation may lead to the prediction that, when there is good functional recovery of the contralesional hand, the patient uses the hand affected by the lesion in a sustained manner. In contrast, when there is poor recovery of the contralesional hand, the patient relies more, if not exclusively, on the ipsilesional hand (unaffected by the lesion) to accomplish most tasks, thus possibly acquiring, through experience, enhanced capabilities in the ipsilesional hand, as compared to the pre-lesion situation or to normal subjects (e.g. Nakayama et al., 1994; Jorgensen et al., 1999b; Liepert et al., 2000a; Cauraugh and Summers, 2005).

From observations of unilateral stroke in human subjects, as well as from unilateral experimental lesion of the motor cortex in monkeys, several mechanisms of cortical re-organization that might underlie recovery have been proposed (e.g. Swayne et al., 2008). For instance, it has been proposed that the ipsilesional premotor cortex (or other territories in the lesioned hemisphere) might contribute to recovery (e.g. Weiler et al., 1993; Seitz et al., 1998; Mima et al., 2001; Carey et al., 2002; Werhahn et al., 2003; Fridman et al., 2004; Luft et al., 2004a for human; e.g. Glees and Cole, 1950; Nudo et al., 1996; Nudo and Milliken, 1996; Liu and Rouiller, 1999; Frost et al., 2003; Plautz et al., 2003; Dancause et al., 2005, 2006; Eisner-Janowicz et al., 2008 in monkeys). Although not mutually exclusive, it is also possible that the intact hemisphere may play a role in the functional recovery of the affected hand, especially in case of large lesion affecting the opposite hemisphere (e.g. Chollet et al., 1991; Netz et al., 1997; Cramer et al., 1997; Seitz et al., 1998; Nelles et al., 1999; Caramia et al., 2000; Johansen-Berg et al., 2002; Feydy et al., 2002; Luft et al., 2004a; Serrien et al., 2004; Takeda et al., 2007; Misawa et al., 2008; Schaechter and Purdue, 2008). The patterns of brain activation associated to hemi-paretic movements are greatly variable, depending on the lesion

location (e.g. cortical versus sub-cortical), the individual degree of recovery, the time interval since lesion and the task demand (see e.g. Luft et al., 2004a; Ward et al., 2007).

The mechanisms that may underlie a contribution of the intact hemisphere to the functional recovery after unilateral lesion of the motor cortex are not well understood (e.g. Netz et al., 1997; Misawa et al., 2008; Swayne et al., 2008), especially with respect to its anatomical substrate (corticospinal projection and/or other, indirect pathways). The role played by the intact hemisphere may depend on the degree of paralysis of the affected hand as a result of the lesion in the opposite hemisphere. When the paralysis is significant, the intact hemisphere is likely to be more engaged in the compensation than in the case of more residual manual performance (Johansen-Berg et al., 2002; Calautti and Baron, 2003; Serrien et al., 2004; Carey et al., 2005). Since the intact hemisphere normally and primarily controls the unaffected hand (referred to below as the ipsilesional hand), depending on the intact hemisphere's contribution to the recovery of the affected hand, the performance of the ipsilesional hand is likely to be influenced. In the case where the intact hemisphere is strongly engaged in the recovery of the affected hand (especially when the paralysis is great), then it may be less available for its "normal" task of controlling the ipsilesional hand, thus resulting in a decrease of motor skill and motor learning ability with the ipsilesional hand.

Following this reasoning, we hypothesize that a permanent unilateral lesion of the motor cortex hand area in monkeys generates, as expected, a loss of manual skills in the contralesional hand, which is then followed by spontaneous, but incomplete, functional recovery. We further hypothesize that, depending on the extent of functional recovery of the contralesional hand, the performance of the ipsilesional hand may also be influenced over the long-term, i.e. over a several months period, considering the slow process of recovery. The aim of the present study was to test the hypothesis that the better the functional recovery of the contralesional hand following unilateral lesion of the motor cortex, the more proficient the ipsilesional hand over the long-term, as observed several months post-lesion. In the present study we assessed the motor performance of the ipsilesional hand not only during the weeks immediately following the lesion but for up to 308 days following the lesion (Table 5.1.1).

	Mk-CE	Mk-JU	Mk-GE	Mk-RO	Mk-VA	Mk-SL	Mk-MO	Mk-AV	Mk-JO	Mk-JA
Treatment	None	None	None	None	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Sham-cell therapy	Cell therapy	Cell therapy
Age at time of lesion (rounded 0.5 year)	4.5	5	5	4	5.5	5.5	5.5	3.5	3.5	4
Weight at time of lesion	3.8	3.6	2.8	3.2	4.9	4.6	5.6	4.3	3.4	4.3
Time window for pre-lesion assessment of manual dexterity (in days)	99	128	70	29	119	77	29	20	29	60
Time window for assessment of long-term deficit of ipsilesional hand post-lesion (in days)	150-287	154-264	78 to 115	48 to 77	153 to 225	99 to 128	70 to 93	100 to 166	140 to 164	260 to 308
Volume of ibotenic acid injected (µL)	40	40	13	18	15.5	18	20	15	15	38*
Nb. of ICMS sites injected with ibotenic acid	21	21	13	12	11	11	20	10	10	38
Total volume of lesion (in mm^3) Gray matter (motor cortex + post-central gyrus)	**112.8**	**63.01**	**48.7**	**14**	**20**	**78.2**	**41.8**	**33.2**	**33.6**	**22.2**
Volume of lesion in post-central gyrus	10.1	0	7.6	0	5.8	1.8	0	0	3.8	2.5
Long-term performance of ipsilesional hand in the modified Brinkman board task "Score" (percentage of pre-lesion score)	**93.9 %**	**92 %**	**100 %**	**124 %**	**119.2 %**	**100 %**	**109.1 %**	**138.5 %**	**105.7 %**	**128.3 %**

* In Mk-JA, nearly the same amount of ibotenic acid was injected as in Mk-CE and Mk-JU. However, in contrast to the other two monkeys, immediately after injection, Mk-JA suffered several epileptic episodes. The monkey was treated with an anti-epileptic drug (Luminal), preventing further episodes. This anti-epileptic drug is known to counteract the excitotoxic effect of ibotenic acid, thus resulting in a smaller lesion volume as compared to the other two monkeys which received comparable volumes of ibotenic acid.

Table 5.1.1: List of monkeys subjected to permanent primary motor cortex lesion and included in the present study with identification code.

5.1.2 METHODS

The present data are derived from 10 adult macaque monkeys (Macaca fascicularis) subjected to a permanent unilateral lesion of the motor cortex. The monkeys were the same as those used in another experiment (see below) and thus, due to specific properties or constraints of the therapeutic protocols applied to some of the monkeys, the time windows during which behavioral assessment took place were not the same across monkeys. In contrast to human studies, our model of experimental motor cortex lesion in the macaque monkey allows us to use each animal as its own control to compare the manual performance before and after the lesion. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by local (Swiss) veterinary authorities.

5.1.2.1 Treatments

As outlined in Table 5.1.1, the 10 monkeys subjected to permanent unilateral lesion of the motor cortex were included in two pilot studies aimed at assessing the possible effect of two treatments: i) anti-Nogo-A antibody treatment; ii) cell therapy with injection of autologous adult progenitor cells, collected from the same animal in the prefrontal cortex (see Brunet et al. 2005). The anti-Nogo-A antibody treatment was tested on monkeys with motor cortex lesions as it was found to significantly enhance functional recovery and sprouting of corticospinal axons after cervical cord injury in macaques (Freund et al. 2006, 2007, 2009). The anti-Nogo-A antibody paradigm was tested on a sub-group of three monkeys (Mk-VA, Mk-SL, Mk-MO) and compared with a subgroup of four monkeys also subjected to a unilateral lesion of the motor cortex but that did not receive any treatment (Mk-CE, Mk-JU, Mk-GE and Mk-RO; see Table 5.1.1). Three additional monkeys (Table 5.1.1) were included in the pilot cell therapy project, two monkeys (Mk-JO and Mk-JA) received an implantation of autologous adult progenitors cells in the vicinity of the cortical lesion, whereas one monkey (Mk-AV) served as sham control animal (infusion of vehicle only). The present study does not address the issue of the efficacy of the treatments; the therapeutic effects of the two treatments on the contralesional hand will be reported elsewhere. The possible impact of the two treatments on the ipsilesional hand is addressed in detail in the discussion section.

5.1.2.2 Behavioral assessment of manual performance

A major consequence of lesion of the hand representation in the motor cortex is the loss of manual dexterity, thus requiring appropriate behavioral tests focused on fine finger movements in order to track the functional recovery (see also Pizzimenti et al., 2007; Murata et al., 2008; Darling et al., 2009 for recent contributions). Monkeys were trained to perform our "modified Brinkman board" test (e.g. Rouiller et al., 1998; Liu and Rouiller, 1999; Schmidlin et al., 2004; Freund et al., 2006, 2009), that requires a reach and grasp motor sequence to retrieve small food pellets from wells while using the precision grip (opposition of the thumb and index finger). Food pellets were made of dried banana powder or glucose powder, compressed in a round shape of about 4 mm in diameter. This test of hand motor capacity was performed on a perspex board (10 cm x 20 cm) containing 50 randomly distributed slots, each filled with a food pellet at the beginning of the test. The dimension of the slots was 15 mm long, 8 mm wide and 6 mm deep. Twenty-five slots were oriented vertically and twenty-five slots horizontally. As outlined in detail in a recent report (Freund et al., 2009), retrieval from horizontal slots was more challenging as it required a postural adaptation of the hand (specifically a forearm rotation) in addition to the precision grip, whereas for the vertical slots, the precision grip can be performed with the hand in its natural posture (in pronation of the forearm). The monkeys were not food deprived: the pellets, which served as positive reinforcement during the tests, were the animals' first access to food in the morning. At the end of the tests, the monkeys received additional food (cereals, fruits). The body weight of the monkeys was checked before each behavioral session. The monkeys had free access to water in the animal room.

Individual testing sessions typically lasted about 60 minutes: they included the time to transfer the monkeys to the primate chair and their transport to and from the animal room to the laboratory, as well as delivery of the additional food at the end of the session. An initial pre-lesion training phase was necessary to bring the monkeys to a stable level of performance that corresponded to a plateau in the reach and grasp score (represented by the red horizontal lines in Fig. 5.1.2A). The pre-lesion plateau, which was achieved within a time frame ranging from 20 to 128 days before the lesion depending on the specific experimental protocol for each monkey (Table 5.1.1), was used to establish the median value of the pre-lesion score (Fig. 5.1.2A). As the goal of the present study was to assess the long-term effect of the unilateral motor cortex lesion on the ipsilesional hand, the post-lesion behavioral data were focused on a time window of several months (Table 5.1.1).

Monkeys performed the reach and grasp task first with one hand and then with the other hand, in 2 to 5 sessions per week during several months before and after the cortical lesion. For each daily session, the entire modified Brinkman board task was performed once with each hand, corresponding to 50 pellets retrieved by the left hand and 50 pellets retrieved by the right hand, in other words 100 pellets in total when the monkey was successful for all slots (this was usually not the case for the contralesional hand following the lesion, due to a considerable deficit of manual performance). The temporal order in which each of the two hands were tested (left hand first or right hand first in a given session) was alternated on each consecutive behavioral session to avoid a possible bias towards one hand or the other. Testing one hand on the modified Brinkman board (retrieval of 50 pellets) took from 1 to 2 minutes. All tests were videotaped. In the present study, two parameters were assessed: 1) The retrieval score defined as the number of pellets successfully retrieved from the slots and brought to the mouth during the first 30 seconds of testing and established separately for the vertical and the horizontal slots; and 2) The contact time, defined as the time of contact (in seconds) between the fingers and the pellet. Specifically, the contact time corresponds to the time interval between the insertion of the first finger (usually the index) into the slot to contact the pellet and the retrieval of the pellet from the slot (grasped in between the index finger and the thumb), as previously reported (Freund et al. 2009). The contact time represents the amount of time it takes to retrieve a pellet from a slot, and thus specifically reflects the manual dexterity. In the present study, the contact time was calculated for the first five vertical slots and the first five horizontal slots targeted by the monkey in an individual session. The manual reach and grasp task as performed on the modified Brinkman board can be seen on the following web page: http://www.unifr.ch/neuro/rouiller/motorcontcadre.htm.

Following the modified Brinkman board task, within the 60 minutes of the behavioral session, the monkeys were also tested with other reach and grasp tasks, such as the rotating Brinkman board task, the hidden Brinkman board task as well as the reach and grasp drawer task (see Freund et al., 2006 and web site above). However, the modified Brinkman board task was the only test performed systematically by all monkeys and on each behavioral session. The inclusion of these other (closely related) tasks in the behavioral sessions did not affect the performance on the modified Brinkman board task considered in the present study. There was no additional rehabilitative training and, importantly, the tests practiced during the behavioral session were always identical for both hands. In other words, there was no attempt to favor practice with the contralesional hand.

5.1.2.3 Surgery

After the monkeys reached a stable pre-lesion performance level (a stable number of pellets retrieved in the first 30 seconds during each session), they were implanted unilaterally (Mk-RO, Mk-SL, Mk-MO, Mk-AV, Mk-JO, Mk-JA) or bilaterally (Mk-CE, Mk-JU, Mk-GE, Mk-VA) with a chronic, stainless steel or tecapeek chamber giving access to the forelimb area in the motor cortex; the dura mater was left in place (see Schmidlin et al., 2004 for detail). Monkeys were sedated with i.m. injection of ketamine (Ketalar, 5 mg/kg) and pre-medicated as previously described, in particular with the analgesic carprofen (Rymadil, 4 mg/kg, s.c.) to reduce pain after surgery (Schmidlin et al., 2005; Wannier et al., 2005; Freund et al., 2006). The surgical intervention itself was conducted under aseptic conditions and profound anesthesia, maintained for several hours by i.v. infusion of propofol (mixture of 1% propofol and 4% glucose in saline, 1 volume of propofol and 2 volumes of glucose delivered at the rate of 0.1 ml/min/kg). Ketamine was added to the perfusion solution, as previously reported (Freund et al., 2007). After surgery, the animals were treated with antibiotics (ampicilin 10%, 30 mg/kg, s.c.) and analgesics (pills of Rymadil mixed with food) for 7-10 days. Chronic chambers were fixed to the skull with titanium screws and orthopedic cement (Palacos). The inside of the chronic chamber was cleaned 2-3 times per week with Betadine and an antibiotic ophthalmic ointment was spread on the dura mater surface to reduce the risk of infection.

5.1.2.4 Electrophysiology: intracortical microstimulation (ICMS)

To guide the lesion procedure, electrophysiological ICMS sessions were first performed to map the primary motor cortex (M1): a tungsten microelectrode (0.1 - 1 MΩ impedance, FHC Inc, USA.) was used to micro-stimulate M1, along penetrations performed at a distance of 1 mm from each others (see e.g. Schmidlin et al., 2004, 2005). Along each electrode track, ICMS was applied below the surface of the dura mater at intervals of 1 mm. When the electrode penetration was located slightly rostral to the central sulcus, ICMS effects were obtained along a distance of up to 10-12 mm, along the rostral bank of the central sulcus forming a band of gray matter perpendicular to the cortical surface (see red dashed line in Fig. 5.1.1B). When the electrode penetration was located more rostral, the cortical layers were oriented parallel to the cortical surface and the distance along which ICMS effects were present was shorter (up to 4-5 mm; see green dashed line in Fig. 5.1.1B). The depth of the ICMS sites were determined with the zero corresponding to the surface of the dura mater, which became progressively thicker with time (it was regularly scratched to facilitate the electrode penetrations every 3-4 weeks). The effect of ICMS was assessed by visual

inspection and/or palpation of the body part (articulation) where a movement was elicited. The minimal current (ICMS threshold) producing this movement was determined at each stimulation site. The repeated ICMS electrode penetrations were performed during several weeks pre-lesion. The ICMS map as seen from the surface was finally represented in the form of an unfolded map of M1 (Supplementary Fig. 5.1.1), as previously reported (Park et al., 2001, 2004) and served as the basis to guide injections of ibotenic acid in order to produce a permanent lesion of the motor cortex, targeting the hand area of M1 (see below).

5.1.2.5 Permanent lesion of M1 hand representation with ibotenic acid

On the day of ibotenic acid injections, selected electrode penetrations were repeated to verify the ICMS effects and the precise depths at which ibotenic acid would be injected. As reported earlier (Schmidlin et al., 2005), there was good reproducibility of the ICMS data derived from electrode penetrations performed several weeks apart. Each site selected for ibotenic acid injection corresponded to a locus where ICMS produced a movement of the digits at low threshold and thus included the hand area of M1. Typically, along a penetration such as that represented by the red dashed line in Figure 5.1.1B, 3 sites were selected for ibotenic acid injection (at 3, 6 and 9 mm deep) whereas, along a more rostral penetration (green dashed line in Fig. 5.1.1B), a single site was selected at the depth of layer V (which exhibited the lowest ICMS threshold).

Ibotenic acid (10µg/µl in phosphate-buffer) was infused using a Hamilton micro-syringe at selected ICMS sites of the hand area in M1 unilaterally, as previously reported in detail (Liu and Rouiller 1999). The number of ICMS sites injected and the total volume of ibotenic acid infused in M1 are indicated for each monkey in Table 5.1.1. The unilateral lesion was performed in the left hemisphere, except in Mk-JU (Fig. 5.1.1A). After a several minutes delay, the ibotenic acid infusion produced a significant paralysis in the contralesional hand.

5.1.2.6 Data analysis

Within the pre- and post-lesion time frame of behavioral analysis defined for each monkey (see Table 5.1.1), the pellet retrieval score was plotted as a function of time in days (e.g. Fig. 5.1.2A). The pre-lesion period was used to establish the reach and grasp performance of reference, indicated by the median value (red horizontal lines in Fig. 5.1.2A). Post-lesion, behavioral sessions were conducted for several months (e.g. Fig. 5.1.2A). To assess the long-term effects of unilateral lesion of the motor cortex on the hand's reach and grasp capacity, the long-term score was also represented by its median value (green horizontal

lines in Fig. 5.1.2A). Finally, the comparison of the two median values (pre-lesion versus long-term post-lesion) allowed a quantitative assessment of the effect of the lesion several months thereafter. The behavioural data were analyzed statistically using an unpaired non-parametric Mann-Whitney test. A similar analysis was conducted on the second behavioural parameter, the contact time. The pre-lesion data used to address the issue of hand dominance (see section 6 in the results) were analyzed using a paired comparison of daily scores obtained for the left and the right hand (paired t-test or Wilcoxon test). The statistical analysis and the related graphs were obtained using the software SigmaStat 3.5 and SigmaPlot 10.0.

At the end of the experiments, the animals were sacrificed with an overdose of pentobarbital sodium (90 mg/kg body weight, i.p.). Transcardiac perfusion with 0.9% saline (500 ml) was followed by fixative (4000 ml of 4% phosphate-buffered paraformaldehyde). The brains were placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection for 3-5 days. Frontal sections (50 μm thick) of the brain were prepared and collected in five series. One series of sections was Nissl stained with cresyl violet whereas a second series was processed to visualize the marker SMI-32, as previously described (Liu et al., 2002; Wannier et al., 2005; Beaud et al., 2008). The epitope recognized by the SMI-32 antibody lies on non-phosphorylated regions of neurofilament protein and is only expressed by specific categories of neurons (Campbell and Morrison, 1989; Tsang et al., 2006). The two series of sections were then used to reconstruct on consecutive sections the position and extent of the permanent lesion in the cerebral cortex, especially using the SMI-32 stained sections (Fig. 5.1.1B), on which the pyramidal neurons in layers III and V are clearly visible. Finally, the lesion was transposed onto a lateral view of the cortical surface of the lesioned hemisphere (Fig. 5.1.1A). Using an ad-hoc function of the Neurolucida software (based on the Cavalieri method; see e.g. Pizzimenti et al., 2007), the volume of the cortical lesion (in mm^3) affecting the cortical gray matter was extrapolated from the reconstructions of the lesion on consecutive histological sections of the brain (see Table 5.1.1).

5.1.3 RESULTS

5.1.3.1 Unilateral lesion of the motor cortex

The unilateral lesion of the motor cortex was produced by infusion of ibotenic acid at multiple sites defined by intracortical microstimulation (ICMS; see Supplementary Fig. 5.1.1). Most ICMS sites selected for infusion of ibotenic acid were located in the rostral bank of the central sulcus, where most of the hand is represented in the primary motor cortex (supplementary Fig.5.1.1; Fig. 5.1.1B middle and right sections). As ibotenic acid was also

injected at a few sites more rostrally, the lesion also extended onto the part of the motor cortex at the brain surface (left section in Fig. 5.1.1B). The infusion of ibotenic acid at multiple sites did not produce a uniform lesion, but rather several distinct zones, the areas of which were added in order to compute the total volume of the lesion in the gray matter (Table 5.1.1). The total volume of the lesion is used to correlate with the behavioral data in order to assess the effect of the lesion size.

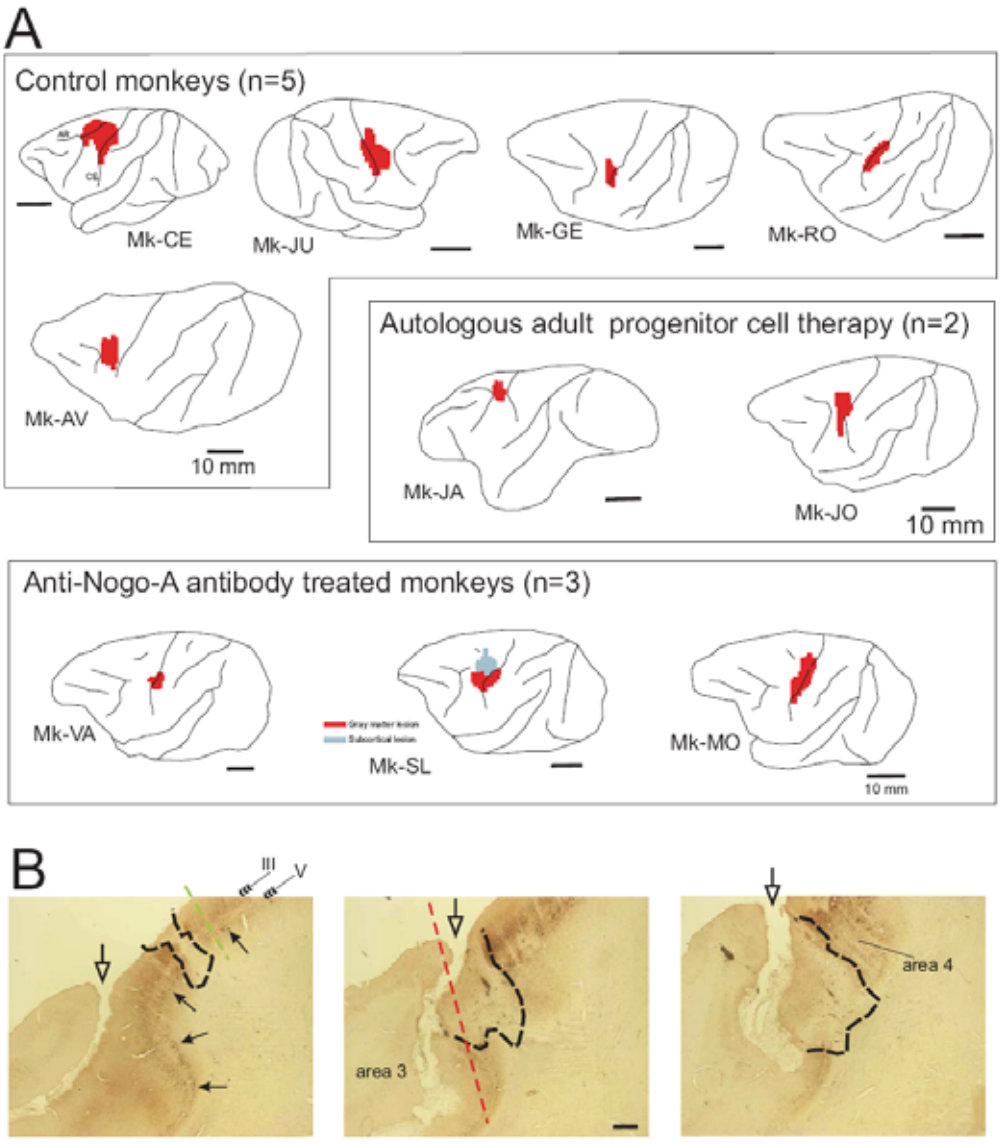


Figure 5.1.1:
A: Location and extent of the permanent unilateral lesion of the hand representation in the motor cortex, as seen on corresponding lateral views of the brain for the 10 monkeys included in the present study (see Table 5.1.1). The lesion area, represented in red, was determined from the lesioned zone of cerebral cortex visible on consecutive frontal histological sections. The red area corresponds to a lesion affecting the gray matter. Spread of the lesion to the subcortical white matter below the gray matter is not represented, except in monkey Mk-SL in which a region of subcortical white matter was lesioned (gray spot), in a zone located medial to the red territory. The motor cortex lesion was performed in the left hemisphere for all monkeys, except in Mk-JU in which the lesion was in the right hemisphere. Of the ten monkeys, five (top panel) were control animals for two pilot treatment studies, three were treated with anti-Nogo-A antibody (bottom panel), and two were subjected to an autologous adult progenitor cell therapy (see methods and Table 5.1.1).

B: SMI-32 stained frontal sections of the left hemisphere of Mk-VA (the most rostral section is on the left). The interval between the left and the middle section is 1 mm, and 0.5 mm between the middle and the right section. The open arrows point to the central sulcus, separating area 3 on the left from area 4 on the right. In area 4, SMI-32 stains the pyramidal cells in layers III and V (triple headed arrows on the left section). The four black arrows point to typical large pyramidal cells in layer V. The area of the lesion in the gray matter is delineated on each section by the dashed line. The red dashed line represents schematically the trajectory of a fictive ICMS electrode penetration, showing that some sites in layer V with low ICMS thresholds are indeed located deep, near the fundus of the rostral bank of the central sulcus. The green dashed line represents schematically the trajectory of a fictive electrode penetration in M1, located more rostral to the central sulcus. Scale bar=1 mm.

The aim of our motor cortex lesions was to permanently inactivate the M1 hand area. The lesion extent was variable from one monkey to another (red areas in Fig. 5.1.1A), corresponding in most animals to an extent of 4-5 mm on surface views of the brain (Fig. 5.1.1A). The lesion area is consistent with the known size of the hand area in macaque monkeys. However, in a few monkeys, along one dimension or another, the lesion extended further, with the largest lesions extending up to 10 mm. In one monkey (Mk-SL), the lesion spread medially to the sub-cortical white matter (Fig. 5.1.1A). There was also limited, but lesser, sub-cortical damage of the white matter in some of the other monkeys (Table 5.1.1), and the damage remained below the gray matter injury (and is therefore not apparent on the brain surface views in Fig. 5.1.1A). In some monkeys, the lesion spread to adjacent areas, including the premotor cortex and/or the somatosensory cortex (S1), as indicated in Table 5.1.1 for the latter area. The impact of the lesion spread in premotor cortex and/or S1 is considered in the discussion section.

5.1.3.2 Modified Brinkman board task: long-term pellet retrieval data for the ipsilesional hand

The number of pellets retrieved by the monkey in 30 seconds from the modified Brinkman board is shown in detail for three representative monkeys (Fig. 5.1.2A: Mk-JU, Mk-MO and Mk-JA), separately for the vertical and the horizontal slots, as well as a total score representing the sum of both slot orientations.

First, focusing on the total number of pellets retrieved, Mk-JU achieved a stable (plateau) pre-lesion retrieval score about 130 days before the lesion. This monkey's median pre-lesion score for the ipsilesional hand was 25 pellets and for the contralesional hand 23 pellets. This monkey's behavioural assessment continued for 264 days after the lesion. A long-term (154 to 264 days after the lesion) post-lesion median score of 23 pellets was obtained for the ipsilesional hand, which represents a manual performance of 92% of the pre-lesion score. This pre- and post-lesion difference for the ipsilesional hand in Mk-JU was not statistically significant (Fig. 5.1.2B). Note however that the contralesional hand of Mk-JU

recovered only incompletely, as the post-lesion retrieval score (median value=9 pellets) represented only 39% of the pre-lesion score, a pre- versus post-lesion difference that was highly significant ($p<0.001$; Fig. 5.1.2B).

Second, in Mk-MO, the median pre-lesion retrieval score was 33 pellets for the ipsilesional hand and 34 pellets for the contralesional hand. Behavioural sessions ended 95 days after the lesion. The long-term post-lesion retrieval score (last 23 days on the plots in Fig. 5.1.2A) showed an enhanced score for the ipsilesional hand (median value of 36 pellets) as compared to the pre-lesion value (thus representing 109% of the pre-lesion score). This pre- versus post-lesion difference for the ipsilesional hand in Mk-MO was statistically significant ($p=0.006$; Fig. 5.1.2B). For the contralesional hand of Mk-MO, recovery was again incomplete with a median post-lesion retrieval score of 26 pellets, representing 76% of the pre-lesion score (the pre- versus post-lesion difference was statistically significant for the contralesional hand as well, but in the other direction: $p<0.001$; Fig. 5.1.2B).

Third, Mk-JA reached a plateau in performance 60 days pre-lesion, with a median retrieval value score of 32 pellets for the ipsilesional hand and 28 pellets for the contralesional hand (Fig. 5.1.2A). Behavioural data was acquired for 290 days post-lesion. For Mk-JA, the long-term ipsilesional hand performance was dramatically enhanced, 128% that of pre-lesion performance, reaching a median value of 41 pellets ($p<0.001$; Fig. 5.1.2B). For this monkey (Mk-JA), the contralesional hand recovered completely from the lesion, achieving a post-lesion retrieval score of 28 pellets, the same score as pre-lesion (thus representing 100% of recovery; Fig. 5.1.2B).

The data shown in Figure 5.1.2 for three representative monkeys suggest that, when the contralesional hand recovered well from the lesion (e.g. Mk-JA), the long-term post-lesion performance in the ipsilesional hand was enhanced post-lesion on the long-term. In contrast (Mk-JU), in the case of poor recovery of the contralesional hand, the manual performance in the ipsilalesional hand is not affected, maintaining a level of performance that is close to or slightly worse than the pre-lesion performance. In between these two extreme cases (Mk-JU and Mk-JA), in the monkey with an intermediate recovery of the contralesional hand (Mk-MO), the ipsilesional hand also exhibited enhanced long-term post-lesion performance, but to a somewhat lesser degree than in Mk-JA, although the pre- versus post-lesion difference was nevertheless statistically significant. As shown in Figure 5.1.2A, the above observations for the total retrieval scores also hold true when considering either the vertical slots or the horizontal slots separately.

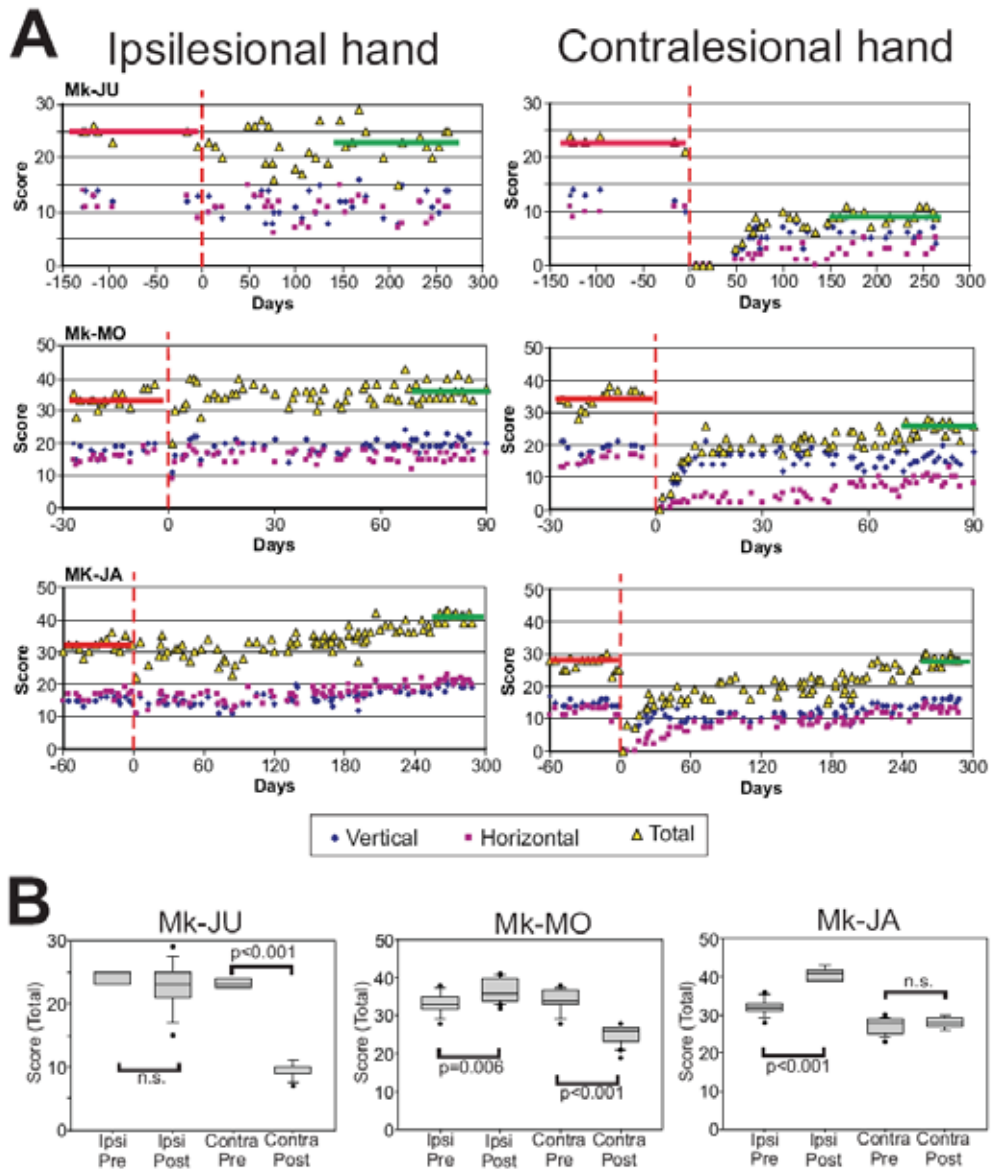


Figure 5.1.2 :

A: Number of pellets retrieved in 30 seconds as a function of time (days) in the modified Brinkman board task for three monkeys (Mk-JU top; Mk- MO middle and Mk-JA bottom). The numbers of retrieved pellets (scores) are indicated separately for the vertical wells (blue diamonds) and the horizontal wells (red squares); the yellow triangles represent the total score determined by summing the vertical and horizontal scores. The vertical red dashed line at time zero is the day of the unilateral motor cortex lesion (pre-lesion days are negative and post-lesion days are positive). Pre-lesion, the median total score is given by the red horizontal line (plateau). Post-lesion, the median long-term total score is given by the green horizontal line (plateau). Pre-lesion, sessions took place earlier than the first day indicated on the abscissa, but the animals' performance had not yet reach a stable plateau. As Mk-JU was generally slower than the other two monkeys (less pellets collected in 30 seconds), note that the maximal value of the ordinate for Mk-JU was 30 (instead of 50), in order to preserve resolution of the individual data points.

B: For the same three monkeys as in panel A, the total score is represented in the form of box and whisker plots for the ipsilesional hand (two left boxes) and the contralesional hand (two right boxes) allowing comparison of the pre- and post-lesion performance for each hand. In the box and whisker plot, the boundary of the box closest to zero corresponds to the 25th percentile, the line within the box is for the median value, and the boundary of the box farthest from zero is for the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Black dots are for outlying points. The result of the statistical comparison pre- versus post-lesion (Mann-Whitney test) is indicated with the corresponding p value (n.s. = not statistically significant at $p>0.05$).

The general trend for the three monkeys shown in Figure 5.1.2 was found to be true when considering the other seven monkeys included in the present study (Fig. 5.1.3). Four out of the seven monkeys in Figure 5.1.3 also showed a long-term enhancement of manual performance (total score) in the ipsilesional hand (Mk-VA, Mk-RO, Mk-JO, Mk-AV), as evidenced by a better post-lesion than pre-lesion retrieval score. In the other three monkeys (Mk-SL, Mk-GE, Mk-CE), the long-term performance in the ipsilesional hand remained at the same level of performance as pre-lesion (Fig. 5.1.3). Note that the latter three monkeys exhibited relatively incomplete contralesional recovery of their manual dexterity post-lesion, as compared to the relatively better recovery of the contralesional hand in the other four monkeys (Fig. 5.1.3).

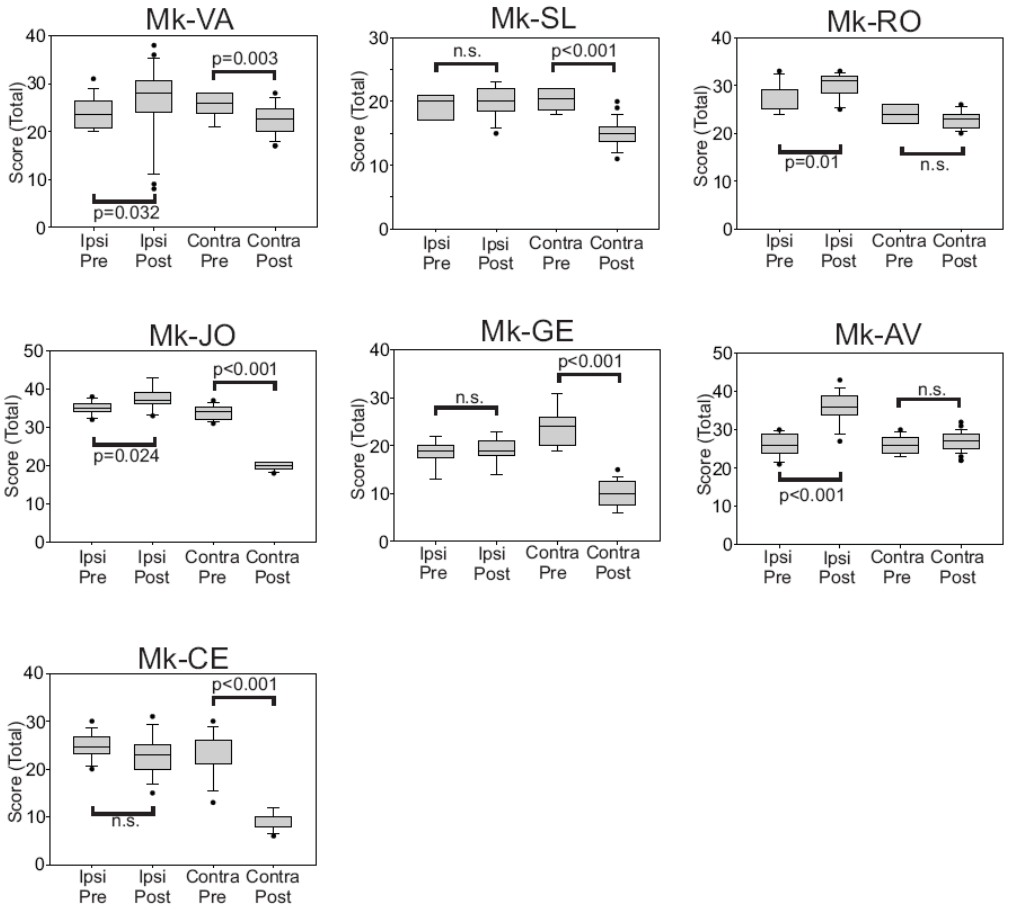


Figure 5.1.3 : Box and whisker plots (as in Figure 5.1.2B) for the other seven monkeys included in the study showing the total score in the ipsilesional hand (two left boxes) and in the contralesional hand (two right boxes) for comparison of the pre- and post-lesion score for each hand. As in Figure 5.1.2, note that the maximal value of the ordinate is not the same for all monkeys, to provide maximal resolution for the comparison of the median pre- and post-lesion values for each hand. The result of the statistical comparison (Mann-Whitney test) is indicated with the corresponding p value (n.s. = not statistically significant with $p>0.05$).

Overall (Figs. 5.1.2 and 5.1.3), long-term post-lesion enhancement of reach and grasp performance in the ipsilesional hand was found in six out of ten monkeys, as assessed by the

total retrieval score in the modified Brinkman board task. In these six monkeys, this enhancement was associated with relatively good recovery of the contralesional hand. To better analyze the dependency between the two hands, the long-term post-lesion manual performance in the ipsilesional hand (expressed in % of pre-lesion score) was plotted as a function of the percent of recovery of the contralesional hand (Fig. 5.1.4). There is a strong correlation between these two parameters, with a coefficient of correlation r=0.932 ($p<0.001$), consistent with the notion that, after unilateral lesion of the motor cortex, a good recovery with the contralesional hand is associated over the long-term with an enhancement of manual performance in the ipsilesional hand.

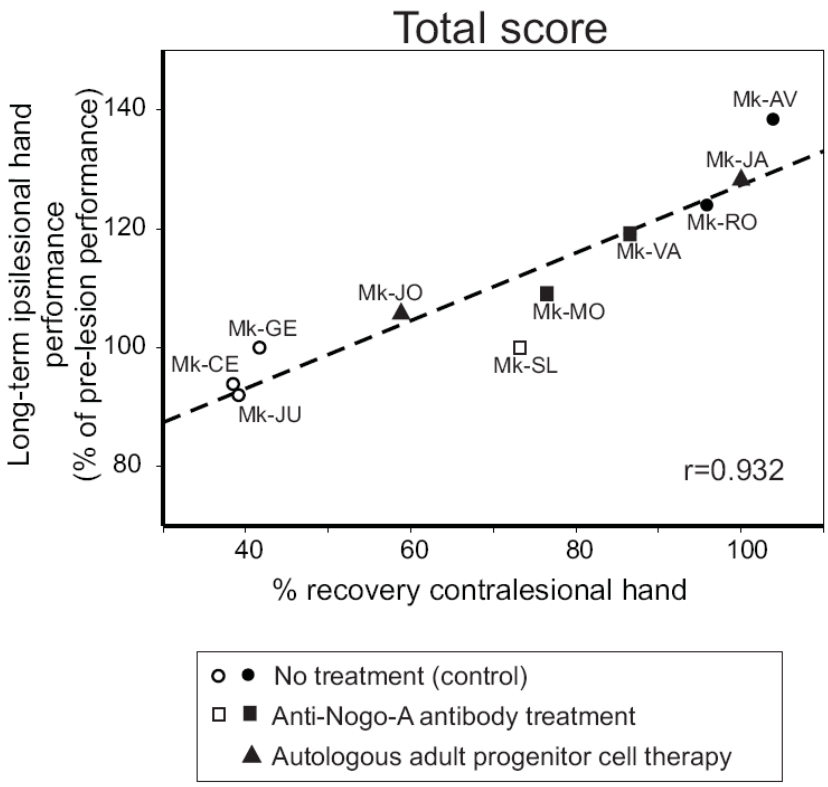


Figure 5.1.4 : Long-term ipsilesional hand performance (expressed in % of the pre-lesion total score in the modified Brinkman board task) was plotted as a function of the percentage of recovery in the contralesional hand (as compared to the pre-lesion total retrieval performance). The dashed line is the regression line representing a significant correlation between these two parameters (r=0.932). The different symbols distinguish the three subgroups of monkeys. Filled symbols are for the six monkeys exhibiting significant long-term enhancement of ipsilesional hand performance (see Figs. 5.1.2B and 5.1.3), whereas there was no enhancement in the four monkeys represented by open symbols.

The above data are based on an analysis of manual performance as assessed by the total retrieval score (sum of vertical and horizontal slots) in the modified Brinkman board task. As the synergy of movements is somewhat different for the vertical and horizontal slots (Freund et al. 2009), it is of interest to analyze the same data considering the vertical and horizontal slots separately (Figs. 5.1.5-6; Supplementary Figs. 5.1.2-3). For the three representative

monkeys (Mk-JU, Mk-MO and Mk-JA depicted in Fig. 5.1.5), the separate data for vertical and horizontal slots are consistent with the total retrieval score data for two monkeys (Mk-JU and MK-JA; Fig. 5.1.2B). For Mk-MO, the vertical slot data lead to the same conclusion as the total score data. Interestingly, contralesional hand performance for Mk-MO was poor when retrieving pellets from the horizontal slots, and this was associated with insignificant long-term enhancement of manual performance in the ipsilesional hand (Fig. 5.1.5). In other words, in Mk-MO considering the vertical and horizontal slots separately, the data are consistent with the notion of enhancement of ipsilesional performance only if recovery of the contralesional hand is complete or at least substantial.

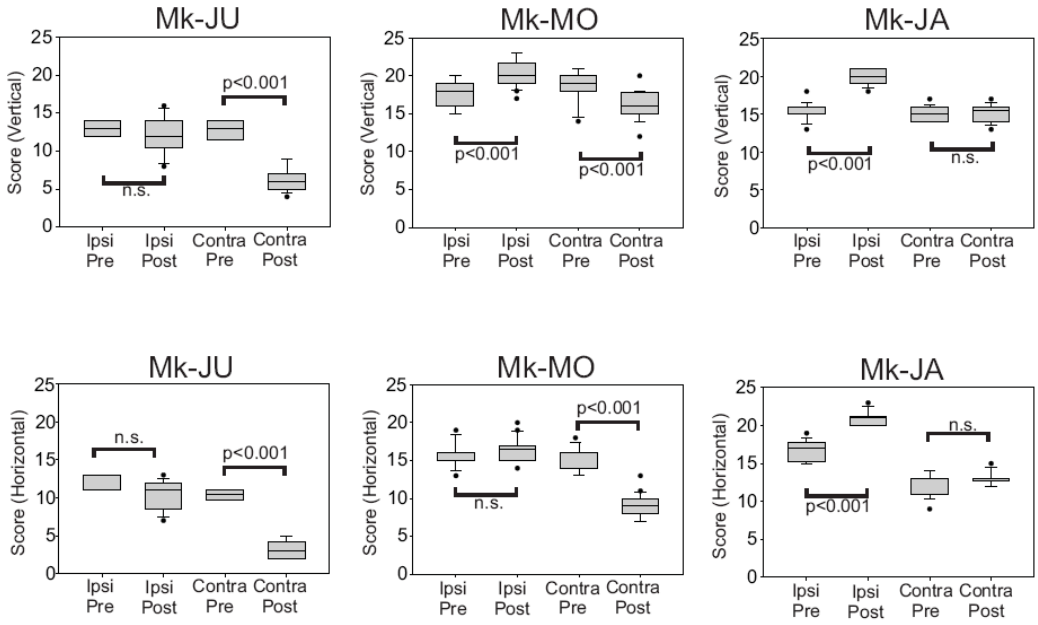
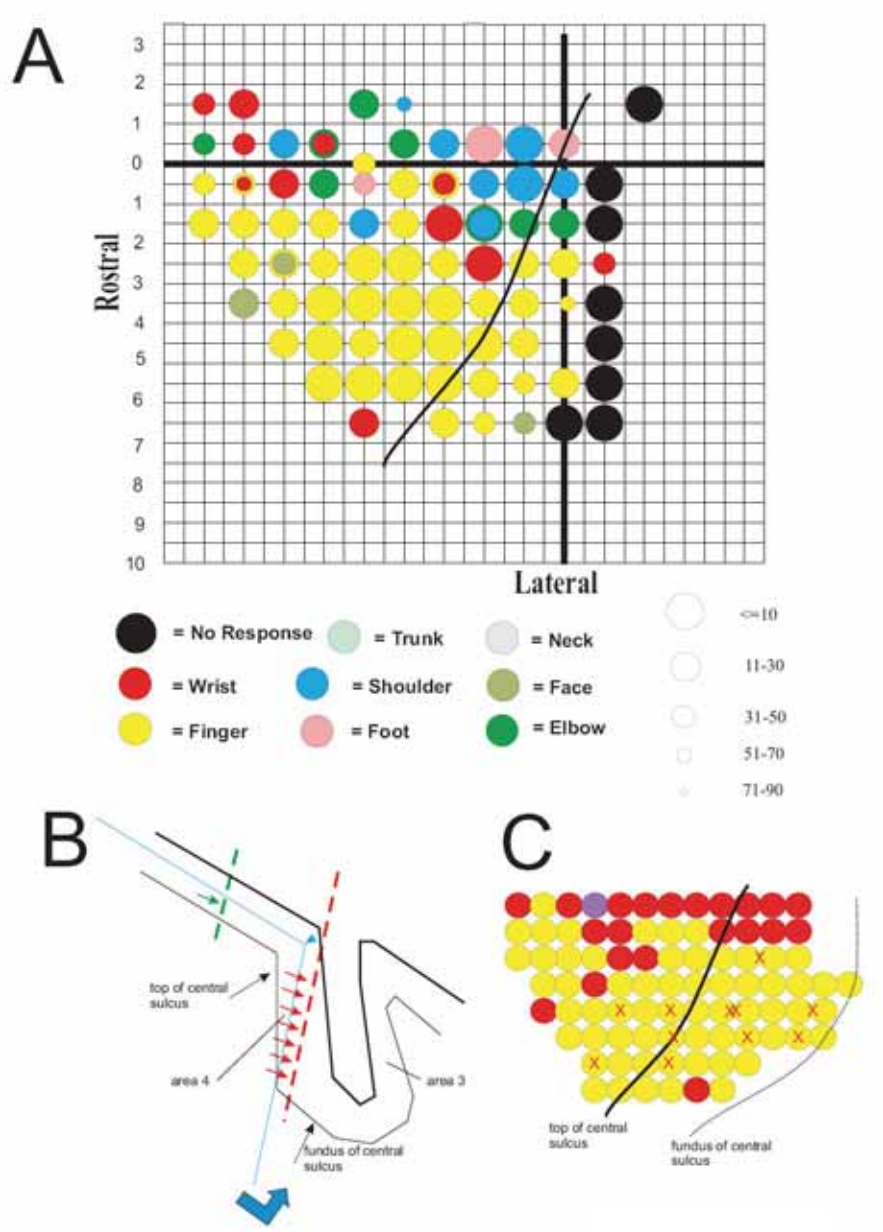
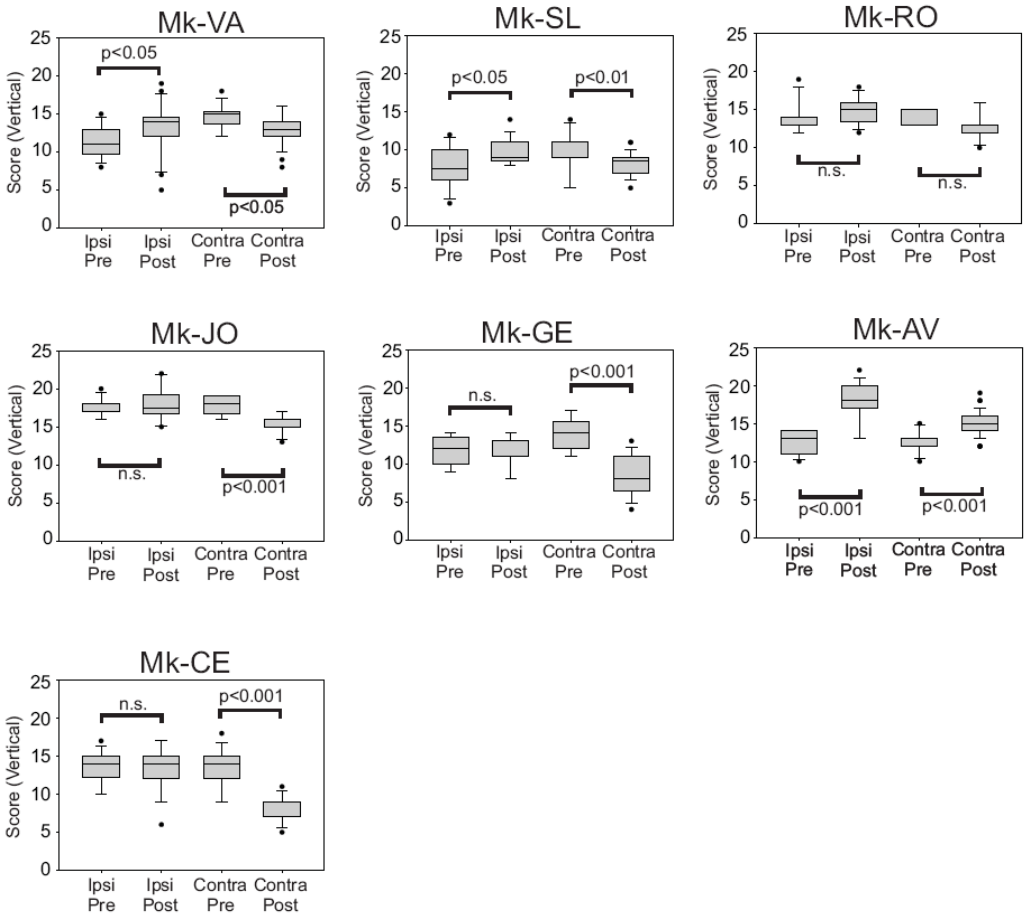


Figure 5.1.5: Same data as in Figure 5.1.2B (Mk-JU, Mk-MO and Mk-JA), but for the two slot orientations (vertical and horizontal) analyzed separately.

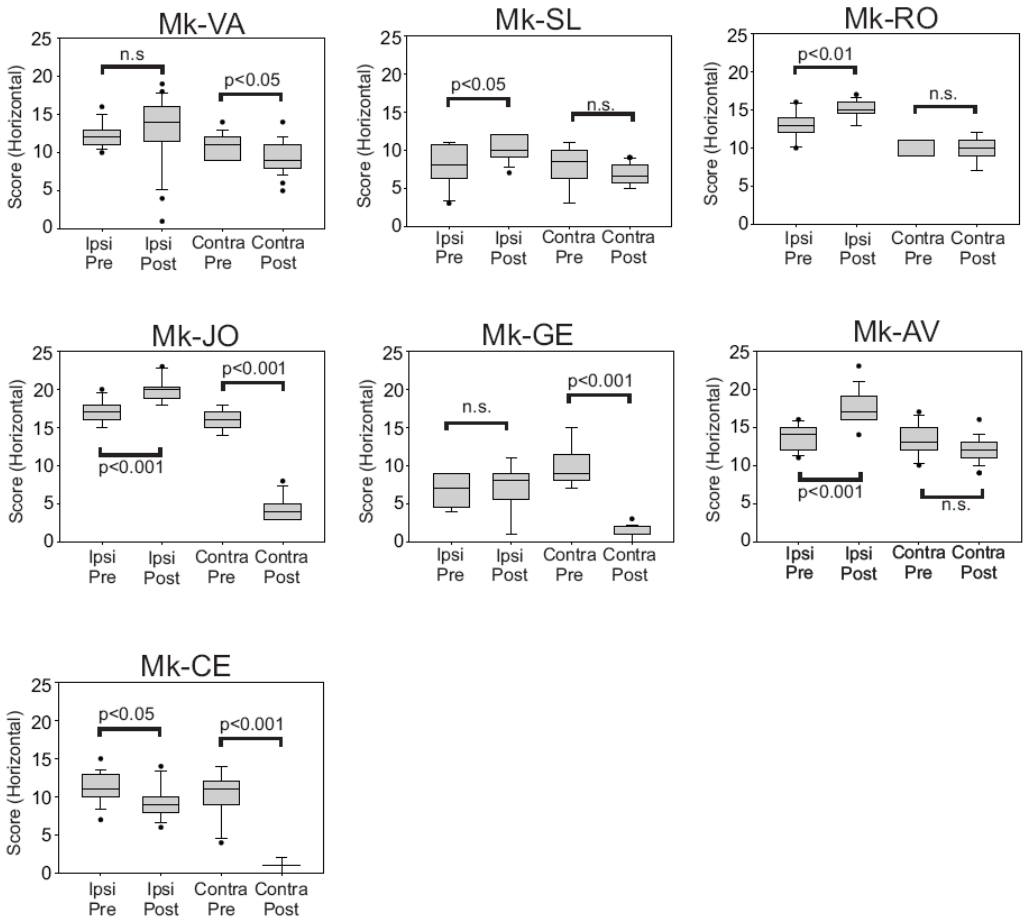


Supplementary Figure 5.1.1:
A: Intracortical microstimulation (ICMS) map in Mk-SL as seen from the surface, in which each circular symbol represents the site of each electrode penetration. The black curve represents the approximate location of the central sulcus. The size of the circles indicates the lowest ICMS threshold (in microAmps) obtained along the corresponding electrode penetration (see table on the bottom right of the panel). The color of the circles represents the body region where the ICMS at threshold elicited a movement on the contralateral side. Finger movements were obtained at the lowest threshold along the electrode penetrations depicted in yellow. The grid (in mm) represents the coordinate system used in the chamber that was chronically implanted in the left hemisphere to register the rostro-caudal and medio-lateral positions of the electrode penetrations (separated by 1 mm from each others).
B: On the surface map shown in panel A, the electrode penetration represented by the dashed green line is represented by a single circle, as the electrode penetration is perpendicular to the cortical layer (only one ICMS site with a low threshold, in principle at the depth corresponding to layer V: green arrow). The electrode penetration represented by the dashed red line is also represented on the surface by a single circle (in panel A), although the penetration is roughly parallel to the cortical layers in the rostral bank of the central sulcus. As a consequence, the surface map does not show the multiple ICMS sites where low threshold can be obtained (red arrows), where the electrode tip is close to corticopsinal neurons in layer V. In order to generate a more complete representation of the hand area in the motor cortex, the rostral bank of the central sulcus was "unfolded", with a rotation to the right (thick blue arrow) using the top of the central sulcus as axis of rotation (small blue arrow). As a consequence, each ICMS site along the penetration represented by the red dashed line appears on the

unfolded map, yielding a more realistic extent for the hand area (yellow circles in panel C). The thin blue line indicates the general orientation of the cortical layers, switching from horizontal to roughly vertical in the rostral bank of the central sulcus.
C: In the unfolded map, ICMS sites eliciting a movement at 30 microAmps of stimulation are represented (as a consequence all symbols have the same size). The red crosses are for the ICMS sites selected for ibotenic acid infusion, corresponding to the sites where low thresholds were observed (usually below 10 microAmps). When two adjacent ICMS sites (separated by 1 mm) had the same threshold, the ibotenic acid was injected at a depth in between these 2 sites. Two adjacent sites of infusion (crosses next to each other) on the map are actually separated along the rostra-caudal axis by 1 mm. The black curve shows the approximate location of the central sulcus, whereas the dashed black curve shows the approximate location of the fundus of the central sulcus.



Supplementary Figure 5.1.2 : Same data as in Figure 5.1.3 (7 monkeys) but the number of pellets retrieved (score) is considered for the vertical slots only. Same conventions as in Figure 5.1.2B.



Supplementary Figure 5.1.3 : Same data as in Figure 5.1.3 (7 monkeys) but the number of pellets retrieved (score) is considered for the horizontal slots only. Same conventions as in Figure 5.1.2B.

As for the total retrieval score, analysis of vertical and horizontal slots separately reveal a correlation between long-term reach and grasp performance in the ipsilesional hand and the percent of recovery of the contralesional hand, although the correlation was less pronounced than for the total score (Fig. 5.1.6). Nevertheless, the correlation was statistically significant for both the vertical slots ($p<0.01$) and the horizontal slots ($p<0.05$).

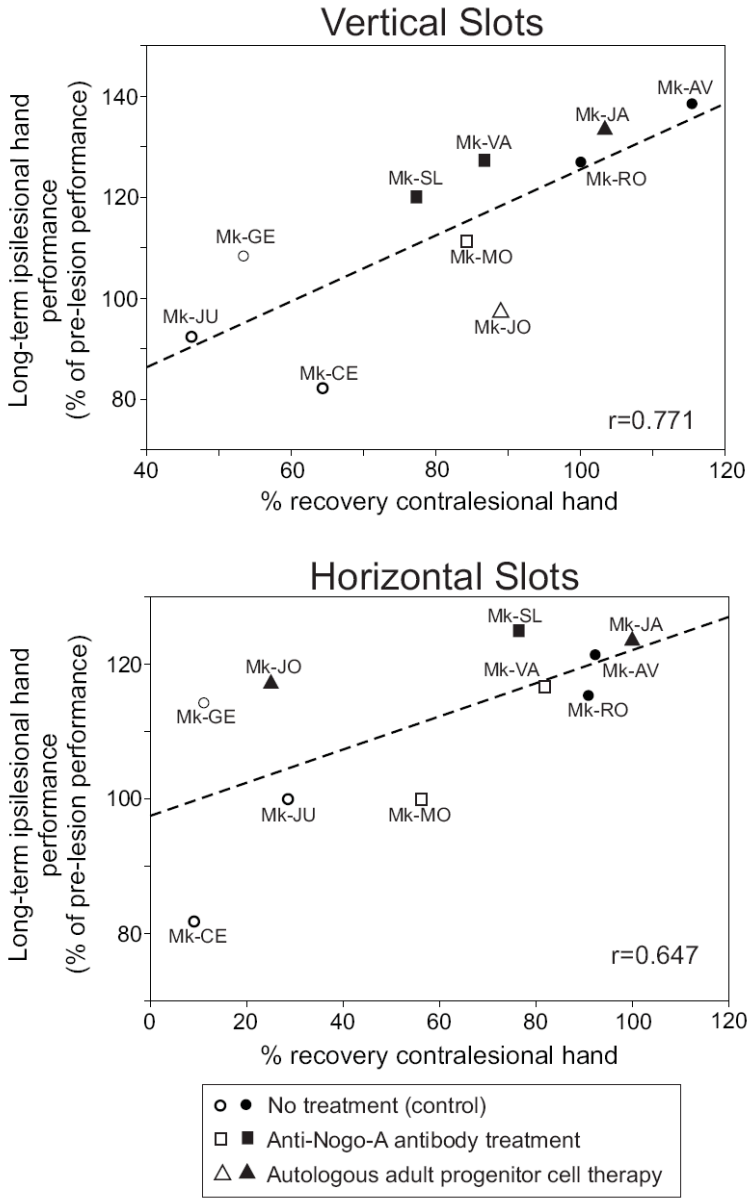


Figure 5.1.6 : Same data as in Figure 5.1.4 (correlation between long-term post-lesional ipsilesional hand performance and % of recovery of the contralesional hand) for the two slots orientations (vertical and horizontal) analyzed separately. Filled symbols are for the monkeys exhibiting significant long-term enhancement of ipsilesional hand performance (see Figs. 5.1.5 and Supplementary Figs. 5.1.1 and 5.1.2), whereas there was no enhancement for the ipisilesional hand in the monkeys represented by open symbols.

5.1.3.3 Correlation between enhancement of ipsilesional performance and volume of motor cortex lesion

The above data indicate that the long-term enhancement of manual performance in the ipsilesional hand is strongly correlated with the degree of recovery for the contralesional hand (Figs. 5.1.4 and 5.1.6). One may wonder whether the same parameter is correlated with the extent of the motor cortex lesion. Manual performance in the ipsilesional hand assessed over the long-term (expressed in percent of the pre-lesion score) was plotted as function of the

volume of the lesion, expressed in mm^3, encompassing primarily the gray matter in M1 and, to a lesser extent, gray matter in S1 in some monkeys (see also Table 5.1.1). As shown in Figure 5.1.7 (top panel), there is a significant inverse correlation (r=-0.735; p<0.01) between the long-term performance in the ipsilesional hand and the volume of the lesion (in the motor cortex and S1).

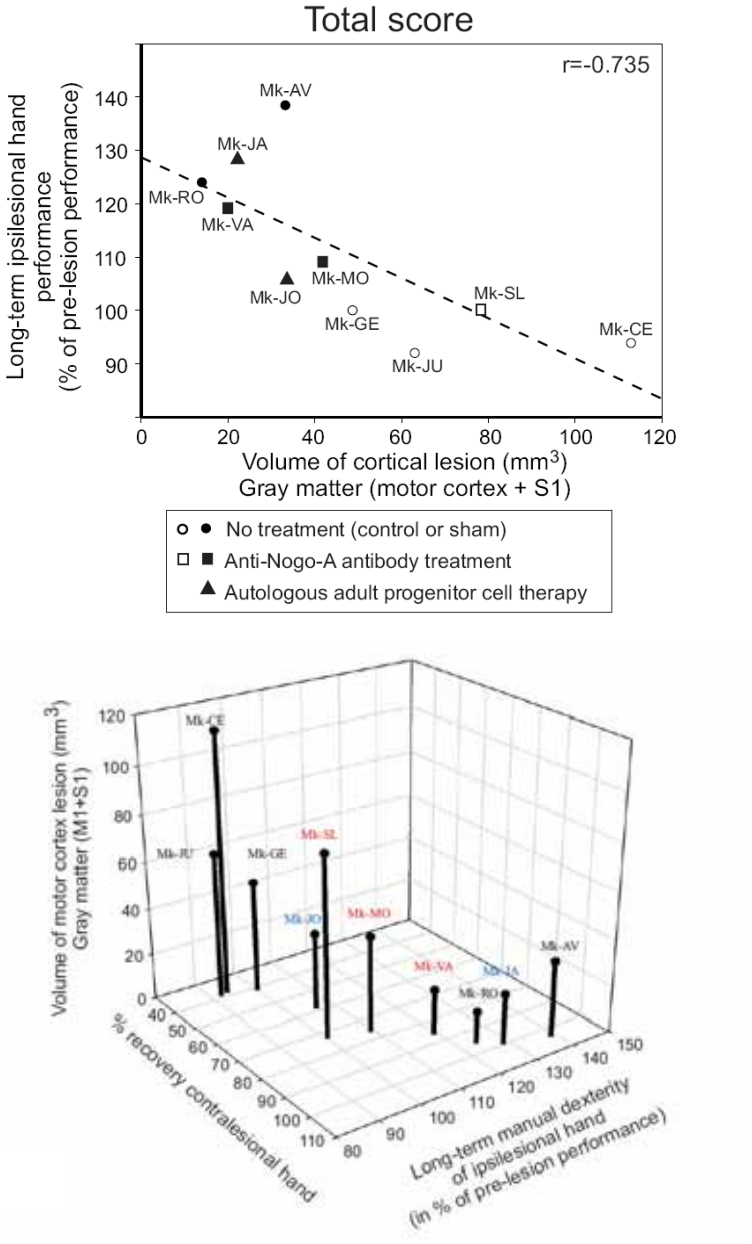


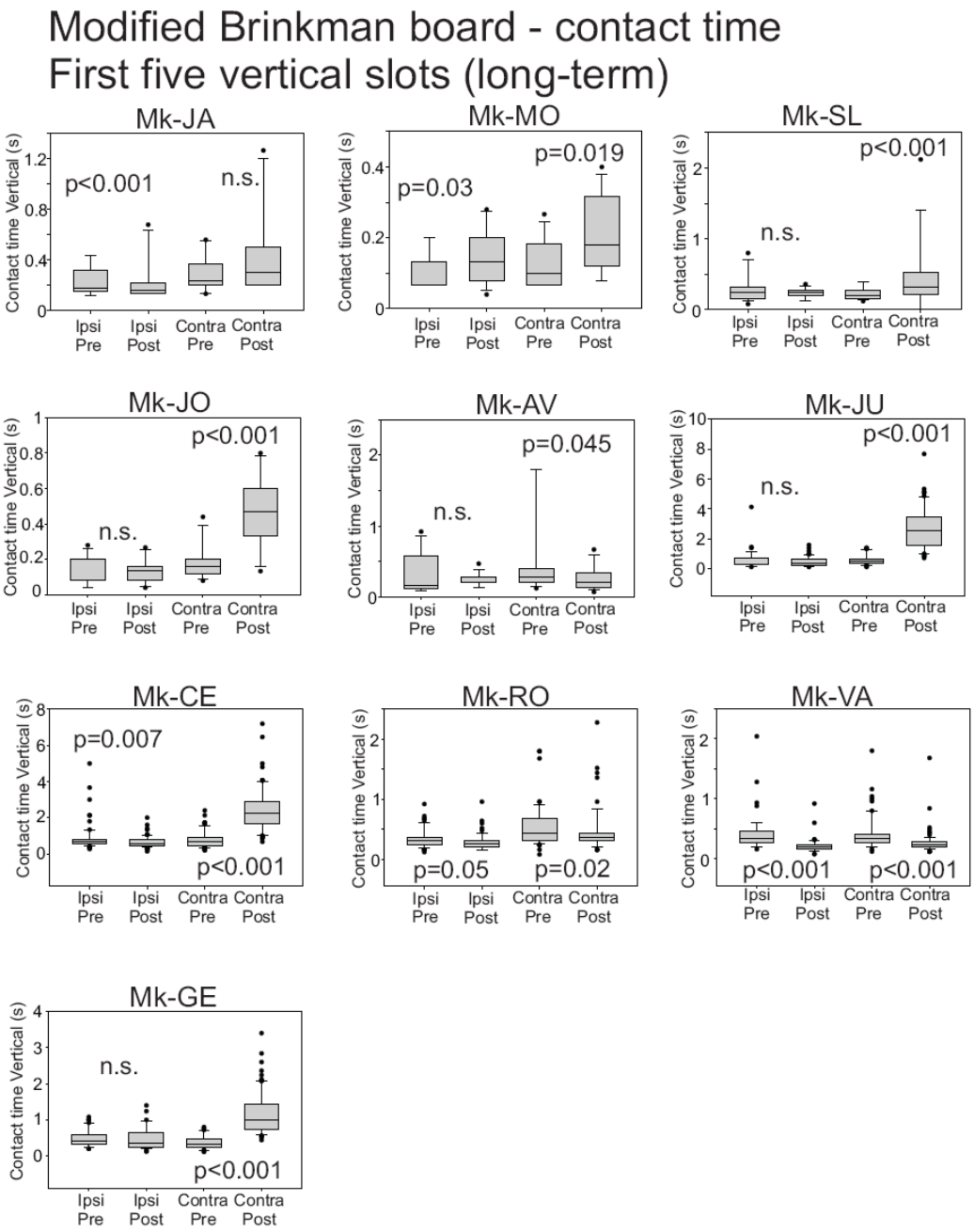
Figure 5.1.7:
Top panel: The post-lesion long-term ipsilesional hand performance (compared to pre-lesion) is plotted as a function of the volume of the motor cortex lesion (corresponding to the volume of gray matter affected by the lesion in the motor cortex and in the primary somatosensory cortex; see Table 5.1.1 for more detail). The dashed line indicates a statistically significant inverse correlation between these 2 parameters (p<0.01). Center inset box gives the symbol code referring to the three groups of monkeys (see text). Filled symbols are for the monkeys exhibiting a significant long-term enhancement of ipsilesional hand performance (see Figs. 5.1.2 and 5.1.3). Open symbols are for the monkeys without such enhancement of ipsilesional hand performance.

Bottom panel: 3D summary representation of the data of the three parameters analyzed in the 10 monkeys, the long-term retrieval performance in the ipsilesional hand, the percent of recovery of the contralesional hand and the volume of the lesion. The coefficient of correlation between the "long-term ipsilesional hand performance" and the "% recovery contralesional hand" is indicated on Fig. 5.1.4. The coefficient of correlation between the "long-term ipsilesional hand performance" and the "volume of motor cortex lesion" is indicated on the top panel. The coefficient of correlation between "% recovery contralesional hand" and the "volume of motor cortex lesion" is r=-0.698.

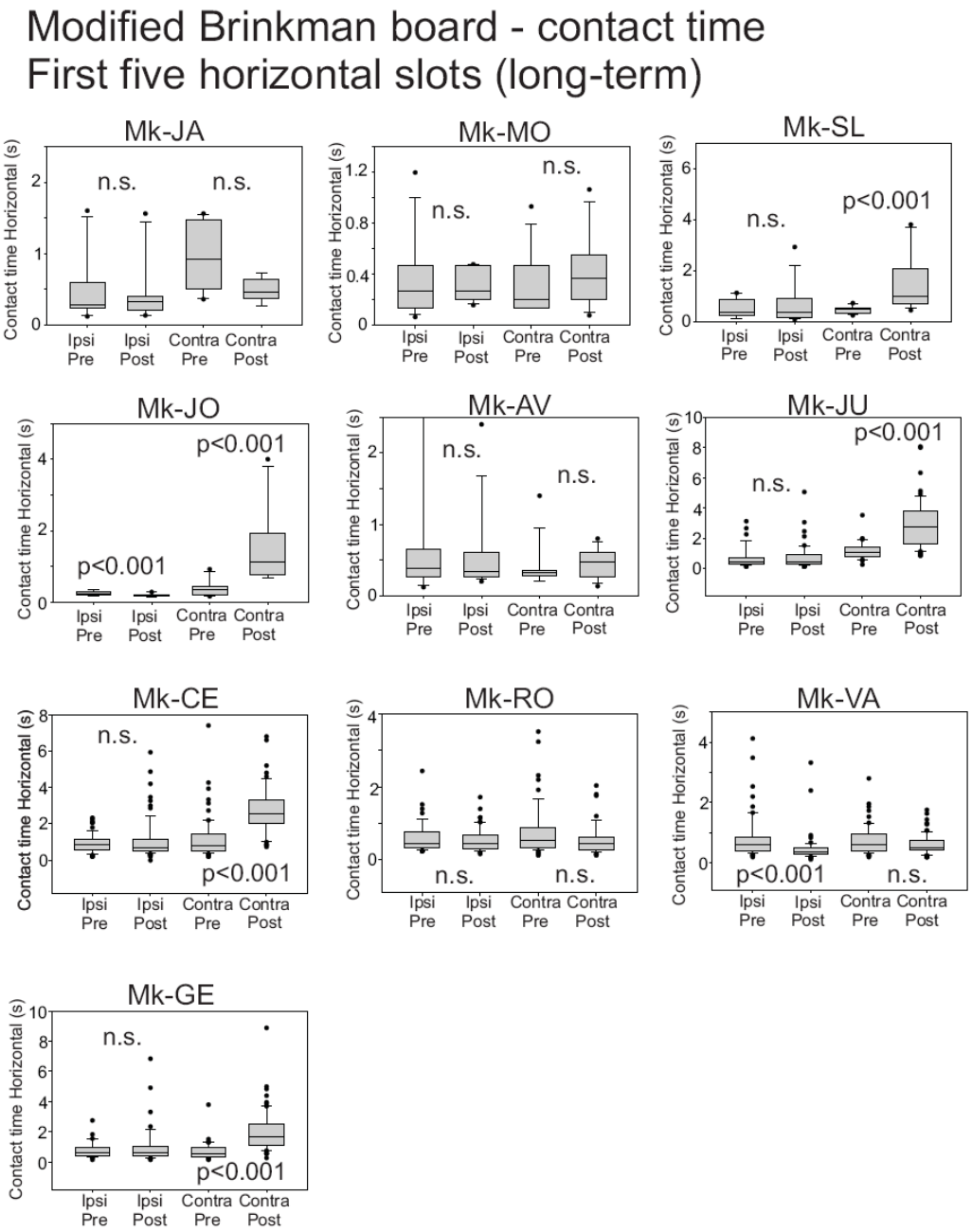
Clearly, the six monkeys with a significant long-term enhancement of manual performance of the ipsilesional hand (filled symbols in Fig. 5.1.7 top panel) had a smaller lesion than the other four monkeys. For a more comprehensive description of the relationship between the 3 relevant parameters, the bottom panel of Figure 5.1.7 shows a 3D plot of the long-term performance in the ipsilesional hand versus the percent of recovery of the contralesional hand and the volume of the cortical lesion.

5.1.3.4 Modified Brinkman board task: contact time data for the ipsilesional hand

The above pellet retrieval score data take into account the entire sequence of movements to collect the pellets (including reaching, withdrawing). In contrast, the contact time parameter is restricted to the time of contact between the fingers and the pellet while it is in the slot (the retrieval time). The contact time reflects specifically the grasping capability during execution of the precision grip, and thus may be a more precise measure of manual dexterity. The contact time was measured during each session for the first five vertical slots and the first five horizontal slots. The data were then cumulated for the pre-lesion plateau period and for the long-term post-lesion period during the same time windows as for the pellet retrieval score data (Table 5.1.1). The contact time data are presented similar as the pellet retrieval score data (see Fig. 5.1.5 and Supplementary Figs. 5.1.1 and 5.1.2), in the form of box and whisker plots and analyzed statistically using the Mann-Whitney test (Supplementary Figs. 5.1.4 and 5.1.5).



Supplementary Figure 5.1.4 : Contact time data for the ten monkeys involved in the study, derived from the first five vertical slots visited by the monkey. The data are presented in the form of box and whisker plots (see legend of Fig. 5.1.2), showing the distribution of contact times obtained from the ipsilesional hand (ipsi) or the contralesional hand (contra), pre-lesion (pre) or post-lesion (post), respectively. The statistics are for the comparison of pre- versus post-lesion contact times for each hand (Mann and Whitney test). Short contact time means good manual dexterity.

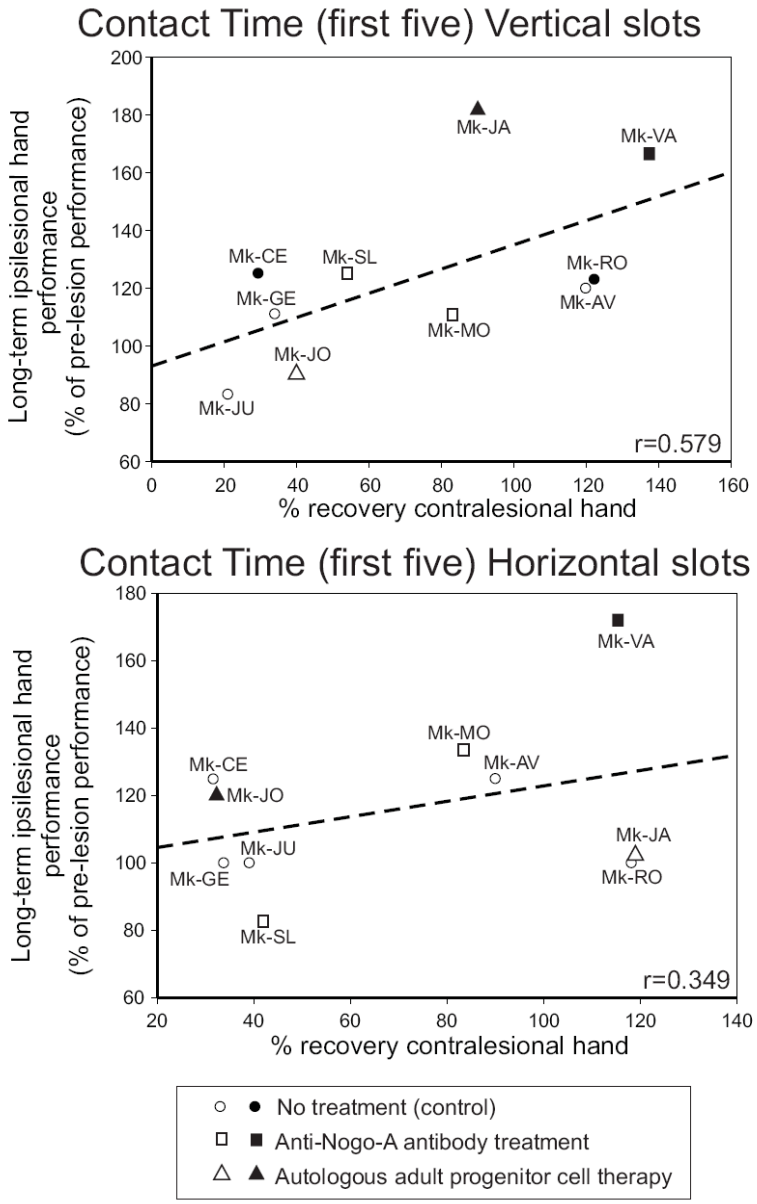


Supplementary Figure 5.1.5 : Contact time data for the ten monkeys involved in the study, derived from the first five horizontal slots visited by the monkey. Same conventions as in Supplementary Figure 5.1.4.

The median contact time for the contralesional hand was largely in line with the retrieval score data. The majority of monkeys for which the long-term retrieval score for the contralesional hand remained significantly lower post-lesion, as compared to pre-lesion, exhibited a consistent long lasting increase in contact time (i.e. more time was needed to grasp the pellet). This was true for the vertical slots for Mk-MO, Mk-JU, Mk-SL, Mk-JO, Mk-CE and Mk-GE (Supplementary Fig. 5.1.4) whereas, for the horizontal slots, this was true for

Mk-JU, Mk-SL, Mk-JO, Mk-GE and Mk-CE (Supplementary Fig. 5.1.5). One monkey (Mk-VA) showed contact times post-lesion which did not increase or even decreased as compared to pre-lesion, inconsistent with a poor post-lesion pellet retrieval score over the long term. The three monkeys (Mk-JA, Mk-RO and Mk-AV) with a complete recovery of retrieval score (>95%) for the contralesional hand exhibited a contact time which was not statistically different pre- versus post-lesion, or was even shorter post-lesion (Supplementary Figs. 5.1.4 and 5.1.5).

As far as the ipsilesional hand is concerned, the contact time data showed less difference between the pre-lesion and the long-term post-lesion periods than did the retrieval score data. An enhancement of post-lesion ipsilesional hand manual dexterity over the long-term, evidenced as a decrease in contact time, was observed in four monkeys for the vertical slots (Mk-JA, Mk-RO, Mk-VA and Mk-CE; Supplementary Fig. 5.1.4) and in two monkeys for the horizontal slots (Mk-VA and Mk-JO; Supplementary Fig. 5.1.5). As observed for the retrieval score data (Fig. 5.1.6), there was also a correlation between the contact time observed over the long-term for the ipsilesional hand and the extent of recovery of contact time for the contralesional hand (Supplementary Fig. 5.1.6), for both the vertical slots ($r=0.579$) and the horizontal slots ($r=0.349$). However, these correlations for the contact time were only a trend as they were not statistically significant ($p>0.05$).



Supplementary Figure 5.1.6: Correlation between long-term post-lesional ipsilesional contact time and % of recovery of contact time of the contralesional hand for the two slots orientations (vertical and horizontal).
The different symbols distinguish the three subgroups of monkeys. Filled symbols are for the monkeys exhibiting a significant long-term enhancement of the manual performance (contact time) of the ipsilesional hand, whereas there was no enhancement of contact time in the monkeys represented by open symbols.

5.1.3.5 Differences with clinical studies

In the present study, each monkey was able to serve as its own control by comparing pre-lesion manual score with post-lesion score, a very sensitive approach that allows the detection of moderate differences between pre- versus post-lesion performance, as presented here for the ipsilesional hand (Figs. 5.1.2B and 5.1.3). Is such long-term enhancement of the ipsilesional hand performance detectable in a clinical study, devoid of available pre-lesion data for the patients (e.g. for instance after a cortical lesion)? Clinical studies rely on group comparisons, intact subjects versus lesioned patients. To address this issue (Fig. 5.1.8), the

post-lesion ipsilesional total retrieval score over the long-term in the group of 10 monkeys included in the present study was compared to a different group of 12 intact monkeys (before they were subjected to spinal cord injury=SCI; see Freund et al., 2006, 2007). As shown in the left part of the plot in Figure 5.1.8, the variability of manual performance as assessed by the total retrieval score in the modified Brinkman board across 12 intact monkeys was large. Plotting on the same graph the long-term ipsilesional total retrieval score observed post-lesion for the 10 monkeys included in the present study (right part of Fig. 5.1.8) yields complete overlap between the two groups, preventing statistical detection of the long-term enhancement of motor performance in the ipsilesional hand in the group of 10 monkeys subjected to the motor cortex lesion (Mann and Whitney test, n.s. p=0.241).

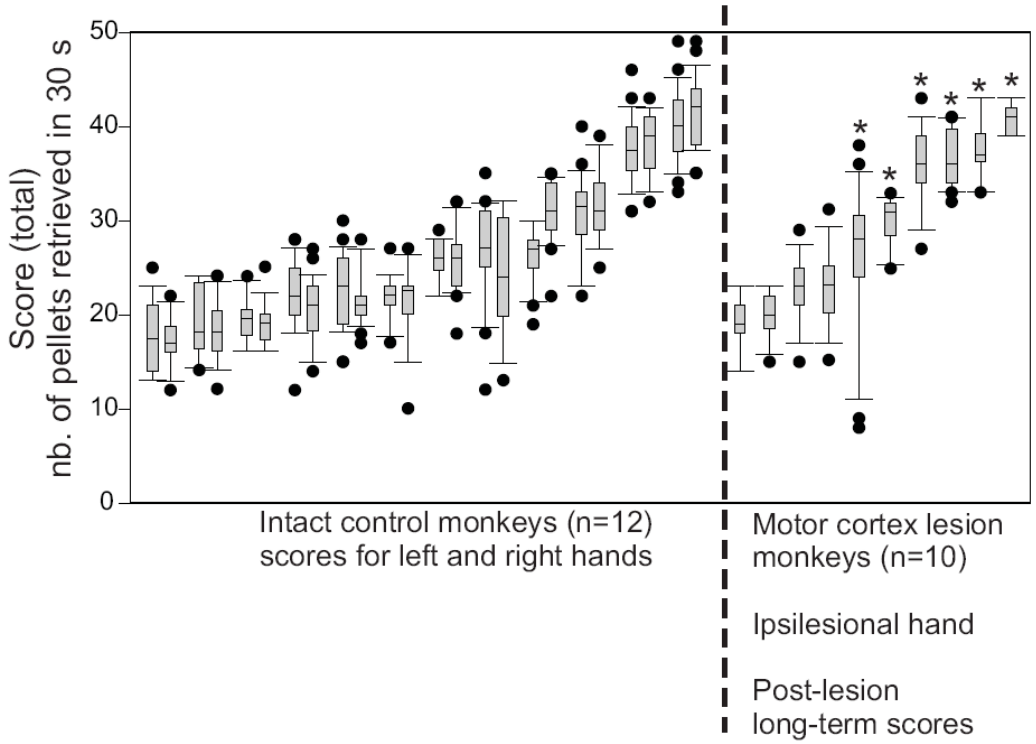


Figure 5.1.8 : Box and whisker plots showing the total number of pellets retrieved (score) in the modified Brinkman board task for two groups of monkeys. On the left, the performance is shown for a group of intact control monkeys (in fact pre-lesion score of monkeys subjected later on to spinal cord injury=SCI; see text). In the control group, performance is shown for each hand for each monkey (box plots are grouped by 2 for each monkey), allowing between hand comparison. The group of monkeys on the right consists of the 10 monkeys included in the present study with their post-lesion total score over the long-term in the ipsilesional hand only. The stars point to the monkeys which were characterized by a statistically significant enhancement of manual performance for the ipsilesional hand over the long-term (see Figs. 5.1.2B and 5.1.3).

5.1.3.6 Hand dominance for the Modified Brinkman board?

The data presented in Figure 5.1.8 are also pertinent to address the issue of whether intact monkeys have a dominant hand when performing the modified Brinkman board task, as assessed by the total retrieval score. In other words, is pre-lesion performance different for the

left hand versus the right hand? Comparing the total number of pellets retrieved for the 12 intact monkeys shown in the left hand panel of Figure 5.1.8 reveals that there was no significant difference in left hand versus right hand performance for 9 out the 12 monkeys (paired t-test or Wilcoxon test: $p>0.05$; range 0.088 – 0.885). In the other 3 intact monkeys, the pre-lesion total retrieval score was significantly higher for one hand as compared to the other hand ($p<0.05$), with a better score for the right hand in 2 monkeys and for the left hand in 1 monkey. Comparing the total number of pellets retrieved pre-lesion by the left or the right hand in the group of 10 monkeys included in the present study confirmed this general trend: three monkeys (Mk-JU, Mk-JA and Mk-GE; see Figs 5.1.2 and 5.1.3) exhibited a statistically significant difference between the left and the right hand ($p<0.05$), whereas in the other 7 monkeys there was no significant difference (the p value was greater than 0.05, ranging from 0.108 to 0.898). In the three monkeys exhibiting hand dominance pre-lesion, two monkeys had a better score for the left hand and one for the right hand. In summary, in a total population of 22 monkeys, only six animals exhibited hand dominance (three for the left hand and three for the right hand). It can thus be concluded that, for the modified Brinkman board task, there was no clear and systematic hand dominance, at least as revealed by the total retrieval score.

5.1.4 DISCUSSION

Based on the retrieval score data and the contact time data, but to a lesser extent for the latter (see below), the results of the present study are consistent with our hypothesis that, after unilateral motor cortex lesion, long-term manual performance in the ipsilesional hand co-varies with the extent of post-lesion recovery of the contralesional hand. This is, to the best of our knowledge, an original observation as most previous studies on unilateral motor cortex lesions focused on the recovery of the contralesional hand and the behavioral assessment was limited to the period immediately following the lesion until a performance plateau was reached. The ipsilesional effect observed here appeared, in some cases, only several months post-lesion, although there is no systematic relationship between the extent of enhancement of manual performance and the time frame in which it occurs (Table 5.1.1).

As expected, the extent of recovery in the contralesional hand is inversely correlated with the lesion volume (Fig. 5.1.7, bottom panel). As the manual performances of the contralesional and ipsilesional hands are positively correlated (Fig. 5.1.4), it follows that the enhancement of manual performance in the ipsilesional hand is negatively correlated with the lesion size (Fig. 5.1.7, top panel). This result contrasts with the observation in rats of a post-

lesion facilitation of motor skill learning in the non-affected hand, an augmentation that parallels increasing lesion size, within a certain range, and as observed 20 days post-lesion (Allred and Jones, 2004). This discrepancy may be related to the different time points (i.e. several months post-lesion in our monkeys) and the very different organization of the corticospinal system between rodents and primates.

The long-term enhancement of manual performance in the ipsilesional hand was found in six out of ten monkeys, specifically those exhibiting the best recovery in the contralesional hand. In the other four monkeys, there was no such enhancement or even a decrease in manual performance in the ipsilesional hand, over the long-term. For example, a decrease in ipsilesional manual performance was observed in the two monkeys with the largest lesions of the motor cortex (Mk-CE and Mk-JU; see Fig. 5.1.4). Data from these two monkeys are thus consistent with data in humans, in which a unilateral lesion of the motor cortex leads to a deficit of manual performance in the ipsilesional hand, although different motor parameters were affected depending on which hemisphere was lesioned (Hermsdörfer et al., 1999a,b; Hermsdörfer and Goldenberg, 2002).

5.1.4.1 Limitations of interpretation

The interpretation of the present results reporting a co-variation between the extent of recovery in the contralesional hand and manual performance in the ipsilesional hand over the long-term after a unilateral lesion of the motor cortex may be limited by confounding factors. The study comprises multiple variables raising some uncertainties about the interpretation of this main finding. In particular, the protocol was disparate to some extent between monkeys, for instance the time windows of behavioral assessment and long-term follow-up period, the lesion size, the precise position of the lesion, as well as the type of treatment. The limited number of monkeys in each group prompted a pooling of all animals to allow a correlation on a sufficiently large number of data points (n=10). The serious ethical concerns for the use of non-human primates in research indeed limits the design of studies based on large groups of animals. One obvious limitation of interpretation of the present study is that the five untreated animals represent extreme values (Fig. 5.1.4). Ideally, a study conducted on a larger pool of untreated monkeys only may have produced more reliable data, although a constraint with the control monkeys is that, above a certain volume of lesion (40 mm^3; see Fig. 5.1.7), the extent of recovery was largely incomplete (around 40%). In the present study, as a result of the two treatments (anti-Nogo-A antibody; autologous progenitor cells' therapy), some monkeys with a fairly large lesion exhibited a substantial recovery, clearly above 40% (Mk-SL, Mk-MO,

Mk-JO). A possible direct effect of the treatments on the enhancement of manual performance in the ipsilesional hand over the long-term after a unilateral lesion of the motor cortex is difficult to evaluate. Among the treated monkeys (n=5), two animals showed a marked enhancement of manual performance with their ipsilesional hand, whereas the other three treated monkeys did not (this depends on the slot orientation; Figs. 5.1.4 and 5.1.6). Thus, there is apparently no systematic relationship between long-term manual performance in the ipsilesional hand and the presence or absence of treatment. Both the extent of functional recovery in the contralesional hand and the manual performance in the ipsilesional hand over the long-term appear to be more dependent on the lesion size than the treatments applied to some of the monkeys. The bottom panel of Figure 5.1.7 emphasizes the interdependency between the three parameters (extent of recovery in the contralesional hand; manual performance in the ipsilesional hand over the long-term; volume of cortical lesion), as well as the limitations of interpretation due to the presence of multiple variables in the present study.

5.1.4.2 Spread of the lesion to cortical areas adjacent to M1.

Although our lesions targeted M1 (see Supplementary Fig. 5.1.1), they sometimes spread into adjacent cortical areas, such as premotor cortex (PM; Mk-CE, Mk-JU, Mk-AV, Mk-JA, Mk-SL) or post-central in the somatosensory cortex (Mk-CE, Mk-GE, Mk-VA, Mk-SL, Mk-JO, Mk-JA). As quantified for the post-central gyrus (Table 5.1.1), the spread of the lesion into the somatosensory cortex was generally limited. However, what is the impact of the lesion's spread in PM or in the post-central gyrus on the present data? In an intact monkey, reversible inactivation of PM had no effect on reach and grasp manual tasks (Liu and Rouiller, 1999; Kermadi et al., 1997). It may however be different in a monkey subjected to a lesion affecting mainly M1, as PM and the somatosensory cortex contribute to functional recovery (e.g. Dancause et al., 2005). The spread of the lesion post-centrally did not impact the present data, as there was no correlation between the enhancement of the ipsilesional manual performance and the spread of the lesion into primary somatosensory cortex (Table 5.1.1). For instance, in two monkeys with comparably reduced post-lesion performance in the ipsilesional hand (Mk-CE and Mk-JU), one had a part of the somatosensory cortex lesioned (10 mm^3) whereas the other monkey did not. At the other extreme, one monkey with enhancement of ipsilesional hand's performance (119%) had a lesion encroaching on the somatosensory cortex, whereas in another monkey (124% performance), the post-central gyrus was not affected by the ibotenic acid infusion. There was also no systematic relationship between the extent of the recovery of the contralesional hand and the

presence/absence or size of lesion affecting the somatosensory cortex. The reasons for this are likely two-fold. First, a lesion of the somatosensory cortex does not necessarily affect the hand representation, and second the monkeys were over-trained on this task, suggesting that the contribution of the somatosensory cortex may be less crucial than during training or during early phases of regular practice or immediately after the lesion. As far as the spread of the lesion in PM is concerned, there is also no correlation with the long-term enhancement of ipsilesional manual performance. In the group of monkeys with spread in PM, some exhibited behavioral enhancement (Mk-JA, Mk-AV), whereas others did not (Mk-CE, Mk-JU, Mk-SL). Note however that the extent of the lesion in PM and in the somatosensory cortex was included in the total volume of the lesion in gray matter considered in the analysis of correlation with the behavioral parameters (Fig. 5.1.7).

Note that the monkey exhibiting the best recovery of the contralesional hand together with the most extensive enhancement of the performance in the ipsilesional hand (Mk-AV; see Fig. 5.1.4) is characterized by a lesion affecting only the rostral part of the primary motor cortex, with spread into PM (Fig. 5.1.1). As expected, for such a lesion position, recovery was better as compared to a lesion including the caudal part of the primary motor cortex.

5.1.4.3 Comparison of score and contact time data

The contact time data specifically reflect the grasping function by measuring the time of manipulation of the pellet with the fingers before successful retrieval. The retrieval score data also reflect this manipulation but, in addition, comprise other facets of the task, such as arm reaching, arm withdrawal and transport of the pellet to the mouth. The observation of long-term enhancement of manual performance in the ipsilesional hand after unilateral motor cortex lesion in six monkeys comes largely from score data (Figs. 5.1.4 and 5.1.6), whereas the contact time data showed only a trend in that direction (Supplementary Fig. 5.1.6). How can it be explained that contact time data are not fully corroborating with the retrieval score data? To address this question, the strategy used by the monkey to perform the modified Brinkman board was investigated. To assess one facet of the strategy, the cumulative distance between consecutive slots was determined, both pre-lesion and post-lesion. If monkeys visit the slots in a systematic manner (e.g. starting at a given extremity of the board and then moving progressively towards the other extremity of the board), then the cumulative distance is smaller than in case of random spatial choice of the slots. For each monkey, the difference of cumulative distance between consecutive slots (post-lesion minus pre-lesion) was calculated. For the ipsilesional hand, there was a significant inverse correlation between the

difference of cumulative distance and the long-term manual performance (not shown). In other words, the monkeys that did not exhibit enhancement of manual performance in the ipsilesional hand had a post-lesion strategy in which they visited slots more randomly. In contrast, monkeys with long-term enhancement of the ipsilesional hand visited the slots in a more ordered sequence pre- and post-lesion. When visiting the slots randomly, subjects exhibit some hesitation before moving to the next slot, resulting in fewer pellets retrieved in 30 seconds. It can be tentatively concluded that the enhancement of manual performance reflects more an improvement of strategy than a better manual dexterity per se. As a consequence, the correlation with the contact time was weaker than with the retrieval score which includes the entire temporal course of the trial, including some strategic aspects. Finally, there was some disparity across monkeys, ranging from a reliable correlation between retrieval score and contact time to an absence of correlation between the two parameters.

5.1.4.4 Comparison with functional recovery in human subjects

From a clinical point of view, a consequence of the present study may be that an efficient therapy aimed at improving the motor control of the contralesional hand, for instance after stroke, is not only pertinent for the affected hand, but also for the fine control of the ipsilesional hand over the long-term, in particular for frequently performed motor sequences. Along this line, constraint induced therapy (e.g. Miltner et al., 1999; Liepert et al., 2000b; Schaechter et al., 2002; Wolf et al., 2006; Sawaki et al., 2008) aimed at immobilizing the non-affected limb to force the use of the affected limb appears to make sense, not only for enhancing the recovery of the contralesional hand by practice, but also for long-term manual performance in the non-affected hand. It has been argued that constraint-induced therapy should not be imposed too early during the recovery phase, nor should it be too severe in order to avoid detrimentally effecting the contralesional limb (e.g. Kozlowski et al., 1996; Leasure and Schallert, 2004). Aggressive constraint-induced therapy may also penalize the ipsilesional hand over the long-term, due to the lack of sufficient motor practice. To avoid a detrimental effect on the ipsilesional hand, bilateral arm training therapies or mirror therapies have been proposed (e.g. Luft et al., 2004b; Altschuler et al., 1999).

5.1.4.5 Potential mechanisms: cortical contribution

Two potential mechanisms will be presented for the observed correlation between the extent of contralesional recovery and the ipsilesional manual performance over the long-term, starting here at the level of the cerebral cortex (see next section for potential subcortical

mechanisms). In human subjects, transient unilateral disruption of the motor cortex with repetitive transcranial magnetic stimulation (TMS) increased excitability of the unaffected motor cortex, resulting in improved motor learning with the hand ipsilateral to the motor cortex disrupted with TMS (Kobayashi et al., 2009). These observations were interpreted in terms of inter-hemispheric competition. Suppression of motor control in M1 on one side may transcallosally disinhibit the contralateral motor cortex, leading to an increase of corticospinal drive onto the motoneurons controlling the muscles of the hand ipsilateral to the lesioned or transiently disrupted motor cortex (e.g. Hummel and Cohen, 2006; Reis et al., 2009). A major difference with the present study is that these observations in humans were conducted immediately after the inactivation (i.e. TMS disruption), whereas the present enhancement of the ipsilesional hand in monkeys was observed over the long-term (several months post-lesion). Furthermore comparison between stroke in humans and the present data in monkeys with restricted lesion focused on M1 is limited by the absence of focal lesion in M1 in humans. Nevertheless, is interhemispheric competition a relevant concept to interpret, at least in part, the present data derived from a restricted unilateral lesion centered on the hand representation in motor cortex? The lesion of the hand representation which is primarily in M1 is expected to have only a minor impact on the callosal connectivity because, as compared to other body representations in M1 or to premotor areas, the hand representation in M1 is only weakly connected with the opposite hemisphere (Jenny, 1979; Rouiller et al., 1994). A possible role played by the callosal projection thus concerns other body representations in M1 or other motor cortical areas (PM, SMA) at the origin of stronger callosal projections. Consistent with a wider recruitment of motor cortical areas, when a movement sequence is executed with more difficulty (e.g. during aging: Ward and Frackowiak, 2003; Heunincks et al., 2008; or e.g. after poor recovery from stroke: Ward et al., 2003, 2004), a more widespread brain area is activated as compared to young human subjects or to patients exhibiting better recovery. The increase of brain activity in the lesioned hemisphere, in the case of poor recovery with persisting motor deficit may be associated with a long lasting increase in callosal inhibition of the intact hemisphere, thus preventing a refinement of motor control on the ipsilesional hand over the long-term. This interpretation however in terms of level of activity in one or the other hemispheres related to the degree of recovery, may actually be complicated by the observation that, as compared to intact human subjects, brain activation is lower in patients with cortical lesion but higher in patients with subcortical lesion (Luft et al., 2004a; Murase et al., 2004; Duque et al., 2005).

5.1.4.6 Potential mechanisms: subcortical contribution

One cannot exclude the possibility of a facilitation of the intact hemisphere on the ipsilesional hand mediated indirectly via the brainstem, involving for instance rubrospinal or reticulospinal neurons. For the red nucleus magnocellularis (RNm), output fibres decussate just after exiting the RNm, thus providing an indirect crossed pathway from the intact motor cortex to the spinal motoneurons of the ipsilesional hand, via the contralesional red nucleus. It has been shown that the rubrospinal projection can re-organize after lesion of the corticospinal tract, presumably to restore function to flexor muscles (Belhaj-Saif and Cheney, 2001). The reticulospinal system projects bilaterally to the spinal cord. Stimulus triggered averaging studies in awake monkeys (Davidson and Buford, 2004, 2006) confirmed bilateral stimulus effects on mostly proximal muscles, with a common pattern of facilitation in flexor muscles and inhibition in extensor muscles ipsilaterally and the opposite effect on the contralateral side. Similar data were obtained when using the spike triggered averaging technique (Davidson et al., 2007). However, the magnitude of the effects was weak and rare (5%). In a more recent study, Riddle et al. (2009) used intracellular recording in anesthetized monkeys to study synaptic connections between the reticulospinal tract and identified cervical motoneurons. The main finding was that the electrical stimulation of the reticulospinal tract activates motoneurons projecting to proximal and distal (wrist and hand) forelimb muscles: out of 140 motoneurons tested, the activation was exerted via direct monosynaptic (13% of the motoneurons) and disynaptic reticulospinal pathways (46% of the motoneurons), indicating that the reticulospinal system may contribute to an enhancement of the motor performance of the ipsilesional hand after unilateral motor cortex lesion.

5.1.4.7 Hand dominance and pertinence of the non-human primate model

The data presented in Figure 5.1.8 support the notion that macaque monkeys do not show a systematic manual dominance for the present task (modified Brinkman board), at least in 16 out of 22 monkeys. It can thus be concluded that the choice of the lesioned hemisphere in the present study did not influence the results. This may be different in human subjects due to the known disparity in motor performance between the dominant and non-dominant hand, but this has not yet been investigated by using a task similar to the modified Brinkman board. A more important conclusion of the data presented in Figure 5.1.8 is the significance of the present experimental model of cortical lesion in monkeys. First, sophisticated manual motor skills are a prerogative of primates (see Lemon and Griffiths, 2005; Lemon, 2008 for review). Second, the observed enhancement of manual performance in the ipsilesional hand after

unilateral motor cortex lesion, though statistically significant, can be observed only in an animal model, where the pre-lesion data are compared with the post-lesion data within the same subject. This is likely the reason why such enhancement of manual dexterity in the ipsilesional hand correlated with the degree of functional recovery of the contralesional hand was not observed in previous clinical studies investigating the possible effect of unilateral stroke on the ipsilesional hand (Sunderland, 2000; Nowak et al., 2005). Therefore, the present study emphasizes the crucial need to maintain animal models of major brain dysfunctions or pathologies (such as the consequences of stroke for instance), especially monkey models as discussed earlier for several neuro-pathologies (e.g. Courtine et al., 2007; Capitanio and Emborg, 2008). The monkey model is pertinent to decipher subtle mechanisms involved in functional recovery after a lesion. Such knowledge, together with the assessment of possible secondary effects of a treatment, represent a solid basis for translating and refining therapeutic strategies to human patients, as recently demonstrated for anti-Nogo-A antibody treatment after spinal cord injury in macaque monkeys (Freund et al., 2006, 2009).

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5.2 DOES THE MOTOR CORTEX LESION AFFECT THE PREHENSION'S STRATEGY OF THE MONKEYS?

5.2.1 PURPOSE

The aim of the present analyses was to assess if the monkeys performed the tasks with a defined strategy, in opposition to a randomly performed task. Then, if it was the case, would the motor cortex lesion induce changes in the monkeys' strategy of prehension?

5.2.2 METHODS

In this study, two motor tasks were considered to assess the strategy used by the monkeys to perform them, namely the modified Brinkman board task and the rotating Brinkman board task. For the first one, the analyses were carried on both hands' performances of five control monkeys (Mk-AV, Mk-CE, Mk-GE, Mk-JU and Mk-RO) and four treated monkeys (Mk-JO and Mk-JA treated with autologous progenitor cells, and Mk-SL and Mk-VA treated with Anti-Nogo A antibody), who had been subjected to an unilateral primary motor cortex (M1) lesion, except Mk-AV, whose lesion was located in the premotor cortx (PM). Analyses were carried out on five parameters : the preferential wrist orientation to grasp the pellets placed in the horizontal wells (abduction, named here « internal » and adduction, named here « external »), the preferential finger (which finger is first introduced in the well, namely the thumb or the index), the picking sequence in terms of vertical and horizontal wells, the cumulative distance crossed to grasp the pellets (assuming that a short cumulative distance would represent an optimal strategy), and the picking sequence on the left-right axis of the board. For the second task, the contralesional hand's performances of one control monkey (Mk-AV) and two treated monkeys (Mk-JO and Mk-JA) were analyzed.

Five parameters were considered: the preferential wrist orientation, the preferential finger, the prehension of two pellets at the same time before bringing them to the mouth, the picking sequence considering the four rings of the board, the area of pellets prehension, when picking in and picking out, and the preferential prehension orientation when picking in and out. All these parameters were described in the "General Material and Methods" part. Analyses were focused on the pre-lesion and post-lesion plateaux, with a glance at the recovery phase in Mk-AV, Mk-JO and Mk-JA (for this monkey, only for the modified Brinkman board task). For the modified Brinkman board task, both hands were considered, whereas only the contralesional hand was taken into account for the rotating Brinkman board task. Furthermore, some aspects, namely the picking sequence in terms of vertical and

horizontal wells, the cumulative distance to perform the task and the picking sequence on the left-right axis of the board for the modified Brinkman board task, and the picking sequence considering the four rings of the board for the rotating Brinkman board task were considered qualitatively. Therefore, only the preferential wrist orientation and the preferential finger for the modified Brinkman board task, and the prehension of two pellets, the area of pellets prehension and the preferential prehension orientation for the rotating Brinkman board task were analyzed quantitatively. The comparisons for the preferential wrist orientation, the preferential finger and the prehension of two pellets were performed by using the non-parametrical statistical test Mann-Whitney, whereas a chi-square test was employed to compare the distributions for the area of pellets prehension and the preferential prehension orientation.

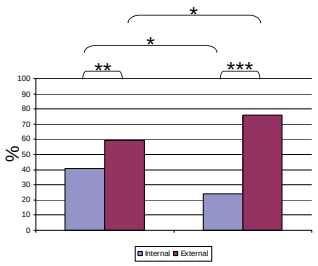
5.2.3 RESULTS

5.2.3.1 Modified Brinkman board task (n=9)

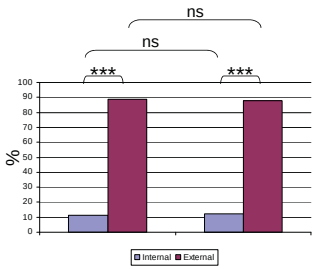
5.2.3.1.1 Preferential wrist orientation: horizontal wells

Control monkeys

Mk-AV Contralesional Hand



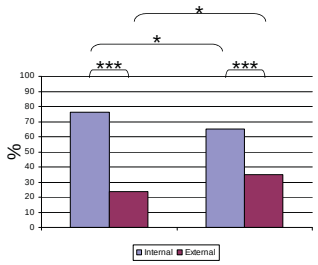
Mk-AV Ipsilesional Hand



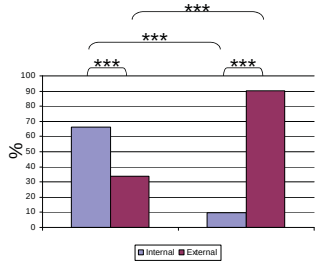
Internal

External

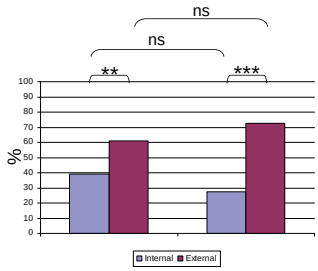
Mk-CE Contralesional Hand



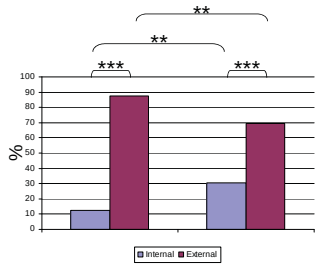
Mk-CE Ipsilesional Hand



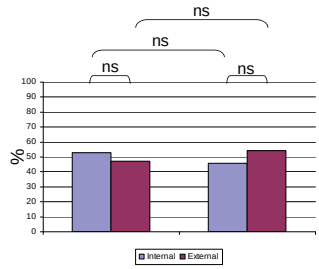
Mk-GE Contralesional Hand



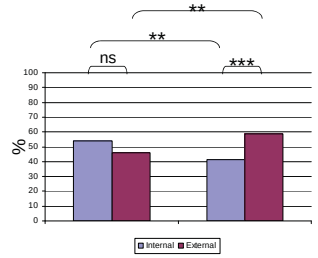
Mk-GE Ipsilesional Hand



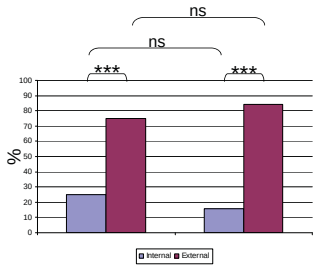
Mk-JU Contralesional Hand



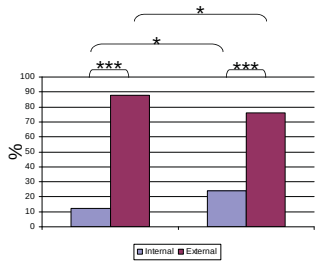
Mk-JU Ipsilesional Hand



Mk-RO Contralesional Hand



Mk-RO Ipsilesional Hand



PRE POST

PRE POST

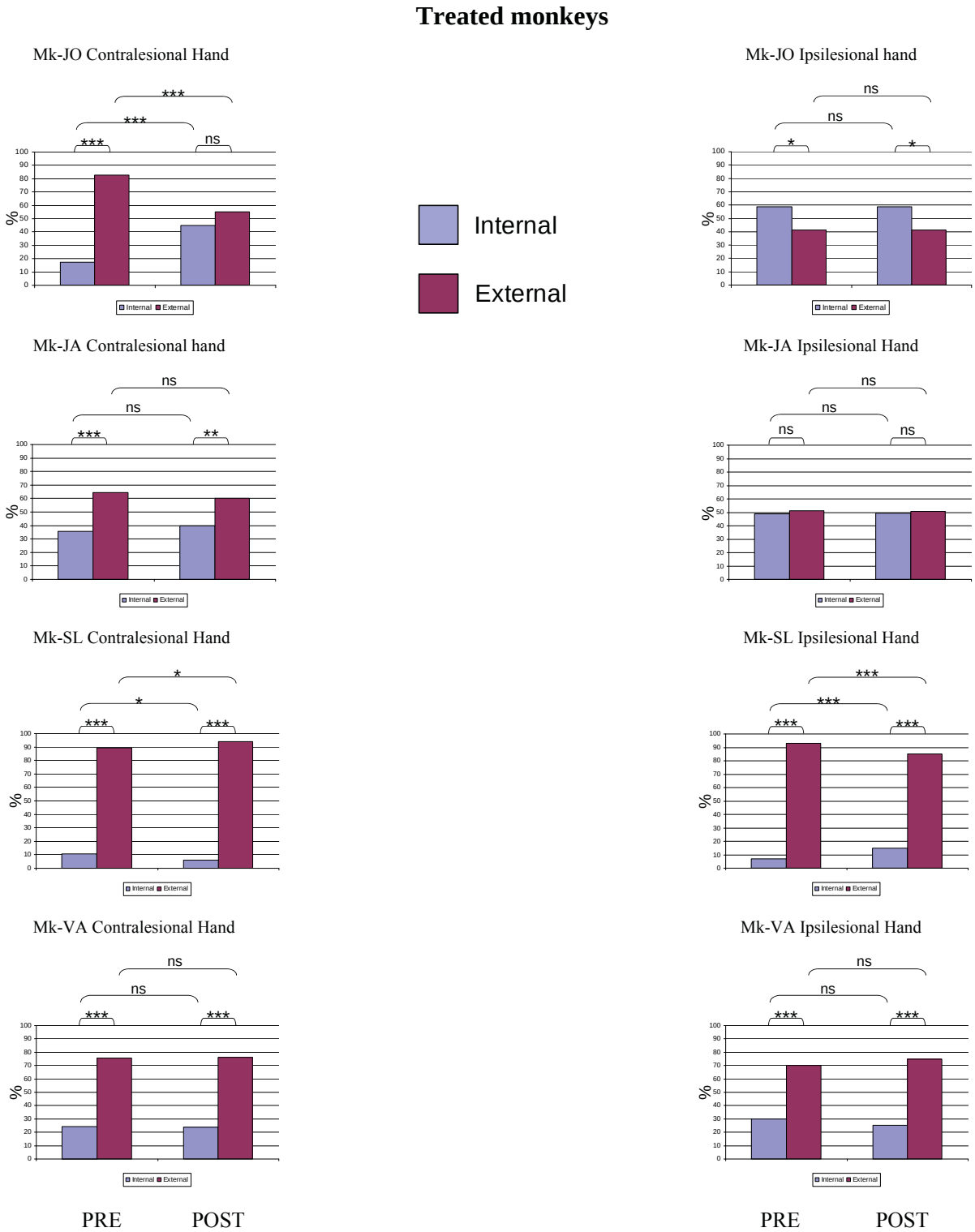


Figure 5.2.1 : Comparisons performed between the pre-lesion and post-lesion plateaux on both hands for each monkey on the two preferential wrist orientations, namely abduction (blue) or adduction (purple), expressed in mean percentage of use. On the left and right sides are represented the contralesional hand and the ipsilesional hand, respectively.

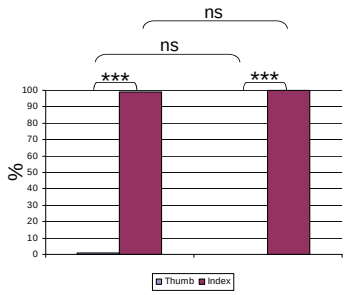
Before the lesion, the preferential use of the abduction (internal) or the adduction (external) to retrieve the horizontal pellets differed from one monkey to another (Figure 5.2.1). This absence of general tendency persisted after the lesion, for both hands. Indeed, after the lesion, three patterns appeared: first, an increase of the adduction and a decrease of the abduction, observed in Mk-AV (control monkey) and Mk-SL (treated monkey) on their contralesional hand, in Mk-CE (control monkey) on both hands and in Mk-GE and Mk-JU (control monkeys) on their ipsilesional hand; second, a decrease of the adduction and an increase of the abduction, found in Mk-RO (control monkey) and Mk-SL (treated monkey) on their ipsilesional hand and in Mk-JO (treated monkey) on his contralesional hand; third, a similar use of each wrist orientation before the lesion, showed by Mk-AV (control) and Mk-JO (treated) on their ipsilesional hand, by Mk-GE, Mk-JU and Mk-RO (control monkeys) on their contralesional hand, and by Mk-JA and Mk-VA (treated monkeys) on both hands.

Therefore, after the lesion, no general trend that could eventually have been linked to the treatment delivery or to the level of recovery was found in relation to wrist orientation used to grasp the pellets in the horizontal wells.

5.2.3.1.2 Preferential finger: horizontal wells

Control monkeys

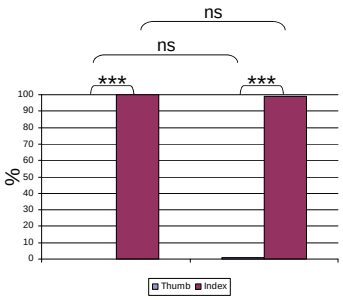
Mk-AV Contralesional Hand



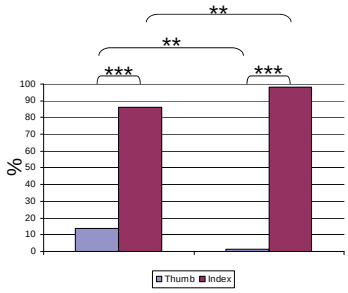
Thumb

Index

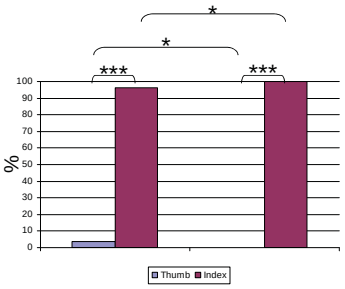
Mk-AV Ipsilesional Hand



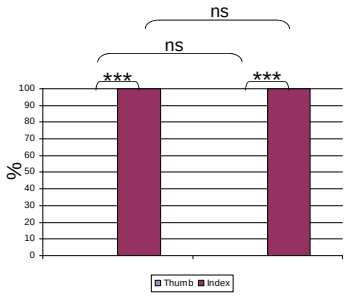
Mk-CE Contralesional Hand



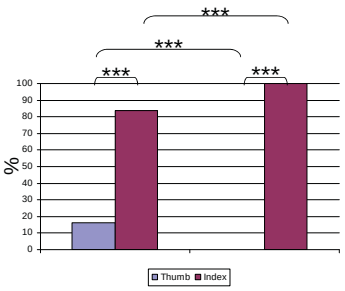
Mk-CE Ipsilesional Hand



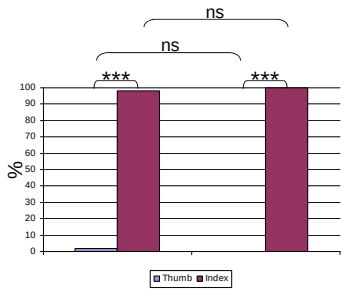
Mk-GE Contralesional Hand



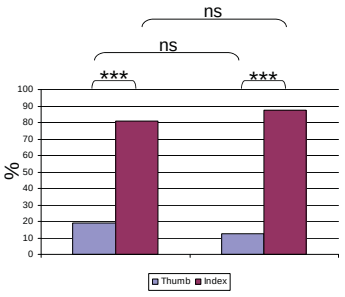
Mk-GE Ipsilesional Hand



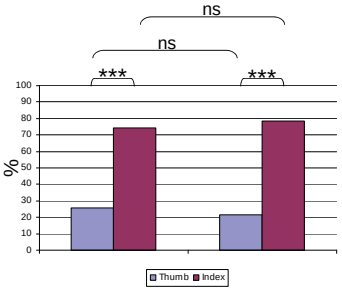
Mk-JU Contralesional Hand



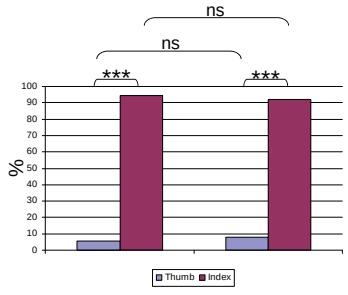
Mk-JU Ipsilesional Hand



Mk-RO Contralesional Hand



Mk-RO Ipsilesional Hand



PRE POST

PRE POST

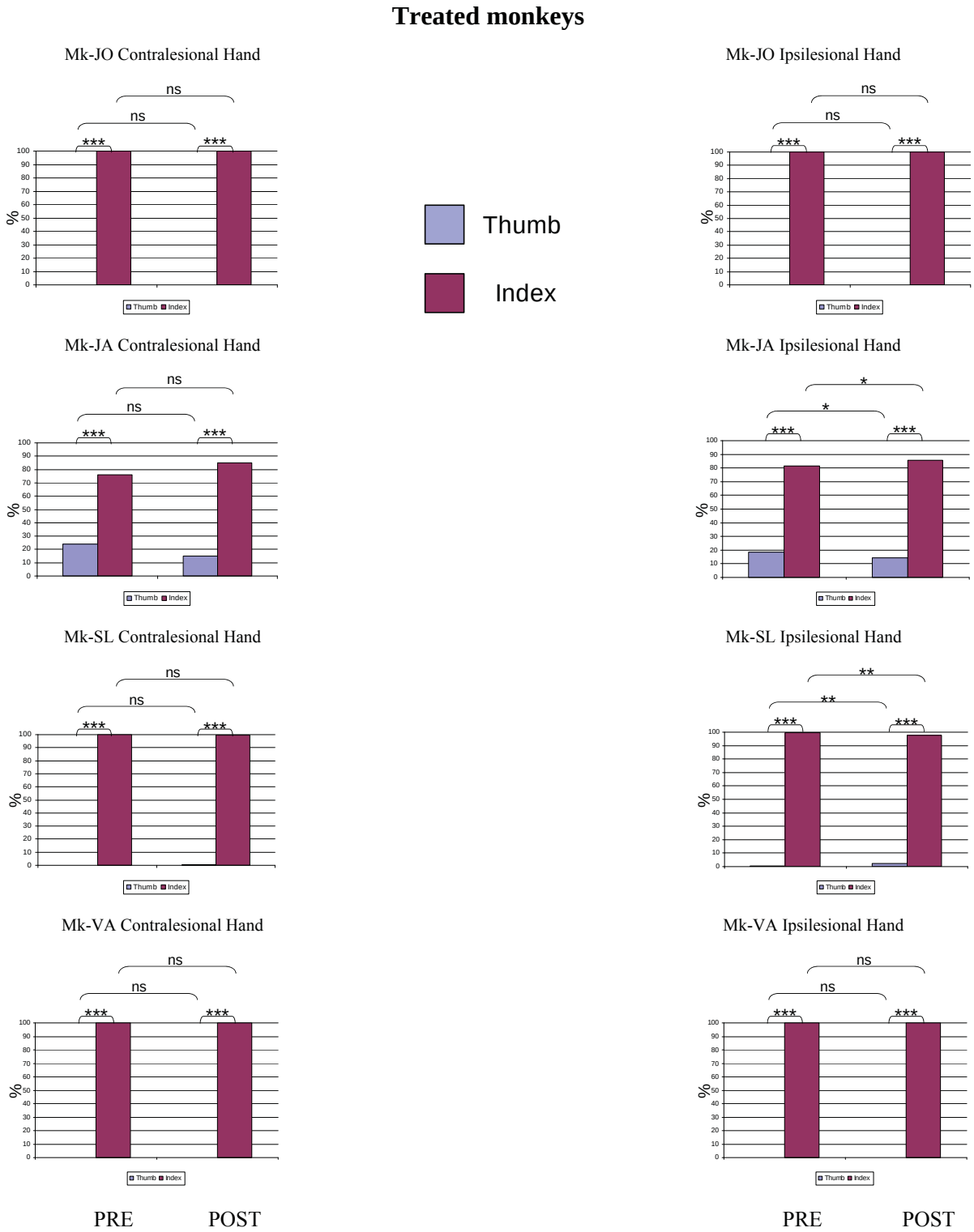
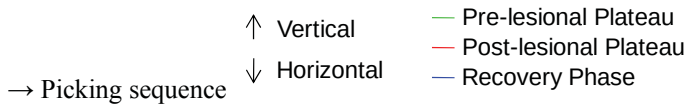


Figure 5.2.2 : Comparisons performed between the pre-lesion and post-lesion plateaux on both hands for each monkey on the two preferential fingers, namely thumb (blue) or index (purple), expressed in mean percentage of use. On the left and right sides are represented the contralesional hand and the ipsilesional hand, respectively.

When looking at the preferential finger used to enter in the well, a main tendency appearing in all the monkeys before the lesion was the few or absence of thumb use (Fig. 5.2.2). After the lesion, on the contralesional hand, no change occurred, except in Mk-CE (control monkey) who showed a significant decrease of the thumb use. On the ipsilesional hand, Mk-CE, Mk-GE (control monkeys) and Mk-JA (treated monkey) had a significant decrease of the thumb use, whereas Mk-SL (treated monkey) showed a significant, even slight, increase of the thumb use. No change occurred in the other monkeys, namely Mk-AV, Mk-JU and Mk-RO (control monkeys), and Mk-JO and Mk-VA (treated monkeys).

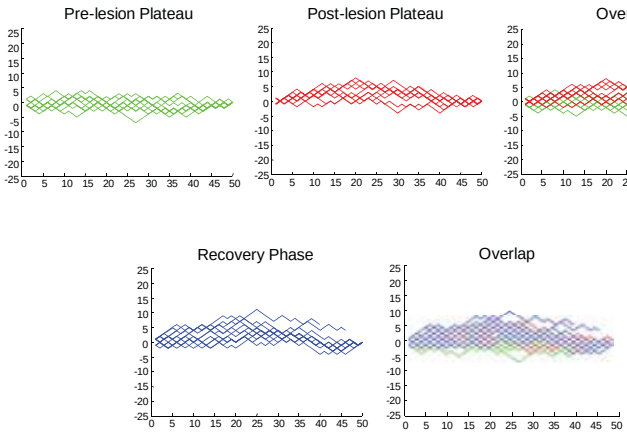
Thus, the thumb was few or even not introduced first in the well, and globally even less after the lesion in the monkeys showing a change pre- versus post-lesion.

5.2.3.1.3 Picking sequence of the vertical and the horizontal wells

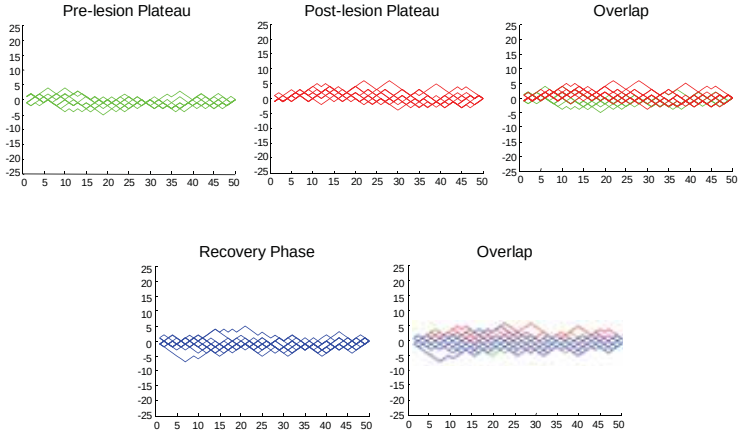


Control monkeys

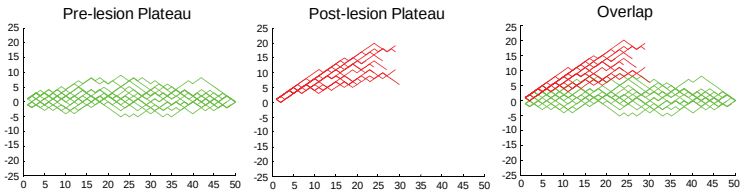
MK-AV Contralesional Hand (Recovery Post: 104%)



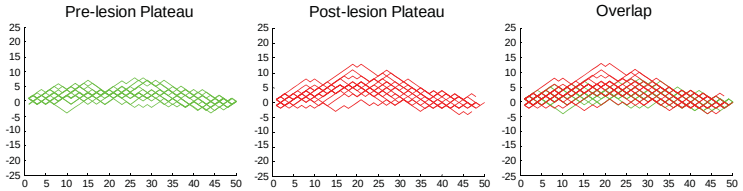
Mk-AV Ipsilesional Hand (Performance Post: 139%)



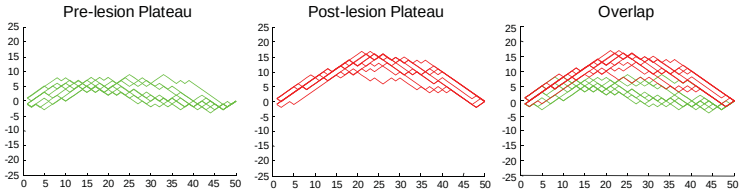
Mk-CE Contralesional Hand (Recovery Post: 42%)



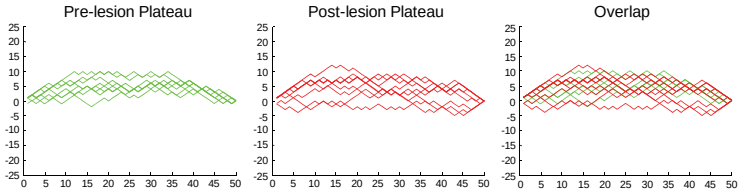
Mk-CE Ipsilesional Hand (Performance Post: 94%)



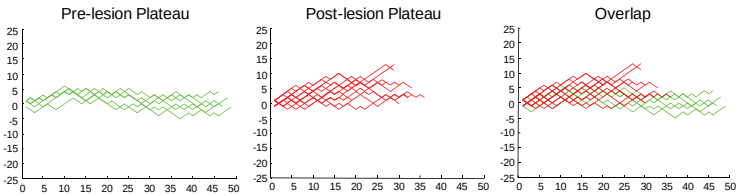
Mk-GE Contralesional Hand (Recovery Post: 38%)



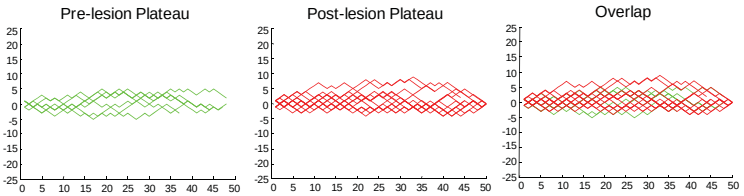
Mk-GE Ipsilesional Hand (Performance Post: 100%)



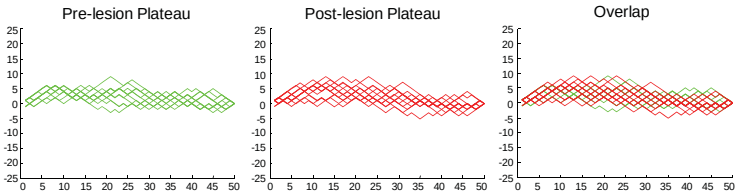
Mk-JU Contralesional Hand (Recovery Post: 39%)



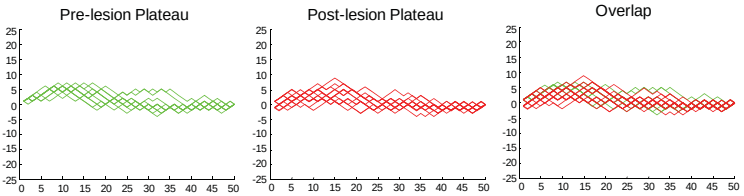
Mk-JU Ipsilesional Hand (Performance Post: 92%)



Mk-RO Contralesional Hand (Recovery Post: 98%)

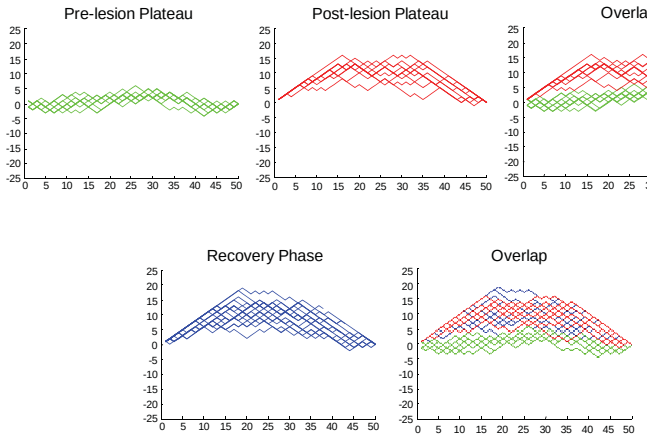


Mk-RO Ipsilesional Hand (Performance Post: 124%)

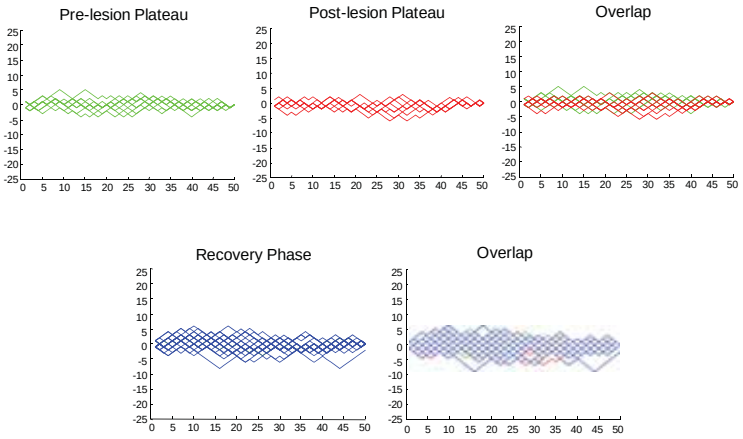


Treated monkeys

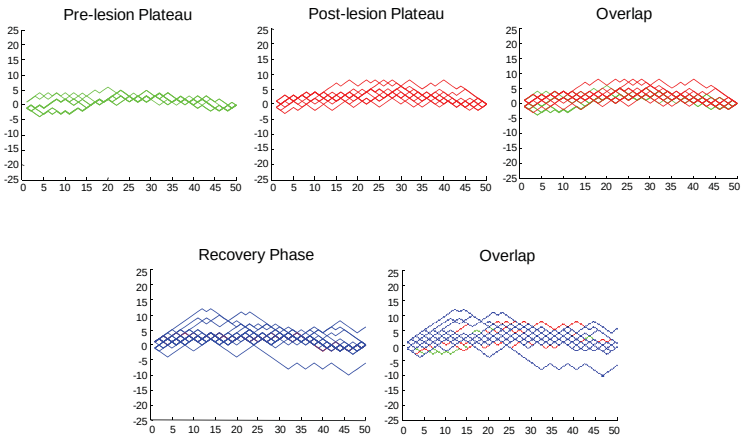
Mk-JO Contralesional Hand (Recovery Post: 59%)



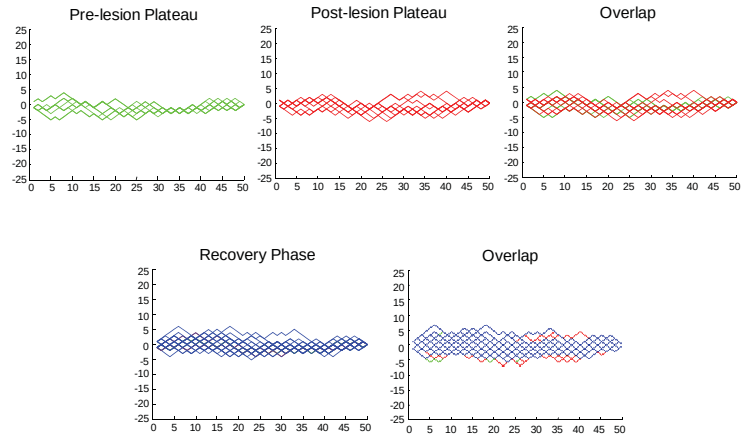
Mk-JO Ipsilesional Hand (Performance Post: 106%)



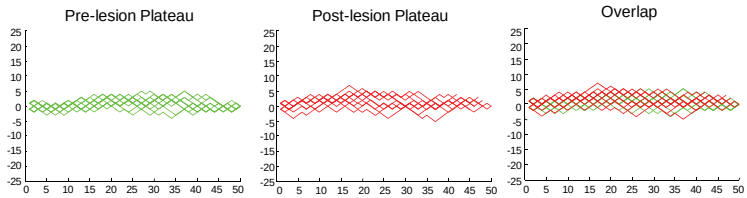
Mk-JA Contralesional Hand (Recovery Post: 100%)



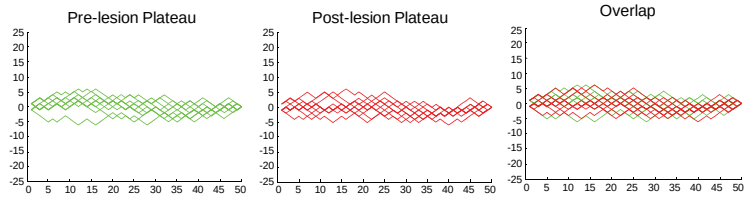
Mk-JA Ipsilesional Hand (Performance Post: 128%)



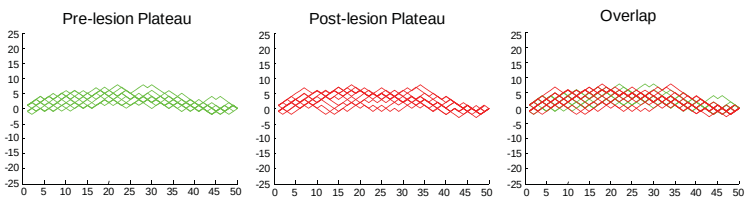
Mk-SL Contralesional Hand (Recovery Post: 79%)



Mk-SL Ipsilesional Hand (Performance Post: 100%)



Mk-VA Contralesional Hand (Recovery Post: 88%)



Mk-VA Ipsilesional hand (Performance Post: 119%)

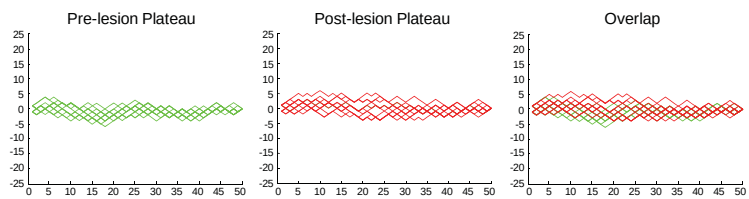


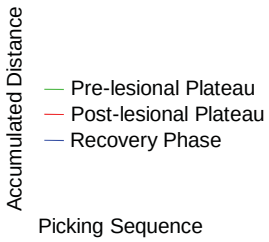
Figure 5.2.3 : Picking sequence of the vertical and the horizontal wells represented for each monkey. X axis represents the picking sequence, whereas on the Y axis, the grasp of a vertical or an horizontal pellet leads to a rise or a fall of 1 unit, respectively. On the left and the right sides are represented the contralesional and the ipsilesional hands, respectively. For each hand, three graphics are presented and correspond to the pre-lesion plateau (green), to the post-lesion plateau (red) and to the overlap. For some monkeys (Mk-AV, Mk-JO and Mk-JA) two other graphics are situated below, representing the recovery phase (blue) and the overlap of the three periods, namely the pre-lesion plateau, the recovery phase and the post-lesion plateau.

When looking at the differences between pre- and post-lesion plateaux on the sequence of prehension in terms of vertical and horizontal wells (Fig. 5.2.3), two main tendencies appeared for the contralesional hand: first, a similar pattern after the lesion as before the lesion, as observed in Mk-RO for the control monkeys and in Mk-JA, Mk-SL and Mk-VA for the treated ones, grasping the pellets as much in the vertical wells as in the horizontal wells along the task ; second, a tendency to grasp more pellets in the vertical wells at the beginning of the task after the lesion, as seen in Mk-AV, Mk-CE, Mk-GE and Mk-JU for the control monkeys and in Mk-JO for the treated ones. For the ipsilesional hand, no difference was observed in the treated monkeys and in Mk-AV and Mk-RO for the control monkeys. Mk-CE and Mk-JU showed some sessions with a tendency to take more pellets in the vertical wells than in the horizontal wells, and there was more variability in Mk-GE after the lesion than before the lesion.

Therefore, these results indicated that, for the contralesional hand, the monkeys recovering less well on the total score -except Mk-AV- showed more differences after the lesion, taking preferentially the pellets in the vertical wells first. For the ipsilesional hand, the differences observed occurred also in monkeys having a post-lesion score performance generally less high, except for Mk-GE.

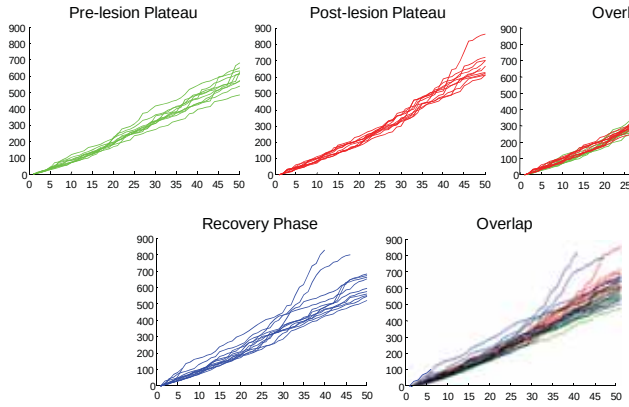
To note that the recovery phase seemed globally very similar to the post-lesion plateau, except for the contralesional hand of Mk-JA, with more variable sessions.

5.2.3.1.4 Cumulative Distance: all wells

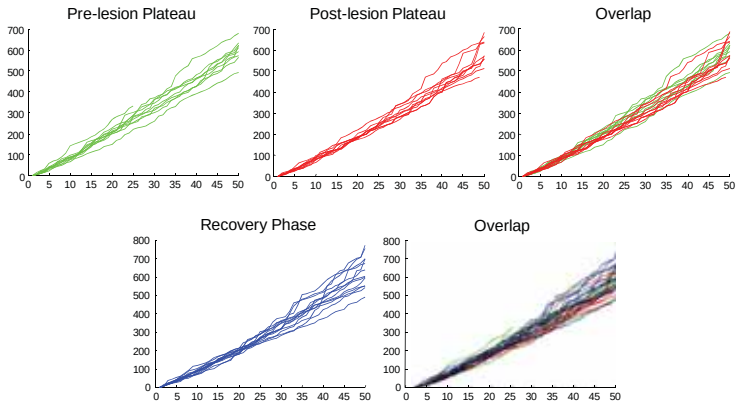


Control monkeys

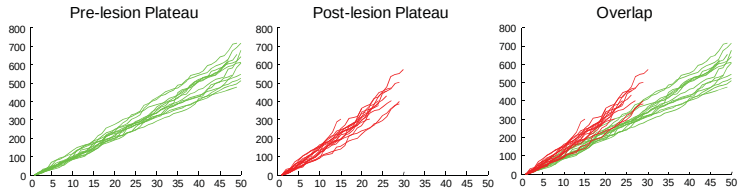
MK-AV Contralesional Hand (Recovery Post: 104%)



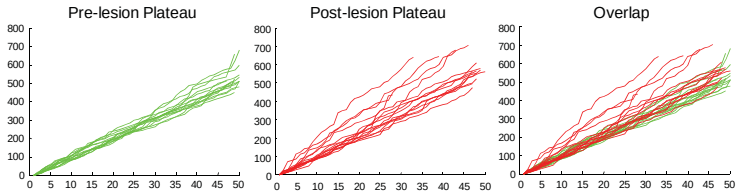
Mk-AV Ipsilesional Hand (Performance Post: 139%)



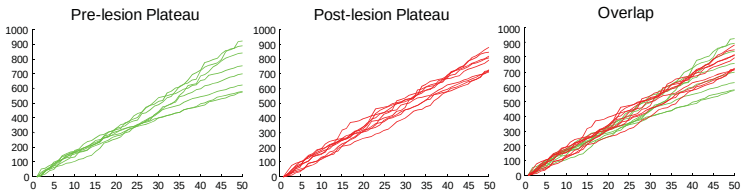
Mk-CE Contralesional Hand (Recovery Post: 42%)



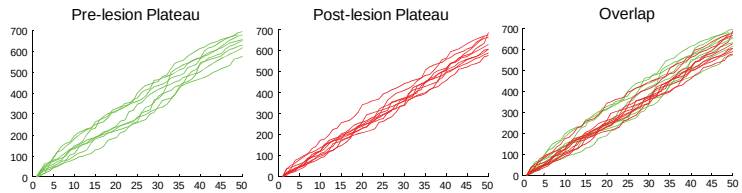
Mk-CE Ipsilesional Hand (Performance Post: 94%)



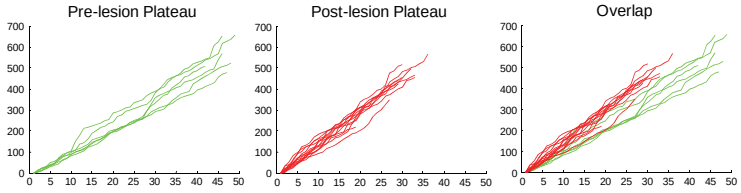
Mk-GE Contralesional Hand (Recovery Post: 38%)



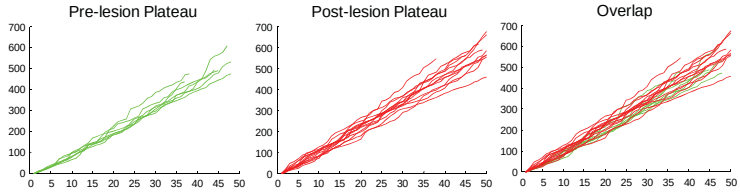
Mk-GE Ipsilesional Hand (Performance Post: 100%)



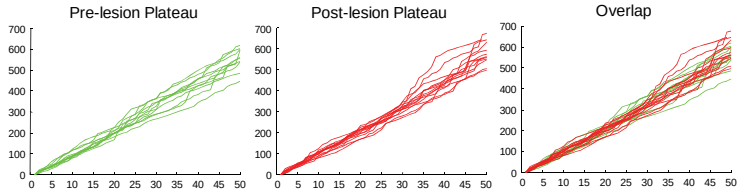
Mk-JU Contralesional Hand (Recovery Post: 39%)



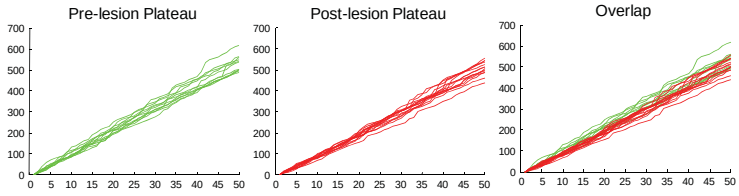
Mk-JU Ipsilesional Hand (Performance Post: 92%)



Mk-RO Contralesional Hand (Recovery Post: 98%)

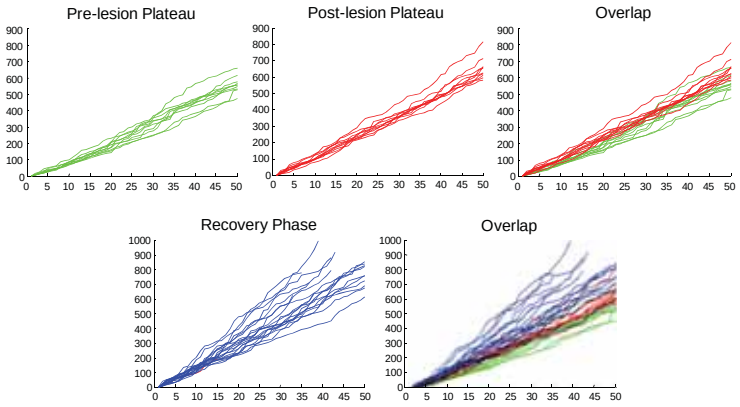


Mk-RO Ipsilesional Hand (Performance Post: 124%)

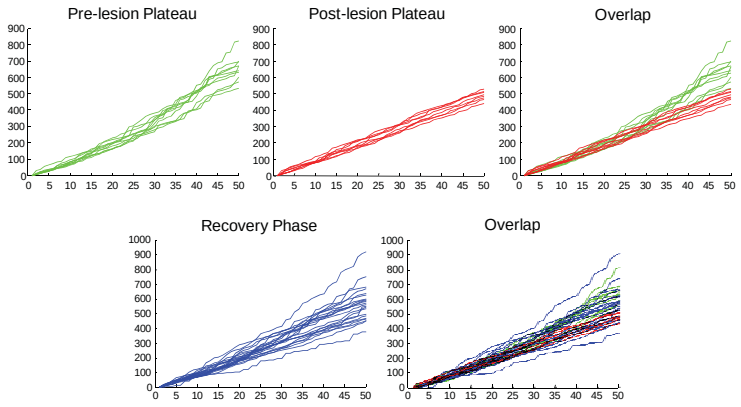


Treated monkeys

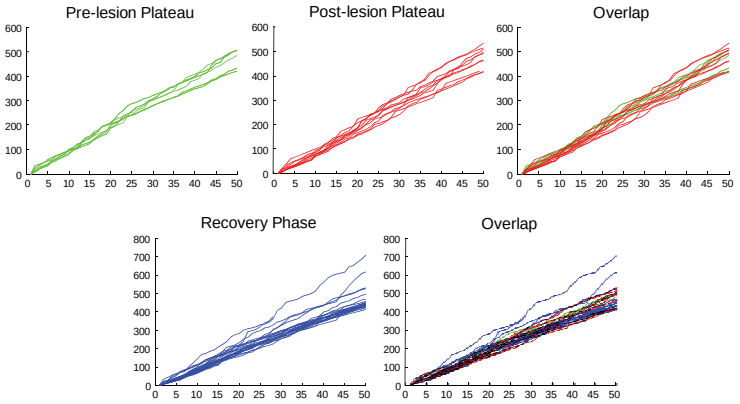
Mk-JO Contralesional Hand (Recovery Post: 59%)



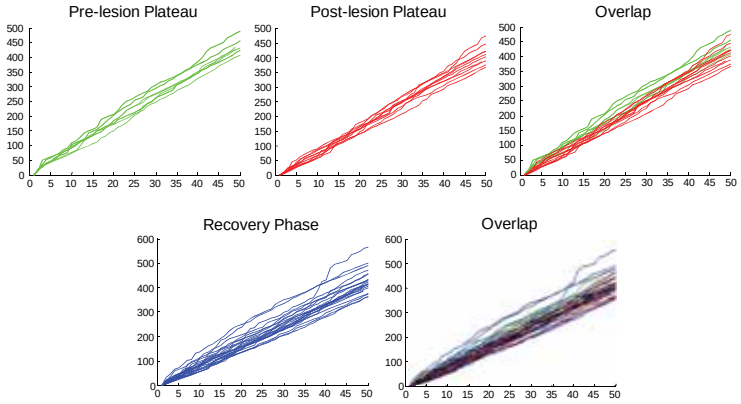
Mk-JO Ipsilesional Hand (Performance Post: 106%)



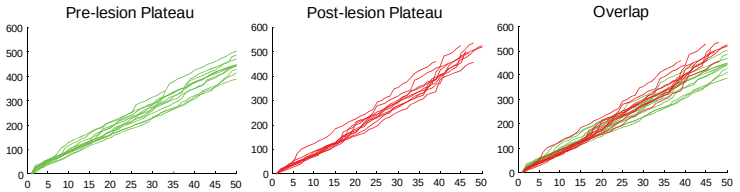
Mk-JA Contralesional Hand (Recovery Post: 100%)



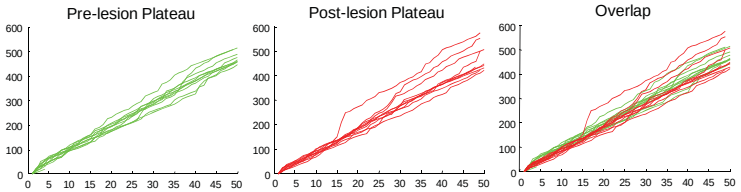
Mk-JA Ipsilesional Hand (Performance Post: 128%)



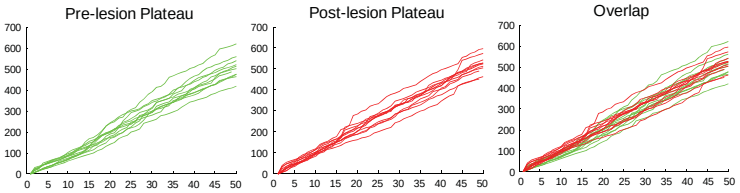
Mk-SL Contralesional Hand (Recovery Post: 79%)



Mk-SL Ipsilesional Hand (Performance Post: 100%)



Mk-VA Contralesional Hand (Recovery Post: 88%)



Mk-VA Ipsilesional Hand (Performance Post: 119%)

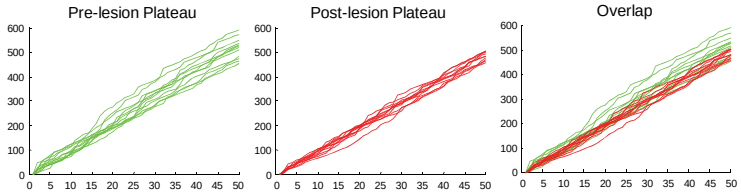


Figure 5.2.4 : Cumulative distance to perform the modified Brinkman board task, represented for each monkey. X axis represents the picking sequence, whereas on the Y axis appears the cumulative distance (mm) when grasping the pellets along the performance of the task. On the left and the right sides are represented the contralesional and the ipsilesional hands, respectively. For each hand, three graphics are presented and correspond to the pre-lesion plateau (green), to the post-lesion plateau (red) and to the overlap. For some monkeys (Mk-AV, Mk-JO and Mk-JA) two other graphics are situated below, representing the recovery phase (blue) and the overlap of the three periods, namely the pre-lesion plateau, the recovery phase and the post-lesion plateau.

Concerning the cumulative distance covered by the hand to perform the task, a similar pattern to the one observed above appeared, namely post-lesion differences in the monkeys recovering less on the total score, in the sense of a less favorable strategy as expressed by longer cumulative distances. Concretely, as shown in figure 5.2.4, with their contralesional hand, Mk-RO (control monkey) and Mk-JA and Mk-VA (treated monkeys) kept similar cumulative distances after the lesion, whereas Mk-AV, Mk-CE, Mk-JU and to a lesser extent Mk-GE (control monkeys) and Mk-JO and Mk-SL (treated monkeys) showed increased cumulative distances on the post-lesion plateau compared to the pre-lesion plateau. For the ipsilesional hand, the monkeys having a higher score performance after than before the lesion, namely Mk-RO, Mk-JO, Mk-JA and Mk-VA, showed an improvement illustrated by shorter cumulative distances, except Mk-AV, whose post-lesion cumulative distances were comparable to the pre-lesion ones, a pattern shared by Mk-GE, having the same 100% post-lesion score performance as Mk-SL, who showed a post-lesion greater variability. Finally, Mk-CE and Mk-JU, whose post-lesion score performances were slightly worse than before the lesion, presented increased cumulative distances after the lesion.

Therefore, these results showed that the monkeys recovering less with their contralesional hand as assessed by the total score showed larger less favorable cumulative distances and thus a less optimal strategy after the lesion (fig. 5.2.5). Furthermore, for the ipsilesional hand, the same pattern was found, with even a tendency to improve the strategy in terms of cumulative distance in the monkeys showing an improvement of their post-lesion score performance (fig. 5.2.5).

To note here that there was a more important variability on the recovery phase, mainly in the sense of a larger cumulative distance to perform the task.

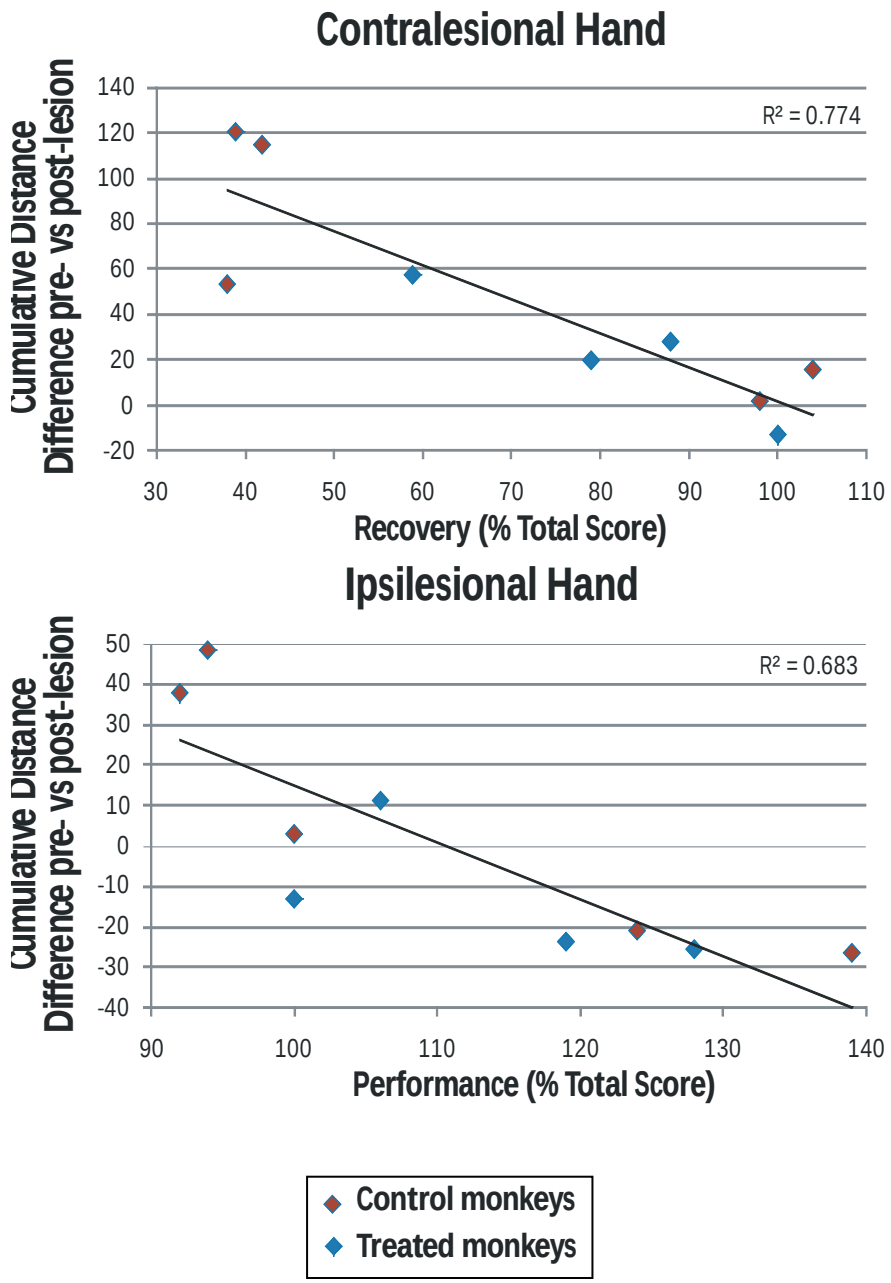
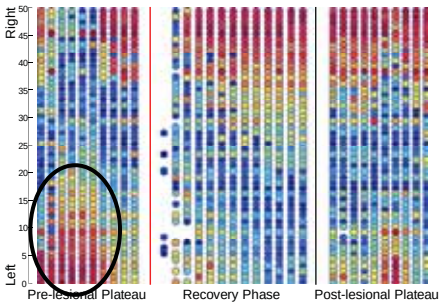


Figure 5.2.5: Plot of the difference between pre-lesion cumulative distance and post-lesion cumulative distance (mm) as a function of the rate of post-lesion manual performance with regard to the pre-lesion one. Red rhombus represent the control monkeys and blue rhombus the monkeys treated either with autologous brain cells or with Anti-Nogo-A antibody.

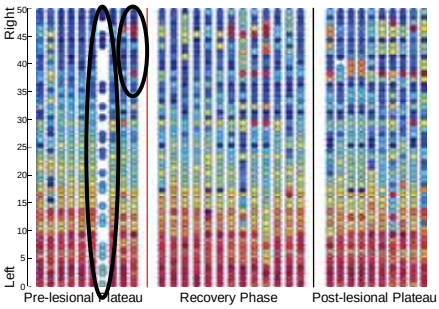
5.2.3.1.5 Picking Sequence on the left-right axis

Control monkeys

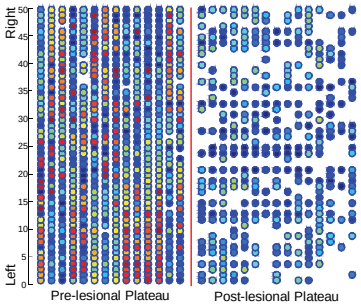
MK-AV Contralesional Hand (Recovery Post: 104%)



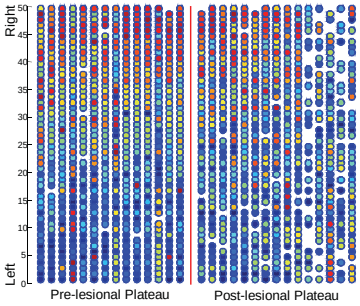
Mk-AV Ipsilesional Hand (Performance Post: 139%)



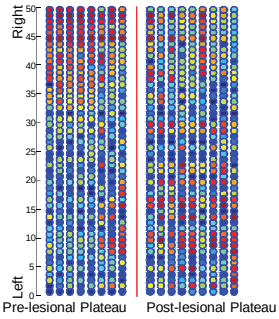
Mk-CE Contralesional Hand (Recovery Post: 42%)



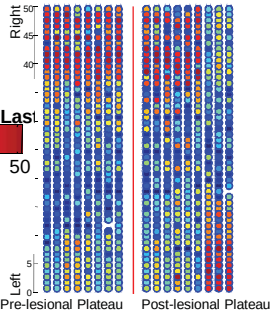
Mk-CE Ipsilesional Hand (Performance Post: 94%)

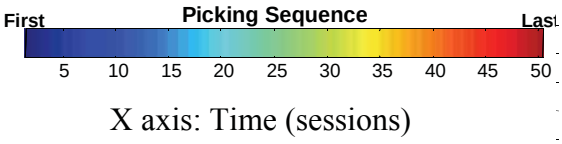


Mk-GE Contralesional Hand (Recovery Post: 38%)



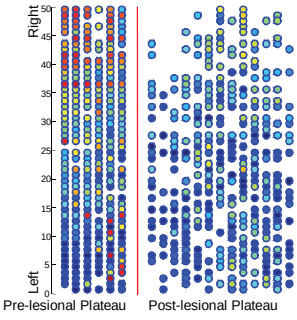
Mk-GE Ipsilesional Hand (Performance Post: 100%)



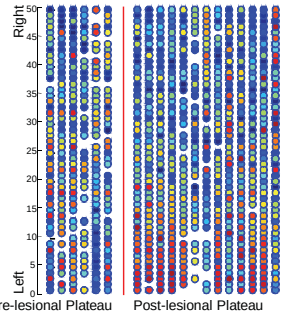


X axis: Time (sessions)

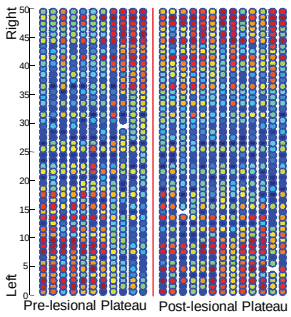
Mk-JU Contralesional Hand (Recovery Post: 39%)



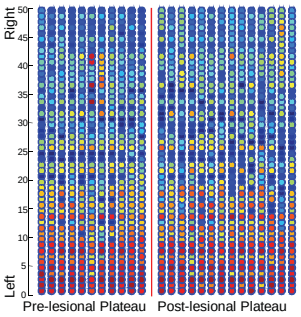
Mk-JU Ipsilesional Hand (Performance Post: 92%)



Mk-RO Contralesional Hand (Recovery Post: 98%)

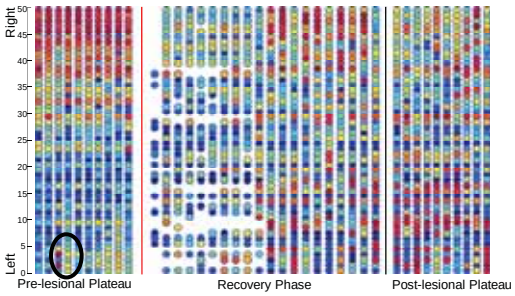


Mk-RO Ipsilesional Hand (Performance Post: 124%)

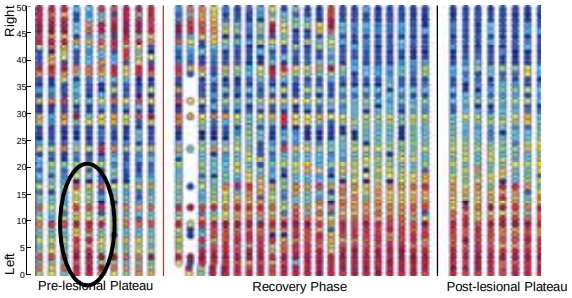


Treated monkeys

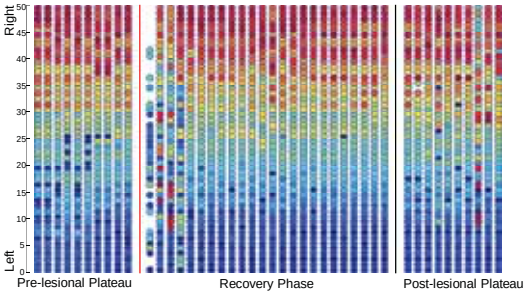
Mk-JO Contralesional Hand (Recovery Post: 59%)



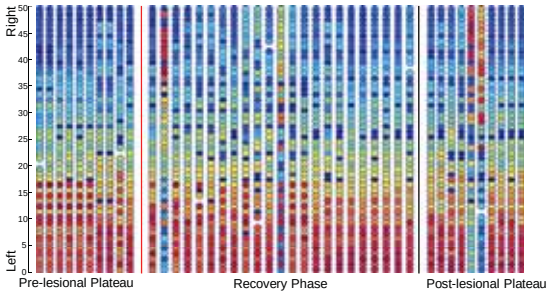
Mk-JO Ipsilesional Hand (Performance Post: 106%)



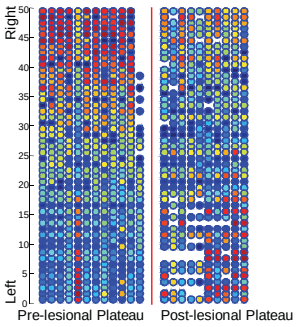
Mk-JA Contralesional Hand (Recovery Post: 100%)



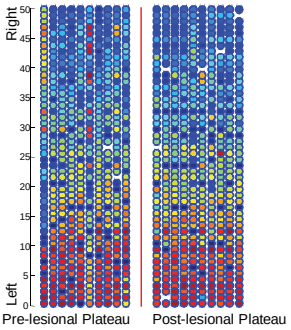
Mk-JA Ipsilesional Hand (Performance Post: 128%)



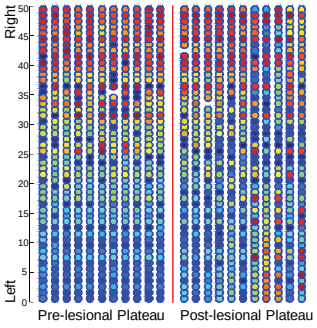
Mk-SL Contralesional Hand (Recovery Post: 79%)



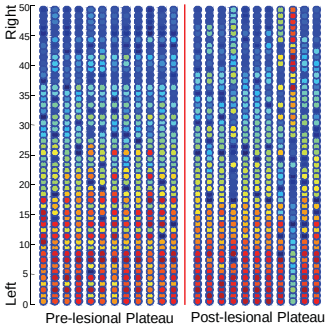
Mk-SL Ipsilesional Hand (Performance Post: 100%)



Mk-VA Contralesional Hand (Recovery Post: 88%)



Mk-VA Ipsilesional Hand (Performance Post: 119%)



⟶Time (sessions)

⟶Time (sessions)

Figure 5.2.6 : Picking sequence on the left-right axis in the modified Brinkman board task for each monkey. The contralesional and ipsilesional hands are placed on the left and right columns, respectively. X axis represents training sessions, one column corresponding thus to one training session. Y axis represents the 50 wells of the board, ordered according to their position on the left-right axis, independently of their position on the up-down axis. Colors indicate the picking sequence, ranging from 1 (blue) to 50 (red). Red vertical lines represent the lesion. For Mk-AV, Mk-JO and Mk-JA, black vertical lines separate the recovery phase from the post-lesion plateau. In Mk-AV and Mk-JO, black ovals indicate the effect of the dlPFC biopsy (see Results chapter 3.3) on the pre-lesion plateaux.

First of all, the present results indicated that before the lesion, almost all monkeys performed the task with a given strategy, except Mk-CE with his contralesional hand, Mk-JU with his ipsilesional hand, and possibly Mk-GE and Mk-JU with their contralesional hand (Fig. 5.2.6). To note the effect of the dlPFC biopsy (see "Does the Right Dorsolateral Prefrontal Cortical Biopsy have an Effect on the Performance of the Monkeys?", in the Results part) on the pre-lesion plateaux in Mk-AV and Mk-JO, indicated by black ovals.

After the lesion, for the contralesional hand, no change in the prehension strategy occurred in Mk-JA and Mk-VA (treated monkeys) and in Mk-AV, Mk-CE, Mk-JU (these two last monkeys did not grasp all the pellets) and Mk-GE (control monkeys). Before the lesion, Mk-RO (treated monkey) showed a picking sequence going either from the left to the right side or from the right to the left side, a strategy that changed after the lesion towards a beginning at the center of the board and an end at the two extreme parts (left and right). Mk-JO and Mk-SL also changed their strategy of prehension, beginning from the left and finishing on the right side before the lesion, and having a randomly distributed order of prehension on the left-right axis after the lesion for Mk-JO and a multiple prehension strategy for Mk-SL, beginning sometimes on one side to end on the other side, sometimes on the center of the board to end on one or the other side, or both.

For the ipsilesional hand, Mk-AV, Mk-CE, Mk-GE and Mk-RO (control monkeys) and Mk-JA, Mk-SL and Mk-VA (treated monkeys) did not show any change in the order in which they grasped the pellets in the left-right axis. Mk-JU had a quite randomly distributed order of prehension before the lesion, changing then to a more defined strategy going mainly from the right to the left side. For his part, before the lesion, Mk-JO grasped first the pellets situated on the center of the board, then those on the left side, ending finally on the right side (except the sessions following the biopsy). After the lesion, on the post-lesion plateau, he grasped the pellets from the right side to the left side of the board.

The recovery phase was similar to the post-lesion plateau in all the monkeys, with the first sessions showing a transitory effect of the lesion on the order of prehension, except for the ipsilesional hand in Mk-AV and Mk-JA on which no change appeared.

Overall, the main tendency showed an absence of change of picking sequence on the post-lesion plateau compared to the pre-lesion plateau. Nevertheless, in Mk-RO (control) and Mk-SL (treated) for their contralesional hand, in Mk-JU (control) for his ipsilesional hand and in Mk-JO (treated) for his both hands, a change occurred after the lesion.

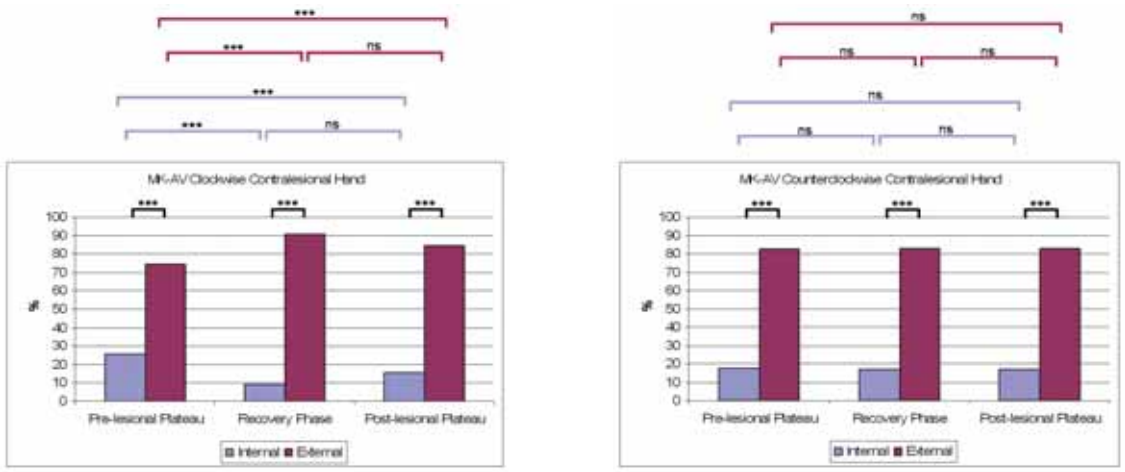
To note that none of the monkeys showed any structured strategy on the up-down axis of the board.

5.2.3.2 Rotating Brinkman board task (n=3)

5.2.3.2.1 Preferential wrist orientation

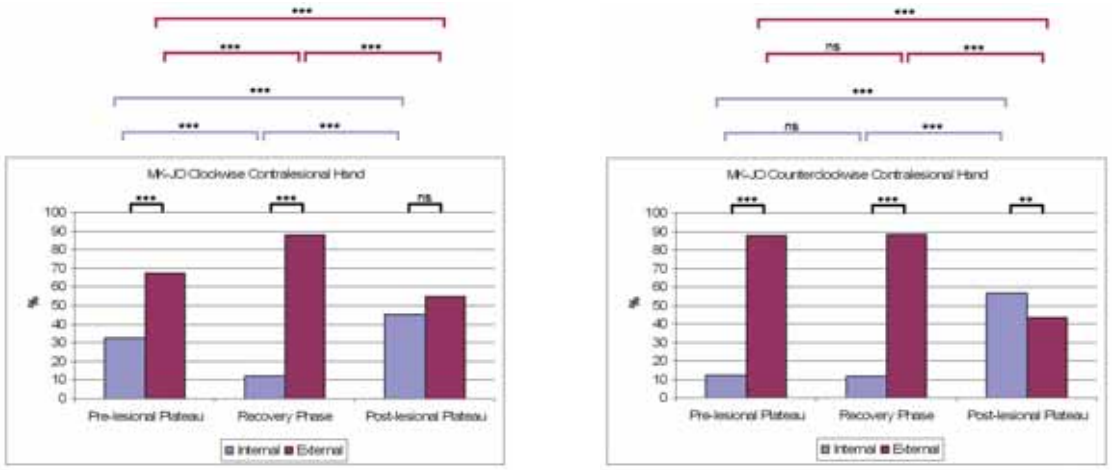
Control monkey

Mk-AV Contralesional Hand



Treated monkeys

Mk-JO Contralesional Hand



Mk-JA Contralesional Hand

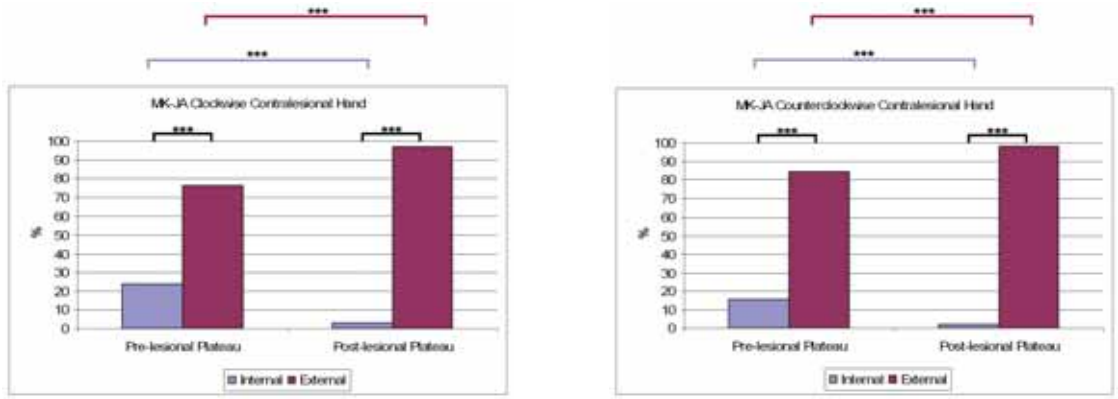


Figure 5.2.7: Comparisons performed for the contralesional hand of each monkey on the two preferential wrist orientations, namely abduction (blue) or adduction (purple), expressed in mean percentage of use, on the pre-lesion and post-lesion plateaux in Mk-AV and Mk-JO and Mk-JA, and on the recovery phase in Mk-AV and Mk-JO. The two orientations of rotation, clockwise and counterclockwise, are represented on the left and right columns, respectively.

For the rotating Brinkman board task, before the lesion, when the board rotated either clockwise or counterclockwise, the preferential wrist orientation was the adduction for all the monkeys, as shown in figure 5.2.7.

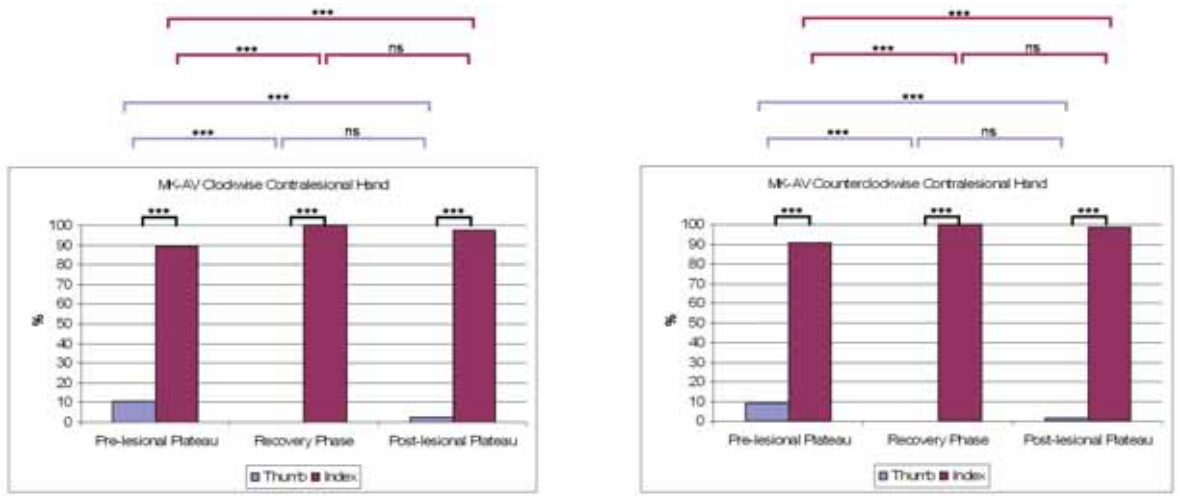
After the lesion, when the board rotated clockwise, the main difference was in the sense of a decrease of the use of the abduction, except in Mk-JO, who showed, after such a decrease on the recovery phase, an increase on the post-lesion phase, leading to a shared use of the two wrist orientations.

When the board rotated counterclockwise, no difference was found in Mk-AV in both the recovery phase and the post-lesion plateau. On his recovery phase, Mk-JO did not show any change, whereas on the post-lesion plateau, there was an increase in his use of the abduction, becoming his preferential wrist orientation. In Mk-JA, the use of the abduction was significantly decreased on the post-lesion plateau.

5.2.3.2.2 Preferential finger

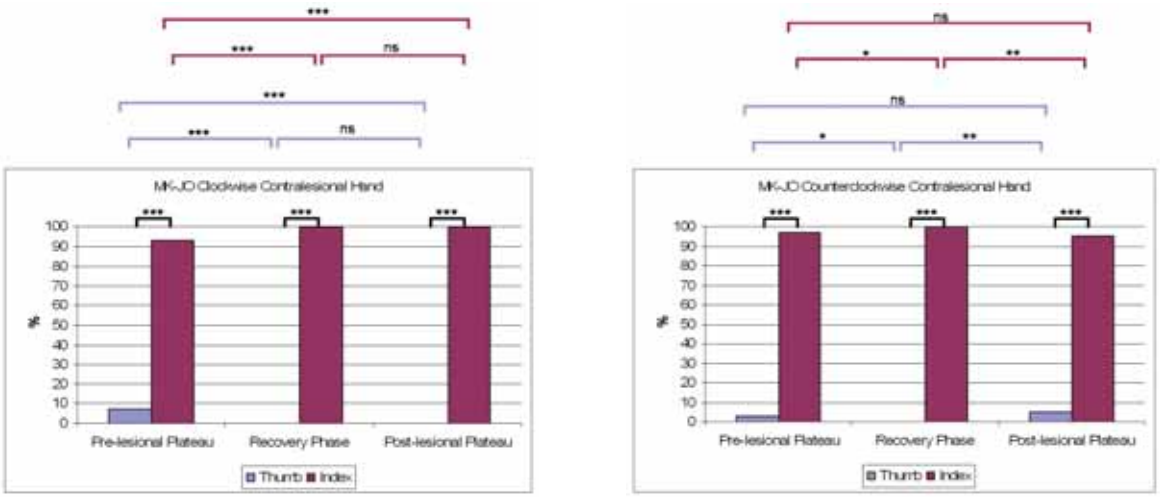
Control monkey

Mk-AV Contralesional Hand



Treated monkeys

Mk-JO Contralesional Hand



Mk-JA Contralesional Hand

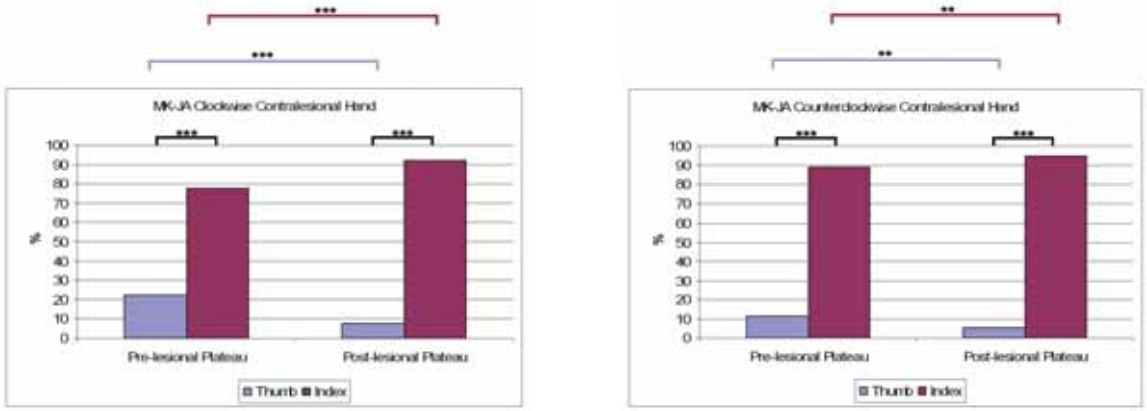


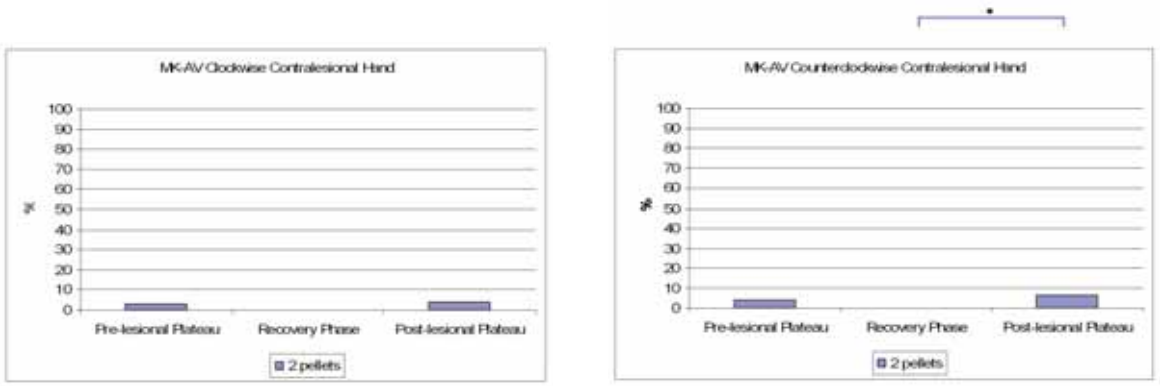
Figure 5.2.8: Comparisons performed for the contralesional hand of each monkey on the two preferential fingers to enter in the wells, namely thumb (blue) or index (purple), expressed in mean percentage of use, on the pre-lesion and post-lesion plateaux in Mk-AV and Mk-JO and Mk-JA, and on the recovery phase in Mk-AV and Mk-JO. The two orientations of rotation, clockwise and counterclockwise, are represented on the left and right columns, respectively.

The main tendency appearing before the lesion for each orientation was a preferential use of the index to enter in the well, pushing then the pellet with the thumb to grasp it (Fig. 5.2.8). After the lesion, a decrease in the introduction of the thumb first occurred in all the monkeys and for each orientation, with an exception in Mk-JO under the form of a return to the pre-lesion level of the thumb use.

5.2.3.2.3 Retrieval of 2 pellets at the same time

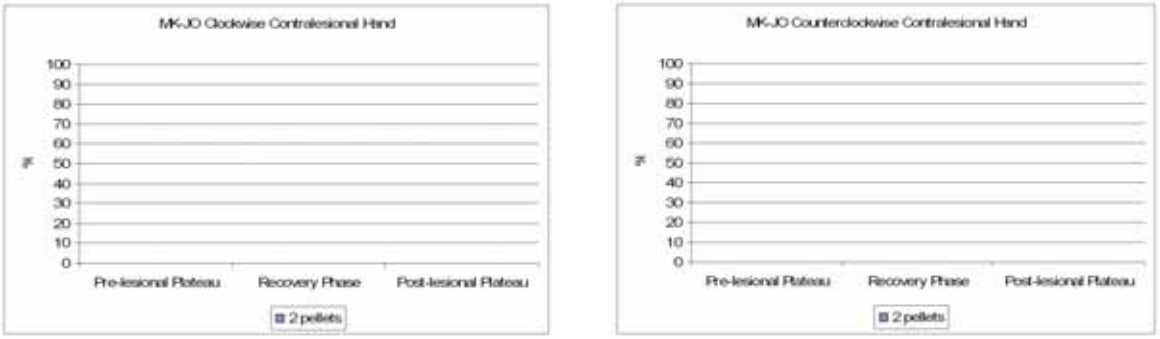
Control monkey

Mk-AV Contralesional Hand



Treated monkeys

Mk-JO Contralesional Hand



Mk-JA Contralesional Hand

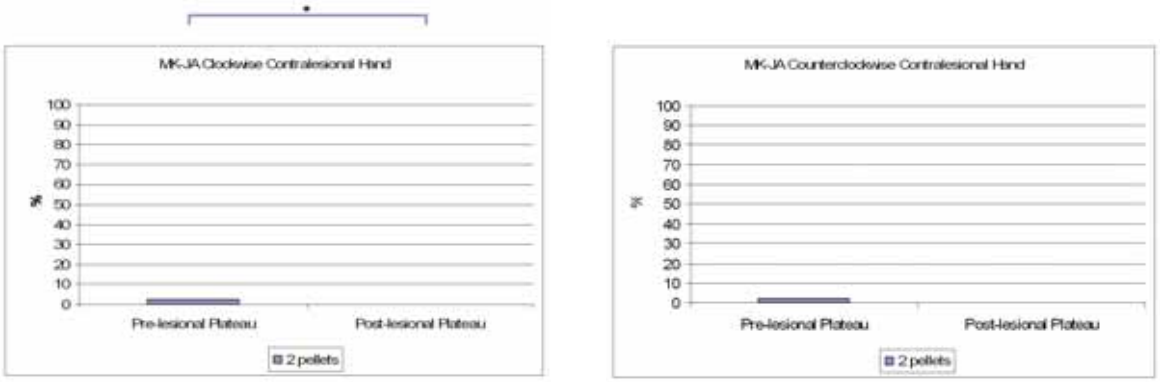


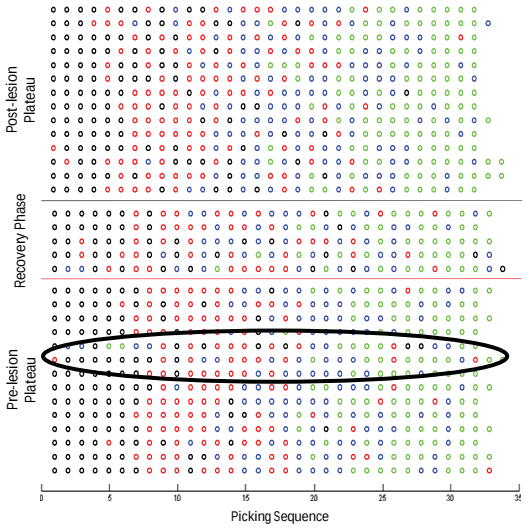
Figure 5.2.9: Comparisons performed for the contralesional hand of each monkey on the grasp of two pellets at the same time before bringing them to the mouth, expressed in mean percentage of occurrence, on the pre-lesion and post-lesion plateaux in Mk-AV and Mk-JO and Mk-JA, and on the recovery phase in Mk-AV and Mk-JO. The two orientations of rotation, clockwise and counterclockwise, are represented on the left and right columns, respectively.

Figure 5.2.9 shows that the prehension of two pellets at the same time before bringing them to the mouth was not used often by the monkeys, even not at all by Mk-JO. After the lesion, Mk-JA never used this strategy anymore, whereas Mk-AV, after a disappearance of this strategy of prehension on the recovery phase, re-used it on the post-lesion plateau.

5.2.3.2.4 Picking sequence on the four rings

Control monkey

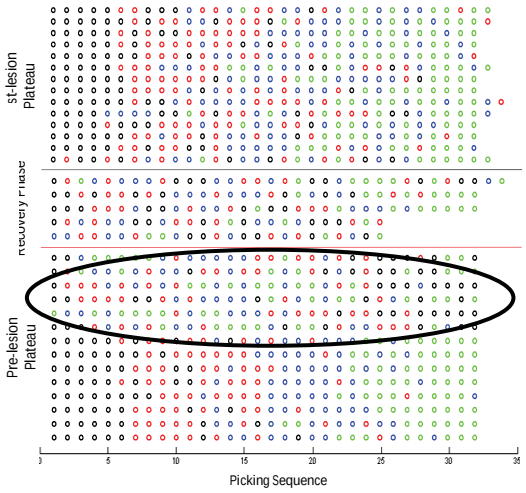
Mk-AV Contralesional Hand Clockwise



↑ Time (sessions)

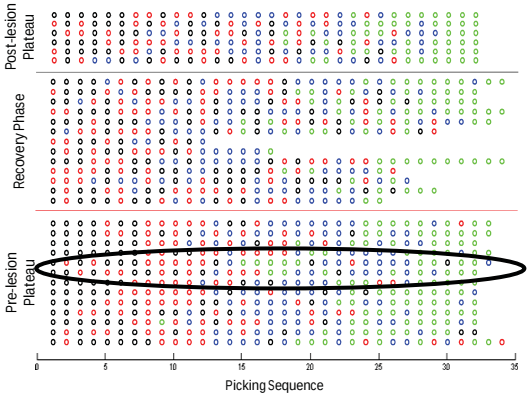
- Ring 1 (most external)
- Ring 2
- Ring 3
- Ring 4 (most internal)

Mk-AV Contralesional Hand Counterclockwise

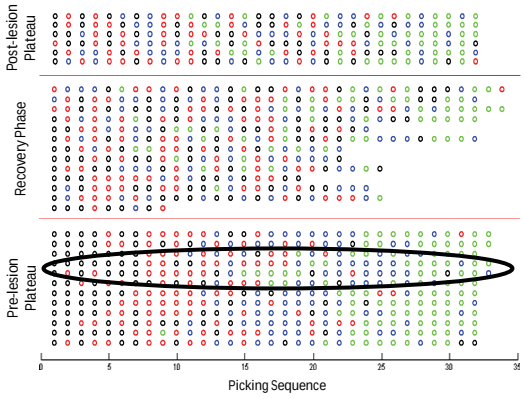


Treated monkeys

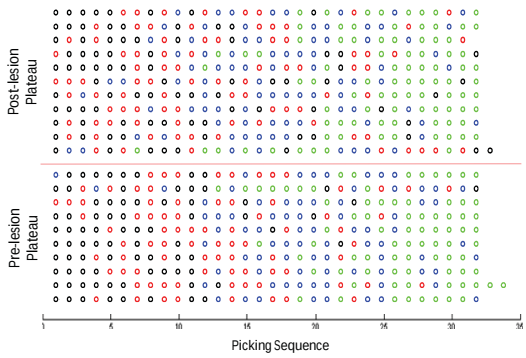
Mk-JO Contralesional Hand Clockwise



Mk-JO Contralesional Hand Counterclockwise



Mk-JA Contralesional Hand Clockwise



Mk-JA Contralesional hand Counterclockwise

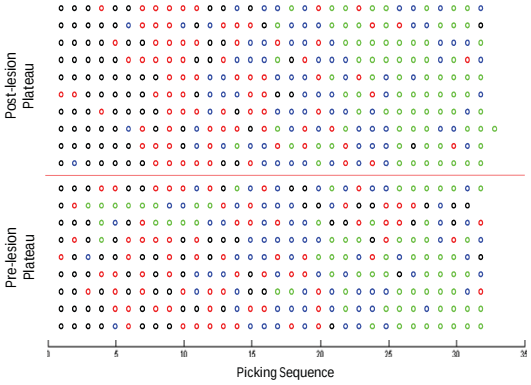


Figure 5.2.10: Picking sequence on the four rings of the rotating Brinkman board task for each monkey. The clockwise and counterclockwise rotation orientations are placed on the left and right columns, respectively. X axis represents the picking sequence, each retrieval being coloured according to the corresponding ring (black for the first, red for the second, blue for the third and green for the fourth). Y axis indicates the training sessions, one line corresponding thus to one training session. Red horizontal lines represent the lesion. For Mk-AV and Mk-JO, horizontal black lines separate the recovery phase from the post-lesion plateau. In Mk-AV and Mk-JO, black ovals indicate the effect of the dlPFC biopsy on the pre-lesion plateaux (see "Does the Right Dorsolateral Prefrontal Cortical Biopsy have an Effect on the Performance of the Monkeys?" in the Results part).

Before the lesion, irrespective of the rotation direction, there was a general tendency to grasp the pellets in a sequence going from the most external ring (1) to the most internal ring (4), meaning that the monkeys took the pellets that were the closest to him (Fig. 5.2.10). As Mk-AV and Mk-JO had some effects on this strategy of prehension after they were subjected to a right dlPFC biopsy (see "Does the Right Dorsolateral Prefrontal Cortical Biopsy have an Effect on the Performance of the Monkeys?" in the Results part), it was a little difficult to distinguish this effect to the effect of the lesion, especially in Mk-AV who had a more long-lasting change after the biopsy when the board rotated counterclockwise.

Nevertheless, it seemed that in Mk-AV, there was no change after the lesion when the board rotated clockwise and no supplementary change to what happened after the biopsy when the board rotated counterclockwise. A slight effect of the lesion was observed in Mk-JO, who grasped pellets from more internal rings while ending to take them in the most external ones, especially when the rotation was counterclockwise. For his part, Mk-JA did not show any change in his picking sequence on the four rings, irrespective of the rotation direction.

5.2.3.2.5 Prehension area

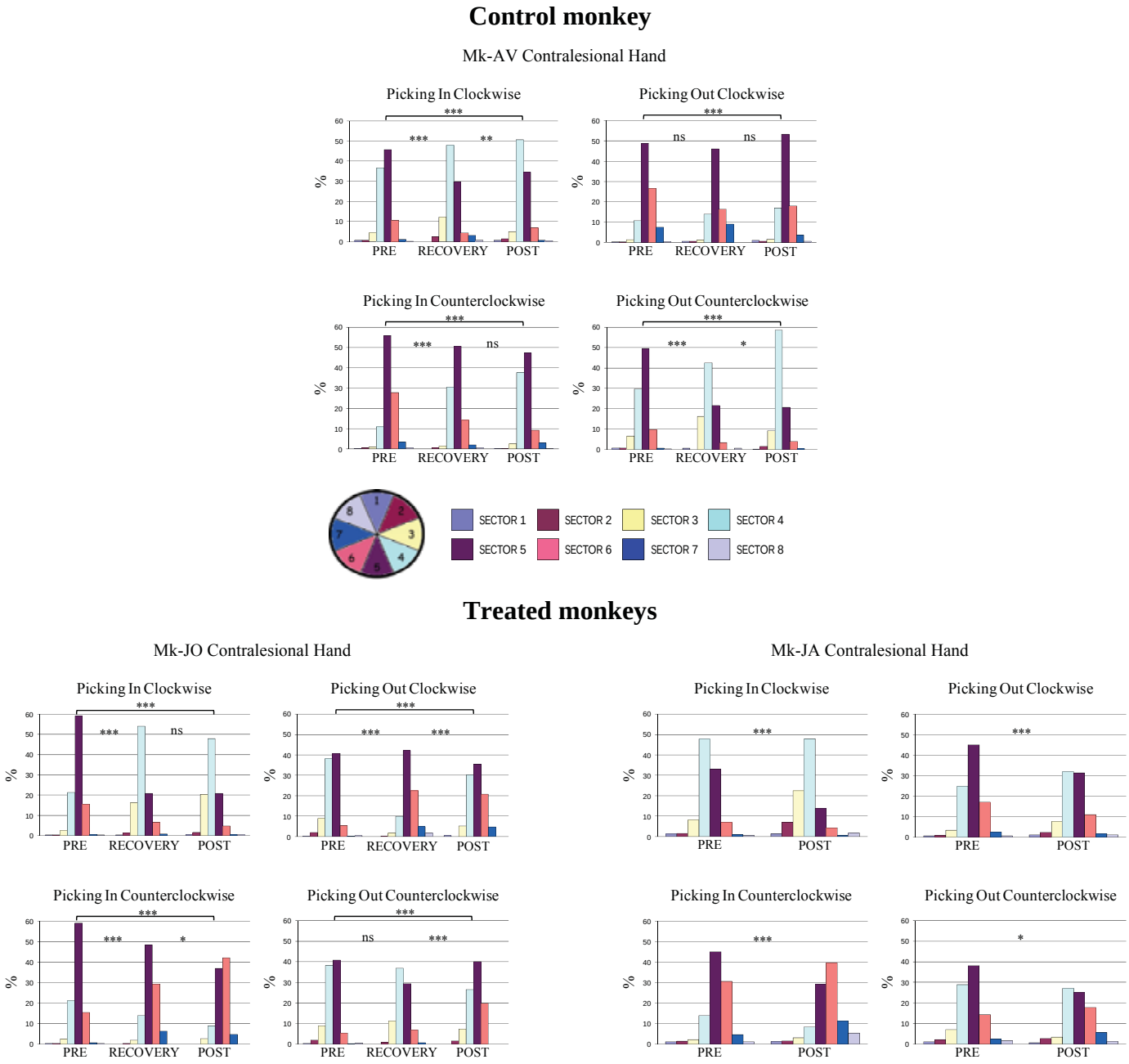


Figure 5.2.11: Comparisons of the distributions of the prehension areas in the rotating Brinkman board task performed for the contralesional hand between pre-lesion plateau and post-lesion plateau for three monkeys. The recovery phase in Mk-AV and Mk-JO is also shown. On the left and the right sides are represented the graphics when the monkey entered his finger in the well and took the pellet out of it, respectively. The top and the down graphics correspond to the clockwise and counterclockwise rotations, respectively. Coloured bars express the mean percentage of prehension areas used, each color representing a given sector, as shown on the coloured circle at the center of the figure.

Concerning the prehension area (Fig. 5.2.11), when the board rotated clockwise, before the lesion, Mk-AV introduced his fingers mainly in the sector 5 and secondary in the sector 4, and took the pellets out mainly in the sector 5 and secondary in the sector 6. On the post-lesion plateau, when entering in the wells, there was a significant switch towards the sector four mainly and the sector 5 secondary, and when bringing the pellets out, there was a switch towards more pick-outs in the sector 5 (main exit sector) and in the sector 4, and a decrease in the sector 6.

Before the lesion, when the board rotated counterclockwise, Mk-AV entered in the wells mainly in the sector 5 and secondary in sector 6, and exited from the wells mainly in the sector 5 and secondary in the sector 4. After the lesion, on the post-lesion plateau, the monkey penetrated into the wells mainly in the sector 5 and 4 and left the wells in the sector 4 and secondary in the sector 5.
For both rotation orientations and both aspects (pick-in and pick-out), the recovery phase was clearly more similar to the post-lesion plateau, even if it sometimes differed from it.
Therefore, after the lesion, in both orientations, there was a switch towards grasping the pellets earlier on the board.

The same pattern was observed in Mk-JO. Indeed, when the board rotated clockwise, a switch was observed in the introduction of the finger from the sectors 5 mainly and 4-6 secondary before the lesion to the sectors 4 mainly and 3-5 secondary after the lesion. In the withdrawal, post-lesion, there was an enlargement of the area used to pick-out the pellet of the wells growing from the sectors 4-5 pre-lesion to the sectors 4-5-6 post-lesion.
When the board rotated counterclockwise, before the lesion, the same areas as clockwise were used, namely the sectors 5 mainly and the sectors 4-6 secondary to enter in the wells, and the sectors 4-5 to exit from the wells. After the lesion, the same move towards a grasp of the pellets earlier on the board appeared, on both the pick-in (sectors 5-6) and the pick-out (sector 5 mainly and sectors 4-6 secondary) aspects.

Concerning the recovery phase, when penetrating in the wells, it was again more similar to the post-lesion plateau, even differing from it when the board rotated counterclockwise. When leaving the wells, the recovery phase was either totally different from the pre- and post-lesion plateaux (clockwise rotation) or similar to the pre-lesion plateau (counterclockwise rotation).

Thus, here too, there was a switch towards grasping the pellets earlier on the board after the lesion in both orientations.

This switch occured also in Mk-JA, either when the board rotated clockwise or when it rotated counterclockwise, and on both pick-in and pick-out aspects. Indeed, when the board rotated clockwise, the pick-in sectors were the sector 4 mainly and the sector 5 secondary before the lesion, moving to the sectors 4 mainly and the sector 3 secondary after the lesion. Similarly, to pick the pellets out of the board, there was a swith from the sector 5 mainly and the sectors 4-6 secondary on the pre-lesion plateau to the sectors 4-5 mainly on the post-lesion plateau. A comparable phenomenon was observed when the board rotated counterclockwise, with a move from the sector 5 mainly and the sector 6 secondary before before to the inverse, namely the sector 6 mainly and the sector 5 secondary, to penetrate in the wells, and a switch from the sector 5 mainly and 4 secondary to the sectors 4-5 mainly and the sector 6 secondary to pick-out from the wells.

Overall, the present results indicated an effect of the lesion on the pellets prehension area towards an earlier grasp of the pellets on the board, especially illustrated by the sectors in which the monkeys introduced their fingers in the wells.

5.2.3.2.6 Prehension orientation

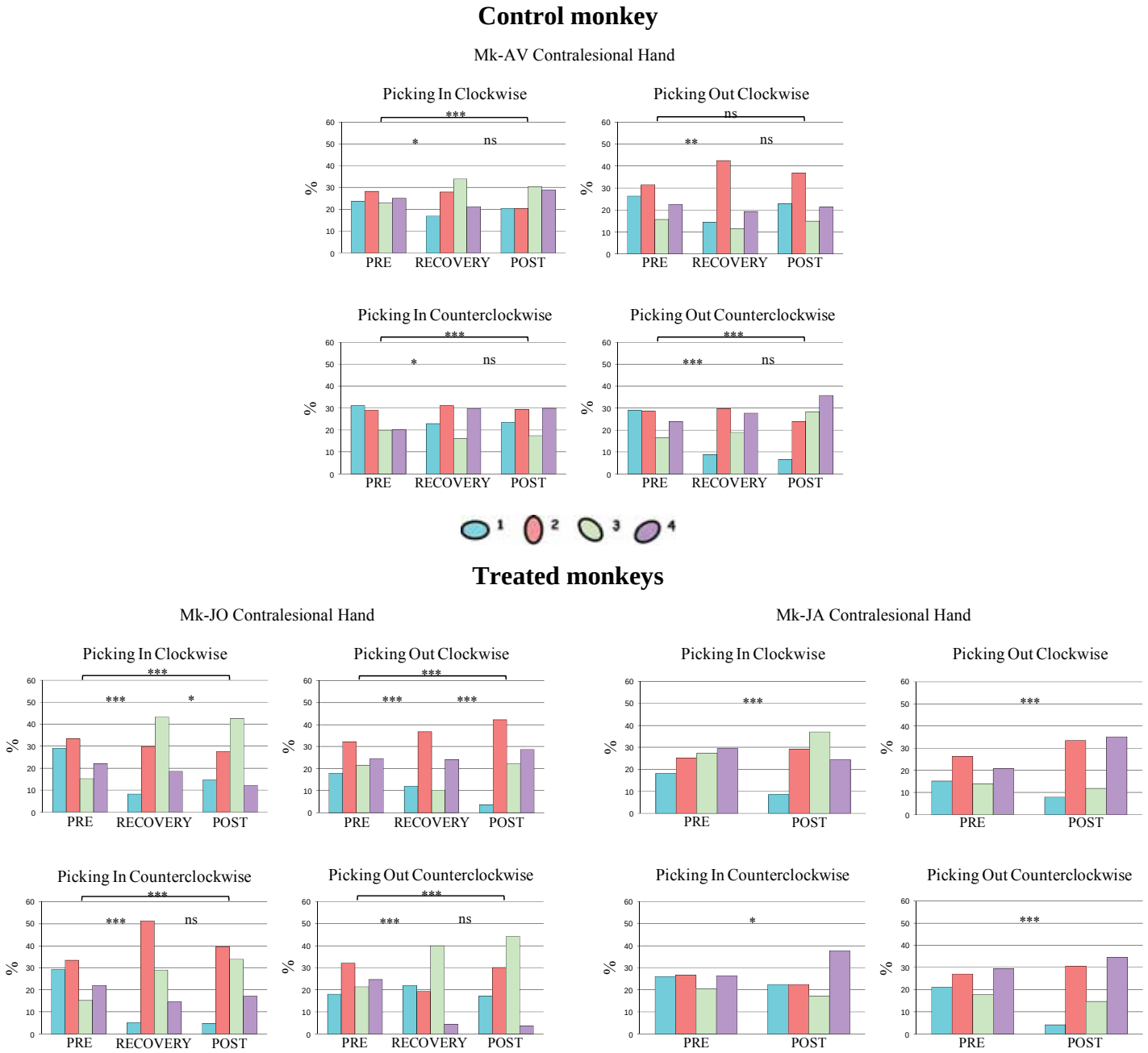


Figure 5.2.12: Comparisons of the distributions of the prehension orientations in the rotating Brinkman board task performed for the contralesional hand between pre-lesion plateau and post-lesion plateau for all the monkeys. The recovery phase in Mk-AV and Mk-JO is also shown. On the left and the right sides are represented the graphics when the monkey entered his finger in the well (pick-in) and took the pellet out of it (pick-out), respectively. The top and the down graphics correspond to the clockwise and counterclockwise rotations, respectively. Coloured bars express the mean percentage of prehension orientations used, each color representing a given orientation, as shown on the coloured ovals at the center of the figure.

Four different orientations of prehension corresponded to the eight prehension sectors previously analyzed (see "General Methods"). As it was previously shown that a slight switch of the prehension area occurred after the lesion, how was it related in terms of preferential orientation? When looking at the pre-lesion plateaux compared to the pre-lesion ones (Fig. 5.2.12), the main observation was, for all monkeys, a decrease of the orientation 1 (which required the most extensive rotation of the wrist), except in Mk-JO on the recovery phase when picking-out in the counterclockwise rotation. To note that in Mk-AV, there was no significant difference between the two plateaux when the board rotated clockwise on the picking-out aspect. This decrease of the orientation 1 was compensated by an increase of different orientations depending on the monkey and on the direction of rotation.

In Mk-AV, when the board rotated clockwise and to enter in the well, the orientations 3 and 4 increased and the orientation 2 decreased, whereas to exit from the well, although non significantly, there was an increase of orientation 2 with the orientations 3 and 4 remaining quite constant. When the board rotated counterclockwise, to introduce his finger in the well, there was an increase of the orientation 4, a slight decrease of the orientation 3 and a similar level of the orientation 2; to take the pellet out of the well, an increase of the orientations 3 and 4 and a slight decrease of the orientation 2 took place. Thus, in Mk-AV, the increases after the lesion were mainly on the orientation 3 and 4.

In the clockwise rotation, Mk-JO showed an increase of the orientation 3 and a decrease of the orientations 2 and 4 to enter the finger in the well, and an increase of the orientations 2 and 4 with the orientation 3 remaining quite constant for pick-out from the well. In the counterclockwise rotation, an increase of the orientations 2 and 3 accompanied by a decrease of the orientation 4 appeared for the picking in aspect, whereas for the picking out, there was an increase of the orientation 3 with a decrease of the orientation 4, the orientation 2 remaining quite similar to its pre-lesion level. Thus, Mk-JO had more post-lesion increases in the orientation 2 and 3.

For these two monkeys, the recovery phase was mainly similar to the post-lesion plateau, except in Mk-JO when the board rotated clockwise, especially when exiting from the well.

When the board rotated clockwise, an increase of the orientations 2 and 3 was observed in Mk-JA to enter in the wells. To pick-out from the well, there was an increase of

the orientations 2 and 4, and a slight decrease of the orientation 3. When the board rotated counterclockwise, Mk-JA showed an increase of the orientation 4 and a decrease of the orientations 2 and 3 to penetrate in the well, whereas to take the pellet out of it, an increase of the orientations 2 and 4, with a decrease of the orientation 3, took place. Thus, after the lesion, Mk-JA showed increases mainly in the orientations 2 and 4.

Therefore, as mentioned above, the trend common to all the monkeys was the decrease of the orientation 1 requiring a more important extensive of the wrist, compensated by the increase of the other three orientations, in various degrees according to the monkeys and the rotation direction.

5.2.4 GENERAL CONCLUSION

The present analyses showed that the motor cortex lesion had an impact on some parameters of prehension's strategy.

In the modified Brinkman board task (n=9, both hands), the introduction of the thumb first in the well, already few used before the lesion, was even lesser used after the lesion by the monkeys employing this strategy. A tendency of the monkeys recovering less to take preferentially the pellets in the vertical wells after the lesion was observed; this can be explained by the greater ease to grasp pellets in the vertical than in the horizontal orientation. Interestingly, larger less favorable cumulative distances and thus a less optimal strategy after the lesion were observed in the monkeys recovering less, and even a tendency to improve the strategy with the ipsilesional hand was found in the monkeys showing an improvement of their post-lesion score performance. On the picking sequence on the left-right axis parameter, a general absence of change of picking sequence on the post-lesion plateau compared to the pre-lesion plateau, except in one control monkey and one treated (Anti-Nogo-A antibody) monkey for their contralesional hand, in one control monkey for his ipsilesional hand and in one treated (autologous brain cells) monkey for his both hands, a change occurred after the lesion. No clear effect appeared on the wrist orientation to grasp the pellets.

In the rotating Brinkman board task (n=3, contralesional hand), after the lesion, different phenomenons were observed in the preferential wrist orientation according to the rotation direction. Indeed, when the board rotated clockwise, the main change was in the sense of a decrease of the use of the abduction, whereas when the board rotated counterclockwise, a monkey did not show any difference, another one used more the

abduction, whereas the last one used less the abduction; therefore, no general tendency could be inferred on this parameter. Concerning the preferential finger to enter in the well, the main tendency was a decrease of the use of the thumb, already less used before the lesion. The prehension of two pellets at the same time before bringing them to the mouth was not used often by the monkeys, even not at all by Mk-JO, both before and after the lesion. This parameter is thus few informative. Before the lesion, irrespective of the rotation direction, there was a general tendency to grasp the pellets in a sequence going from the most external ring to the most internal ring, meaning that the monkeys took the pellets that were the closest to him. Globally, no change occurred post-lesion, except a slight effect in one monkey, especially when the rotation was counterclockwise. The main changes were observed on the prehension areas and on the prehension sectors, with a shift towards an earlier grasp of the pellets on the board, especially to enter the fingers in the wells, corresponding to a decrease of pickings in and pickings out in the wells when they were completely horizontally oriented, requiring the most extensive rotation of the wrist.

5.3 QUALITATIVE ASSESSMENT ("SCORING") OF GENERAL BEHAVIOUR AND HEALTH CONDITIONS OF THE MONKEYS BEFORE AND AFTER MOTOR CORTEX LESION

5.3.1 PURPOSE

One can suppose that being subjected to an unilateral motor cortex lesion leading to a loss of manual dexterity represents a stressing or traumatizing situation that could activate depression and/or anxiety reactions, which could be accentuated by the fact that dominant monkeys subjected to the kind of lesion practiced in the present study often lose their hierarchical position. To investigate these aspects, the general behavior and health state of the monkeys before and after the lesion were assessed.

5.3.2 METHODS

The assessment of the monkeys' general behaviour and health state in the experimental room and in the housing room was performed on 4 monkeys, all subjected to a unilateral motor cortex lesion. The comparisons were carried out on three phases, namely the pre-lesional plateau, the post-lesional recovery phase and the post-lesional plateau, three times per week on average, based on the following "clinical" scoring:

	Shivers	Scratching (self or other)	Rotations in the chair	Chair exit movements	Distress shouts	Sum
Score (0-4)						

A

	Loss of perseverence	Distraction	Refusal to perform the task	Loss of reactivity to pleasant stimuli	Motor immobility Dumbness	Sum
Score (0-4)						

B

	Prostration	Social isolation	Loss of reactivity to pleasant stimuli	Motor immobility Dumbness	Stereotypies	Distress shouts	Bizarre voluntary movements	Flight reaction	Motor excitement	Sum
Score (0-1)										

C

	Observations in the detention room (C)	Anxiety (A)	Humor (B)
Total mean score	0-9	0-20	0-20

D

	Banana pellets	End-task rewards	Pellets	Banana	Apple	Other
Yes-No/ Quantity						

E

Table 5.3.1: Qualitative observations. A. Observations in the experimental room: anxiety. B. Observations in the experimental room: humor. C. Observations in the detention room. D. Total observation scores. E. Observations regarding appetite.

Different criteria were chosen on the basis of qualifications in Lickert and bipolar scales (Gendre and Capel, 2000) used with humans to quantifiy their anxiety and humor state. Concretely, in the experimental room, two different tables reporting on general behavioural observations were used; one concerned anxiety (Table 5.3.1A) and the other concerned humor (Table 5.3.1B). For both, five different criteria were noted from one to four, giving a final score ranging from zero to twenty. The noted criteria for anxiety were the occurence of "shivers", of "scratching", of "rotations in the primate chair", of "chair exit movements" and of "distress shouts". For humor, the observed criteria were the "loss of perseverance", the "distraction", the "refusal to perform the task", the "loss of reactivity to pleasant stimuli" and the "motor immobility or dumbness". In the detention room, another table (Table 5.3.1C) was used to assess the animals' well-being. It contained nine dichotomic criteria, namely "prostration", "social isolation", "loss of reactivity to pleasant stimuli", "motor immobility or dumbness", "stereotypies", "distress shouts", "bizarre voluntary movements", "flight reaction" and "motor excitement". These criteria were noted zero (absence) or one (presence), giving a final score ranging from zero to nine. Together, the scores in these three tables

(anxiety, humor and observations in the detention room) led to three total mean scores indicating the state of the monkey (Table 5.3.1D). Another important observation was done on the appetite of the monkey, which is also an indicator of the monkeys' state (Table 5.3.1E). Along this line, the body weight of the monkeys was checked before every behavioural session.

5.3.3 RESULTS

	DETENTION ROOM (max.9)			**ANXIETY** (max.20)			**HUMOR** (max.20)		
Mk-JO	0	0	0	2.7	11***	4.9**	0.6	6.1***	1.6*
Mk-AV	0.3	1.1*	0.3	0.7	0.7	0.2	0.4	0.9	0.4
Mk-JA	0	0	0	0.6	1.1	0*	1.2	4.7***	4.5***
Mk-WI	0	0.9***	0.7***	1.1	4.6***	3.2***	1.5	3.5***	3.2***

Table 5.3.2: Mean total scores obtained for each monkey on the detention room observations, on the anxiety and on the humor. To note that a "0" score represents the best score for an optimal state and that the maximal score in parenthesis would be the score for the most perturbated state. In each cell, the three numbers correspond to the pre-lesional plateau, the recovery phase and the post-lesional plateau respectively. Red stars indicate significant enhancements in comparison to the pre-lesional plateau; black stars correspond to significant diminutions as compared to the pre-lesional plateau.

Table 5.3.2 resumes the mean total scores obtained for each monkey on the three types of observations, namely in the detention room, and on the anxiety and the humor in the experimental room. In each cell, the three numbers correspond to the mean values during the pre-lesional plateau, during the recovery phase and during the post-lesional plateau respectively, with indication of the significant differences as compared to the pre-lesional plateau. The following part describes each type of observation in more details.

5.3.3.1 Anxiety

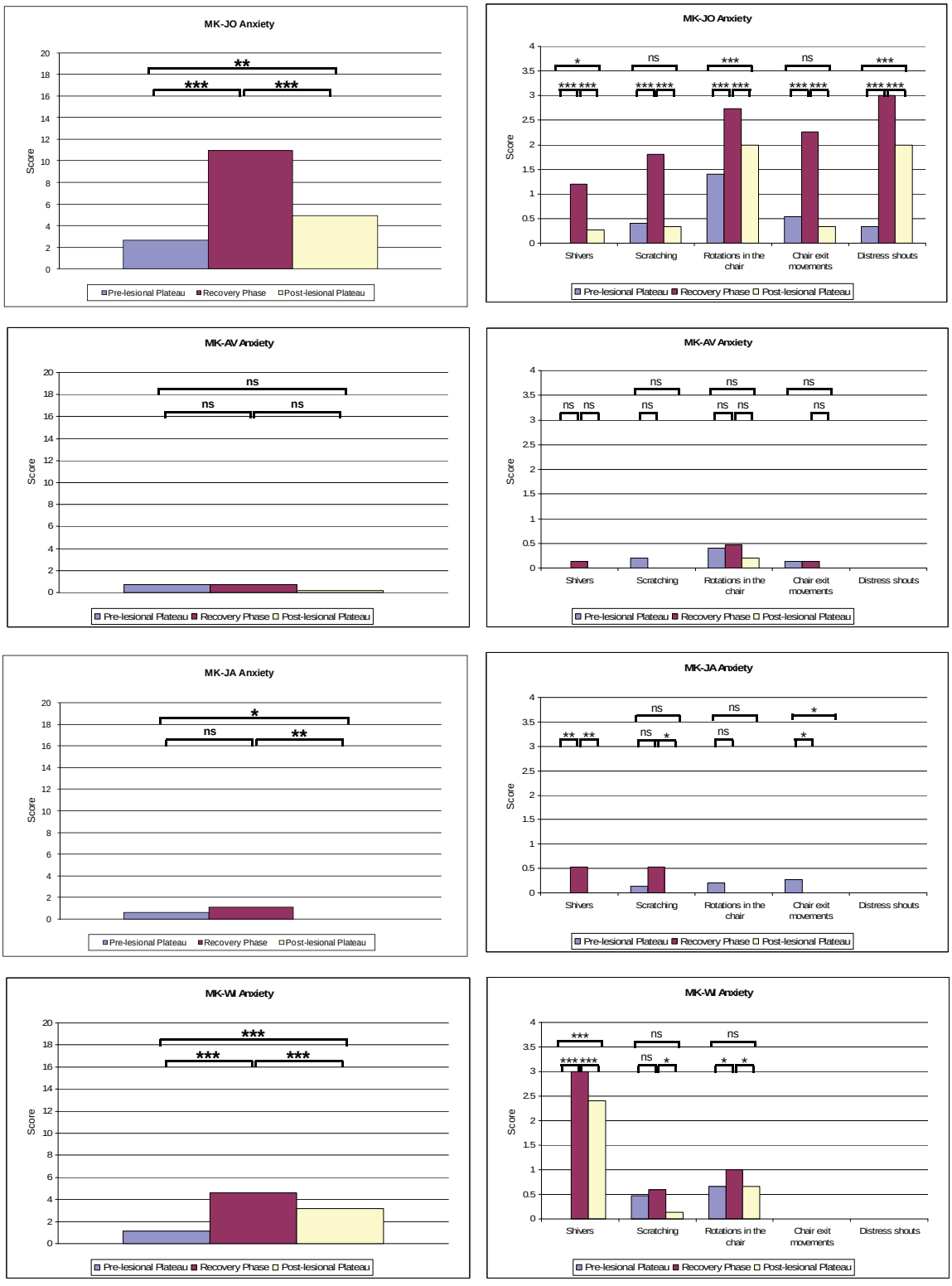


Figure 5.3.1: Comparisons for each monkey of the anxiety parameters between pre-lesional plateau, recovery phase and post-lesional plateau. On the left side is represented the mean total score. On the right side figures the mean score for each criterion.

Observations on the global score of anxiety indicate significant enhancements in two monkeys, namely Mk-JO and Mk-WI, both on the recovery phase and on the post-lesional plateau (Fig. 5.3.1 and Table 5.3.2). To note however that the global score on this latest period was significantly lower than on the recovery phase. The detailed criteria enhancing after the lesion differed between the two monkeys. More precisely, in Mk-JO, all five criteria enhanced strongly during the period following the lesion and diminished significantly on the post-lesional plateau, reaching a similar score to the pre-lesional plateau for the "scratching" and "chair exit movements" criteria, and staying higher than before the lesion for the "shivers", "rotations in the chair" and "distress shouts" criteria, this latest having undergone the stronger enhancement after the lesion and later on the post-lesional plateau. In Mk-WI, the "shivers" which were absent before the lesion enhanced strongly after the lesion and stayed at a high score after recovery. This monkey showed also a provisory enhancement in his tendancy to "rotate in the chair", which returned to its pre-lesional level. On the "scratching" criterion, no difference was found after the lesion, but a slight decrease appeared on the post-lesional plateau, whereas the "chair exit movements" and "distress shouts" criteria stayed at zero level. On their total score, Mk-AV and Mk-JA did not show any enhancement after the lesion (Fig. 5.3.1 and Table 5.3.2); furthermore, Mk-JA diminished signficantly his global score on the post-lesional plateau. Concretely, Mk-AV stayed constantly at low or even zero scores on each specific criterion. For his part, before the lesion, Mk-JA had low scores on the "scratching", "rotations in the chair" and "chair exit movements" criteria and zero scores in the "shivers" and "distress shouts" criteria. On the recovery period, he showed a significant enhancement on the "shivers" criterion and a light and non-significant enhancement on the "scratching" criterion. Finally, on the post-lesional plateau, all the criteria were at zero level.

5.3.3.2 Humor

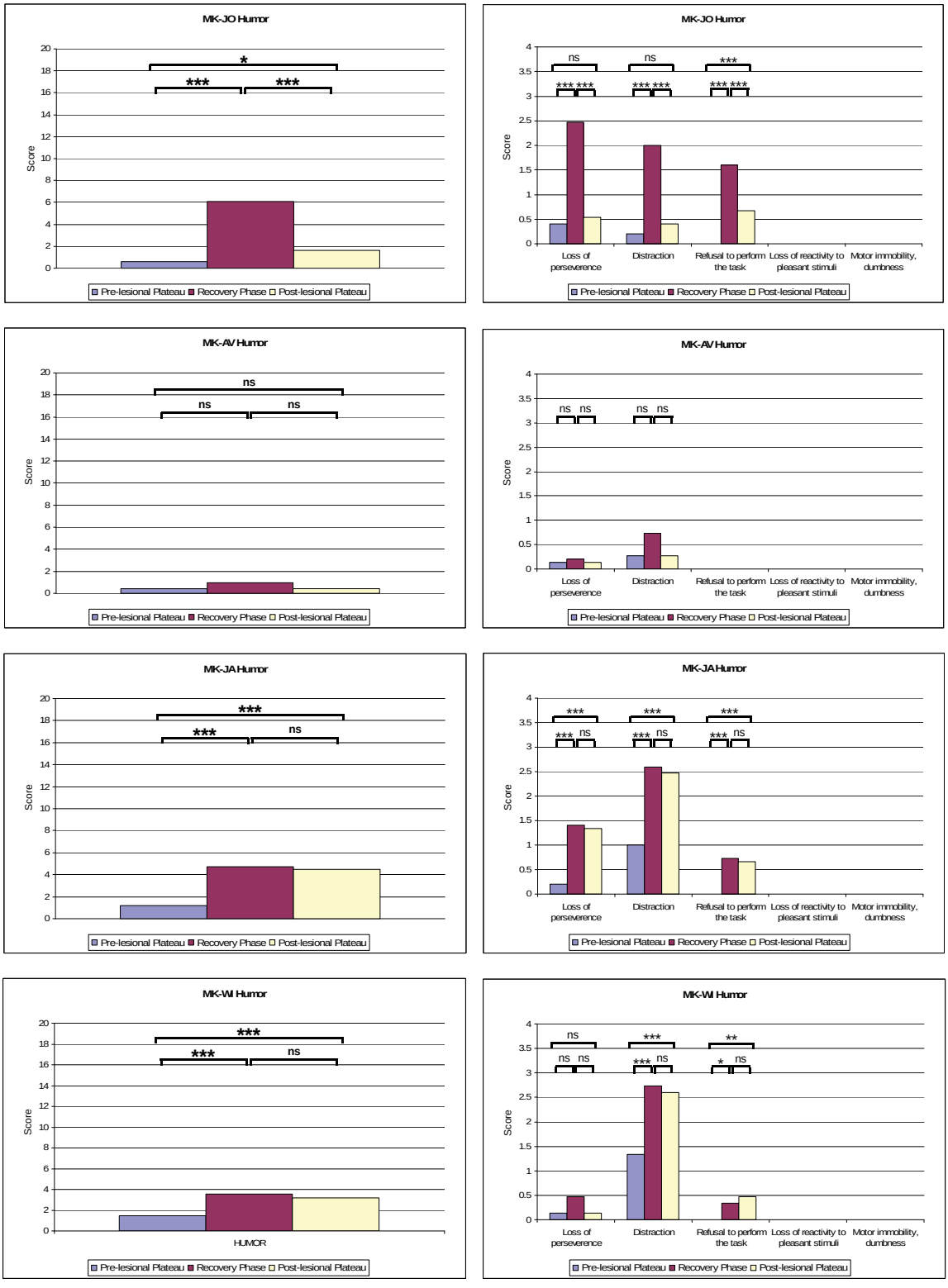
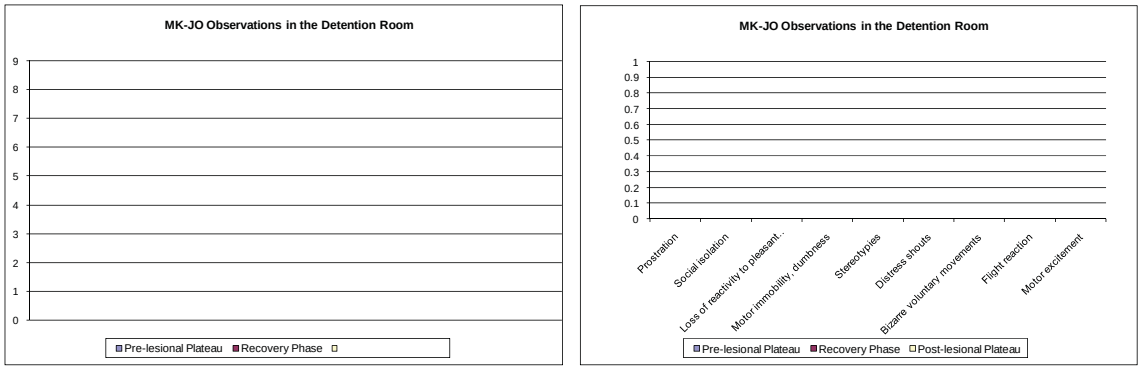


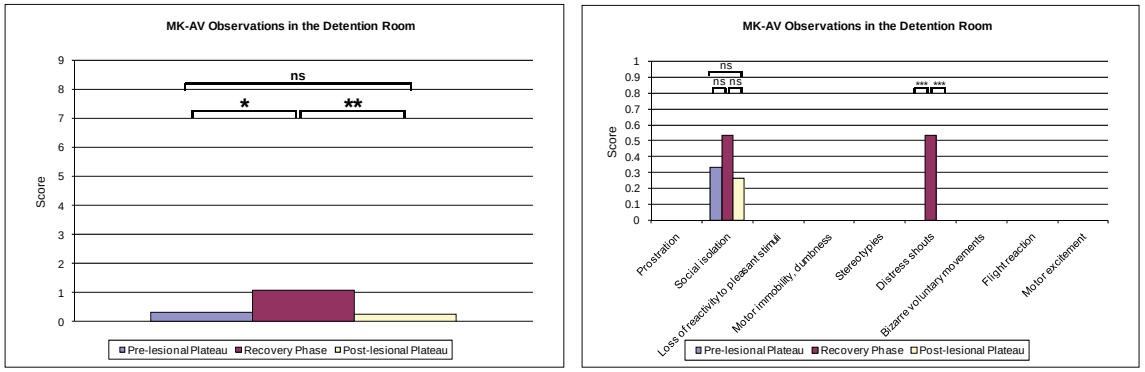
Figure 5.3.2: Comparisons for each monkey of the humor between pre-lesional plateau, recovery phase and post-lesional plateau. On the left side is represented the mean total score. On the right side figures the mean score at each criterion.

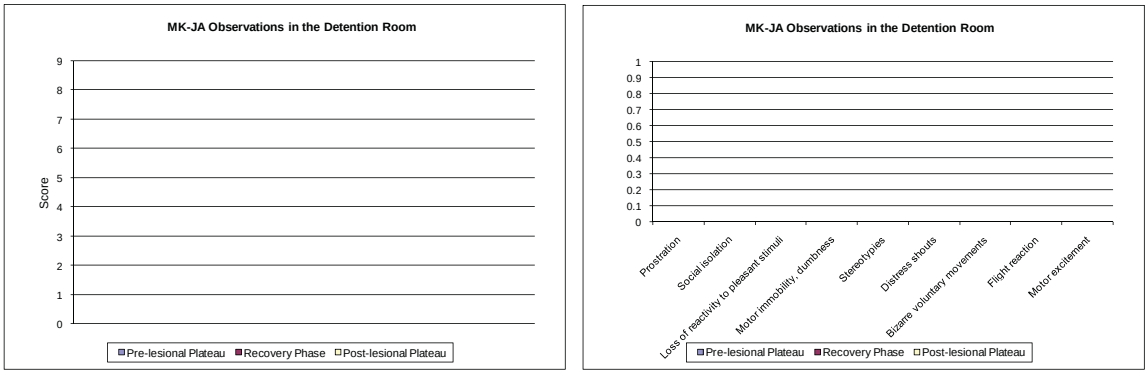
Concerning the humor total score, three on the four evaluated monkeys showed a significant enhancement after the lesion, with scores staying at a similar level on the post-lesional plateau for two of them (Mk-JA and Mk-WI), whereas for one (Mk-JO), the post-lesional plateau score diminished, but stayed significantly higher than the pre-lesional plateau score. For the last monkey (Mk-AV), no significant difference was observed (Fig. 5.3.2 and Table 5.3.2). When looking more precisely at the five evaluated criteria, this monkey had scores of zero for the criteria "refusal to perform the task, "loss of reactivity to pleasant stimuli" and "motor immobility or dumbness", whereas the criteria "loss of perseverance" and "distraction" stayed at a low level. For the three other monkeys, the criteria "loss of reactivity to pleasant stimuli" and "motor immobility or dumbness" were also constant at zero. Mk-JO showed significant enhancements on the criteria "loss of perseverance", "distraction" and "refusal to perform the task" after the lesion, the two first reaching a similar level than before the lesion on the post-lesional plateau and the third, even diminished, staying at a higher level. For Mk-JA, the same significant enhancements on the criteria "loss of perseverance", "distraction" and "refusal to perform the task" occurred after the lesion and stayed at similar higher levels on the post-lesional plateau. A comparable pattern was observed in Mk-WI on the criteria "distraction" and "refusal to perform the task", whereas the criterion "loss of perseverance" was constantly low.

5.3.3.3 Observations in the detention room



Values at 0





Values at 0

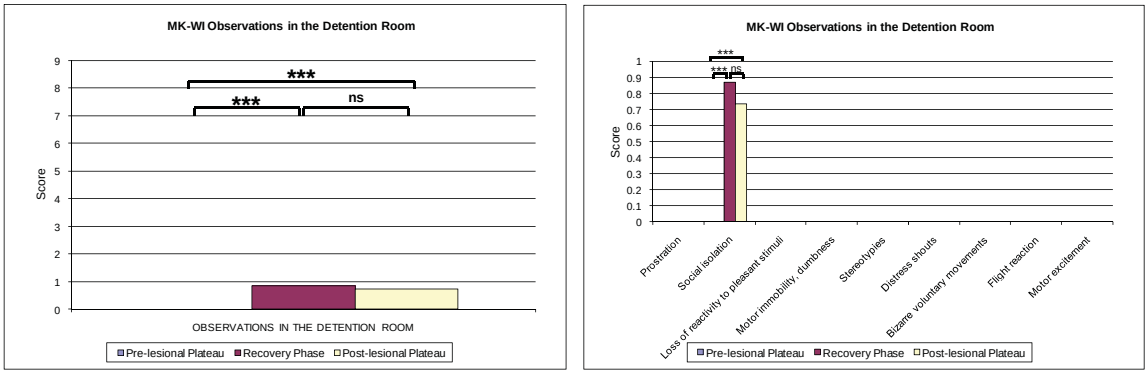


Figure 5.3.3: Comparisons for each monkey of the observations in the housing room between pre-lesional plateau, recovery phase and post-lesional plateau. On the left side is represented the mean total score. On the right side figures the mean score at each criterion.

When looking at the global results on the observations in the housing room (Fig. 5.3.3 and Table 5.3.2), it appeared that two monkeys (Mk-JO and Mk-JA) were systematically at a zero score, none of the nine criteria having been observed, neither before nor after the lesion. One monkey, Mk-WI, showed a significant enhancement after the lesion, remaining constant on the post-lesional plateau. This enhancement was due to the strong enhancement on the "social isolation" criterion, all the other critera staying at zero. The last monkey, Mk-AV, had a total score significantly enhanced on the recovery phase and returning to the pre-lesional score on the post-lesional plateau. The enhancement on the recovey phase was due to significantly higher scores on the criteria "social isolation" and "distress shouts", the latest being at zero on the pre- and post-lesional plateaux.

5.3.4 GENERAL CONCLUSION

Different observations were drawn from these results. First, the scores pre-lesion did not predict the changes occurring after the lesion, although on the anxiety parameter, the monkeys having a higher score before the lesion showed more important changes after the lesion. Second, almost no effect was observed in one dominated monkey (Mk-AV), whereas changes occurred in the three other monkeys, one dominated (Mk-WI) and two dominant (Mk-JO and Mk-JA). Thus, the hierarchical rank of the monkeys and its loss after the lesion did not seem to play a specific role in the post-lesion changes. Third, few effects were observed on the observations in the detention room parameter (no effect in two monkeys and few effects, although significant, in the two other monkeys).

Importantly, the obtained results showed that the changes that occurred after the lesion stayed at very low score levels, both on the anxiety, on the humor and on the observations in the detention room parameters.

5.4 MANUAL DOMINANCE

5.4.1 PURPOSE

As nearly 90% of humans are right-handed, the question of the evolutionary origins of this trait arose. To try to answer it, Papademetriou et al. (2005) performed meta-analyses on 62 studies of primate handedness published since 1987 that provided individual data (representing 31 species: 9 prosimians, 6 New World monkeys, 10 Old World monkeys, 2 lesser apes, and 4 greater apes). Their results did not confirm the evolutionary point of view regarding handedness, as although some evidence of a population-level left-handed bias for prosimians and Old World monkeys was found, no population-level handedness appeared for apes and New World monkeys, contrarily to the expected right one especially for apes.

Various studies showed different results on hand preference in non-human primates according to the species and the type of tasks assessed (e.g. Deuel and Dunlop, 1980; Fletcher, 2006; Hopkins, 1993; Hopkins et al., 2006; Lonsdorf and Hopkins, 2005; Meunier and Vauclair, 2007; Schmitt et al., 2008; Westergaard and Soumi, 1996; Westergaard and Lussier, 1999).

The main conclusion drawn from these investigations was that non-human primates lacked any species-typical, directional bias for using a preferred hand, although individuals of some species often had hand preferences, left and right in approximately equal numbers. Furthermore, it was concluded that non-human primates preferred to use different hands for different tasks (Annett and Annett, 1991; Marchant and McGrew, 2007; Hopkins et al., 1993; Schmitt et al., 2008; Warren 1977). At the individual level, some studies showed that individuals often exhibit a consistent hand preference (Hook and Rogers 2000 in marmosets; Hopkins et al. 2004 in captive chimpanzees; Lonsdorf and Hopkins 2005 in wild chimpanzees), whereas others suggested an inconsistent hand preference across tasks within an individual, although a preference is exhibited for many different tasks (McManus, 2002; Warren, 1977). Population biases to use a preferred hand were shown to be more common when the task is complex in terms of postural, perceptual and cognitive demands, or when novel bimanual tasks are used (Blois-Heulin et al., 2007; Fagot and Vauclair, 1991; King and Landau 1993; Spinozzi et al., 2004). But a study on chimpanzees showed that a highly complex task (ant fishing) revealed no hand preferences at the population level (Marchant and McGrew, 2007), indicating a great degree of variability between species and tasks.

The aim of the present investigation was to assess if there is a hand dominance, probably reflecting a hand preference, in the macaque monkeys when performing the fine manual prehension “modified Brinkman board” task.

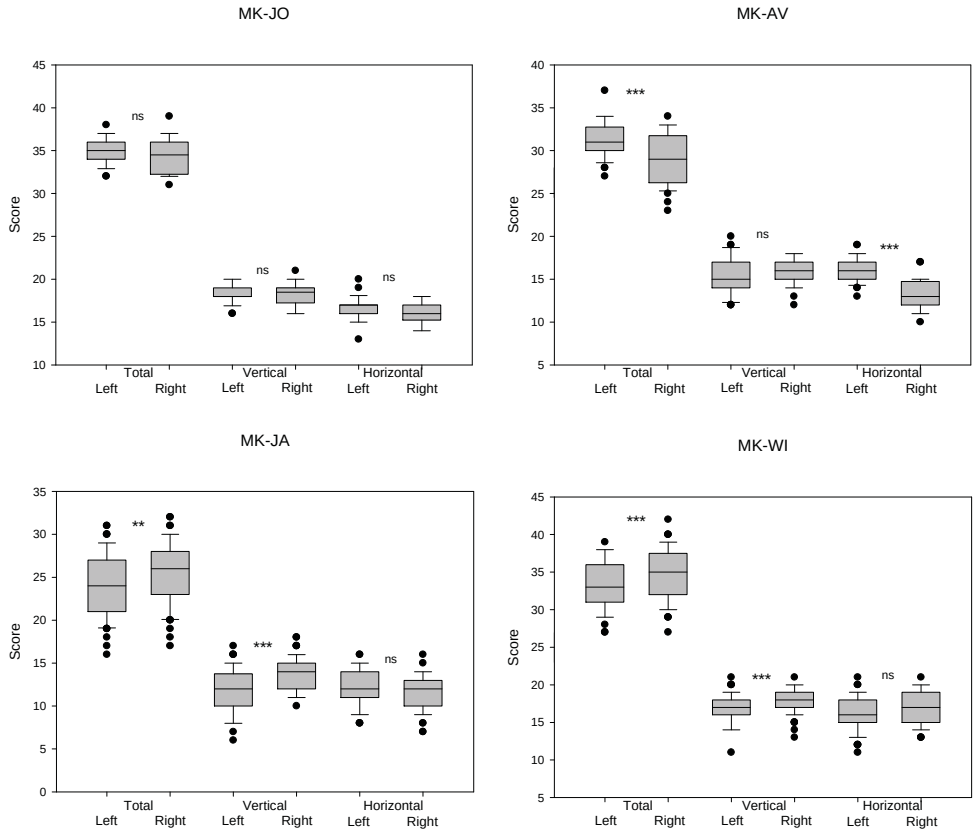
5.4.2 METHODS

The manual dominance has been observed on 10 monkeys on the modified Brinkman Board task. The parameters considered were the number of pellets retrieved in 30 seconds, the contact time, the percentage of errors committed and the percentage of retrieval of 2 pellets at the same time before bringing them to the mouth. Comparisons between the left and the right hands were carried on the behavioural plateau phase preceeding the unilateral primary motor cortex lesion.

For the statistical analysis, the Wilcoxon non-parametrical statistical test was used for the score, and the Mann-Whitney non-parametrical statistical test was used for the contact time and the other parameters.

5.4.3 RESULTS

5.4.3.1 Score



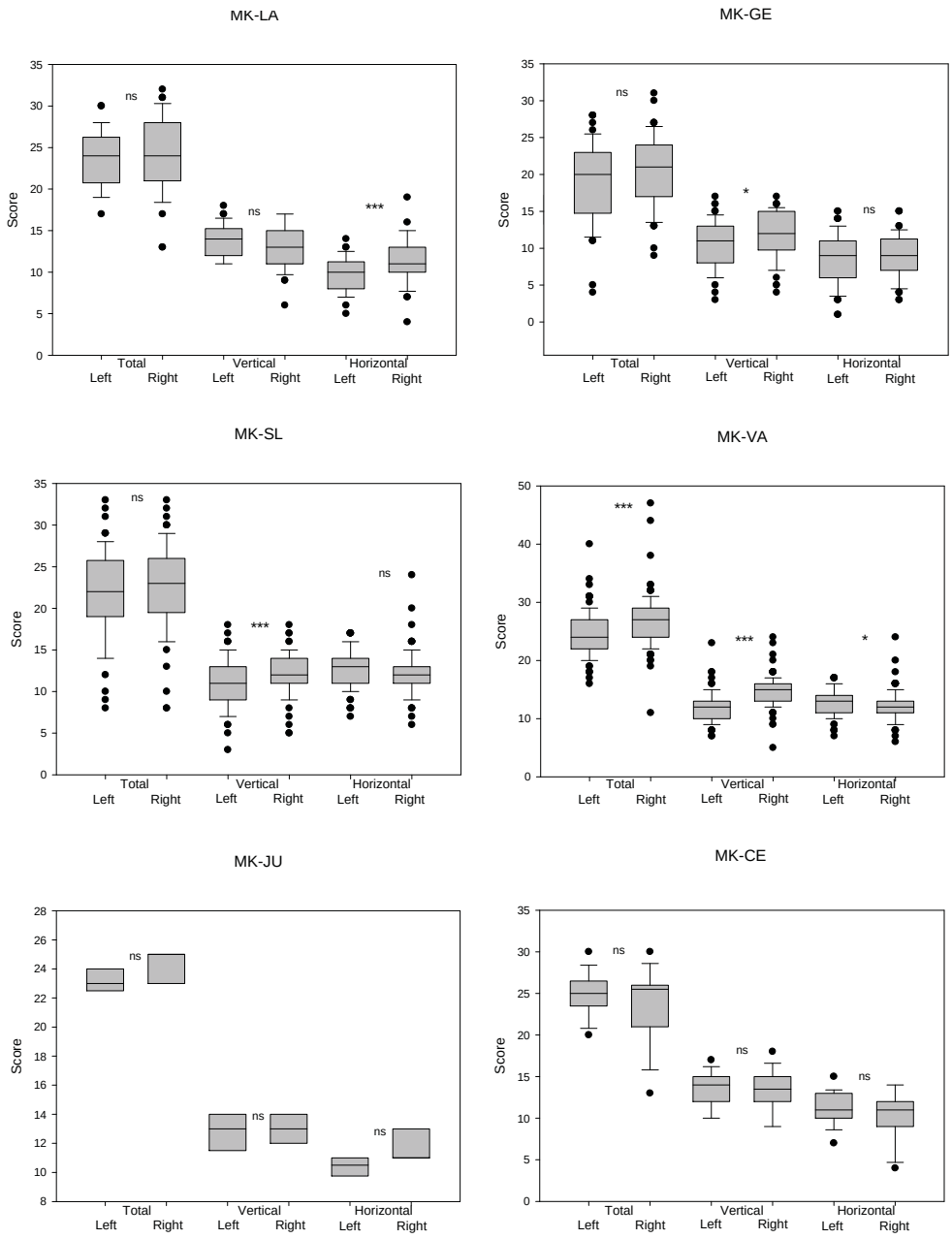
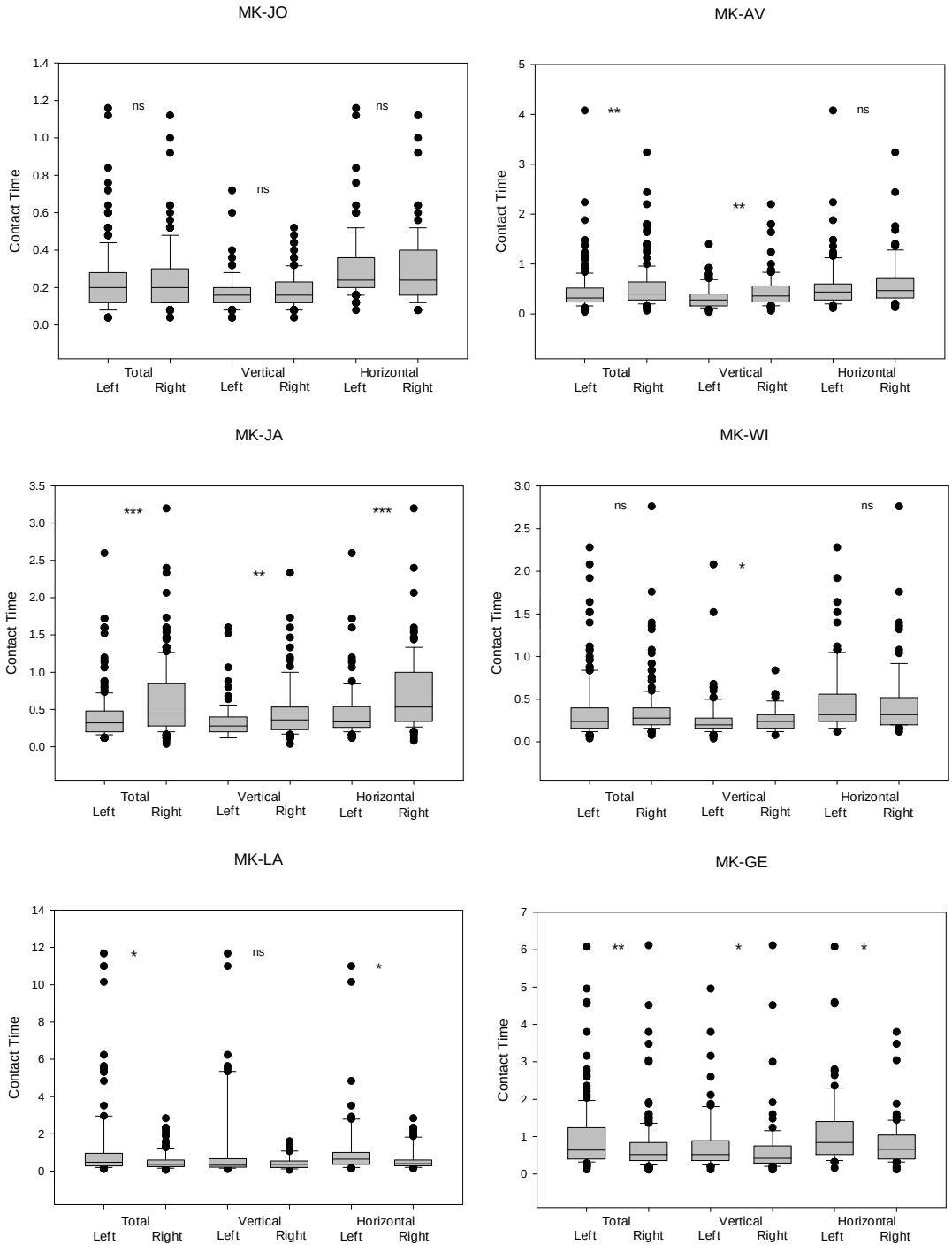


Figure 5.4.1 : Comparisons for each monkey of the right and left hands based on the score at the modified Brinkman board task, considering the total wells, and the vertical and horizontal wells separately.

When looking at the score (Fig. 5.4.1 and Table 5.4.1), namely the number of pellets taken in 30 seconds, no manual dominance appeared for three monkeys (Mk-JO, Mk-JU and Mk-CE), even when considering the vertical and horizontal wells separately. The same pattern was found on the total wells for three other monkeys (Mk-SL, Mk-LA and Mk-GE), but differences occurred either on the vertical wells in favour of the right hand for Mk-SL and Mk-GE, or on the horizontal wells with a better performance with the right hand for Mk-LA. Two monkeys (Mk-JA and Mk-WI) showed a right manual dominance on the total and vertical wells but not on the horizontal ones, whereas one monkey (Mk-VA) had the same right hand dominance on total and vertical wells with a left better performance on the

horizontal wells. Finally, a left manual dominance was found in one monkey (Mk-AV) on the total and horizontal wells, but not on the vertical ones.

5.4.3.2 Contact Time



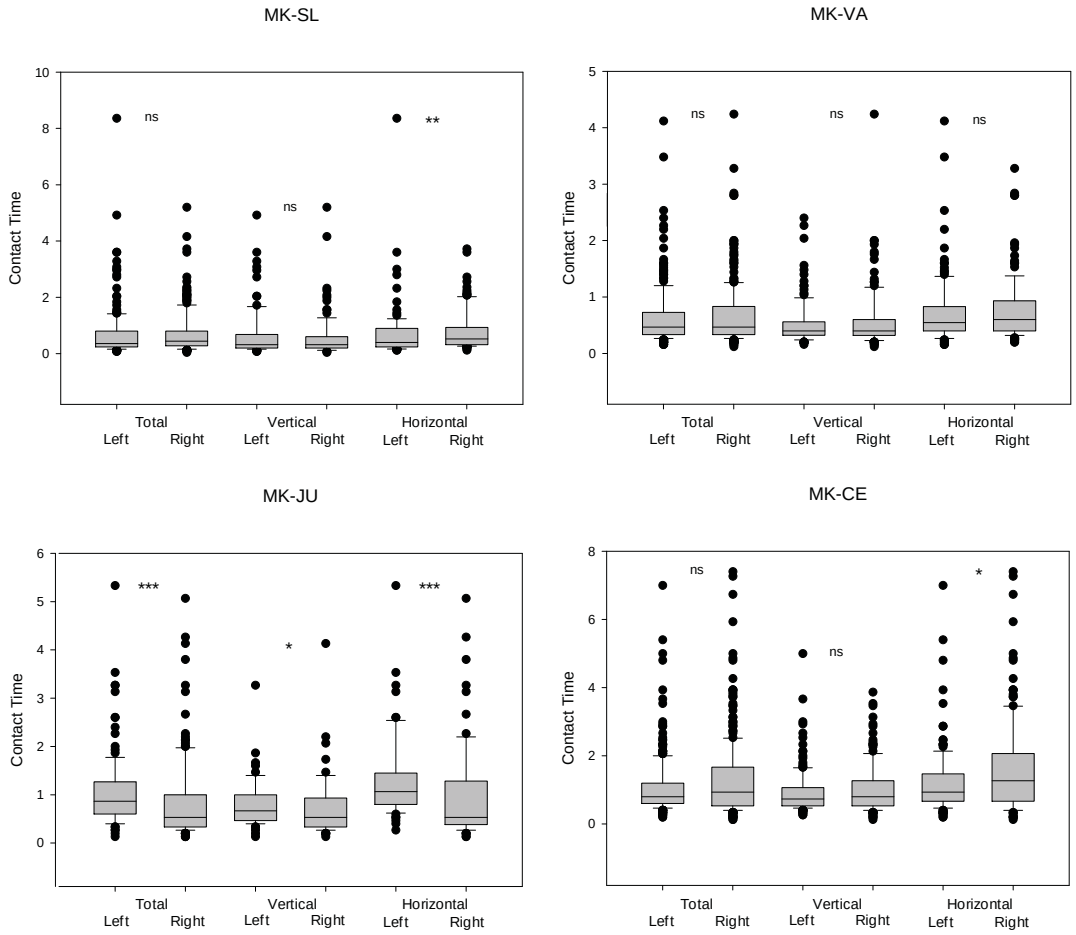


Figure 5.4.2 : Comparisons for each monkey of the right and left hands on the contact time at the modified Brinkman board task, considering the total (n=20 per session) wells and the vertical (n=10 per session) and horizontal (n=10 per session) wells separately.

In terms of contact time, no better manual performance of one hand or the other was found in two monkeys, namely Mk-JO and Mk-VA (Fig. 5.4.2 and Table 5.4.1). In the other eight monkeys, diverse patterns appeared. Mk-JA was quicker with his left hand on all the wells. Mk-AV showed the same left hand dominance, but not on the horizontal wells. For their part, Mk-SL and Mk-CE had a better performance with their left hand only on the horizontal wells, whereas it was on the vertical wells for Mk-WI. A right hand dominance was found on all the wells in Mk-JU and Mk-GE. Mk-LA showed the same quicker performance with the right hand, but not on the vertical wells.

5.4.3.3 Other parameters

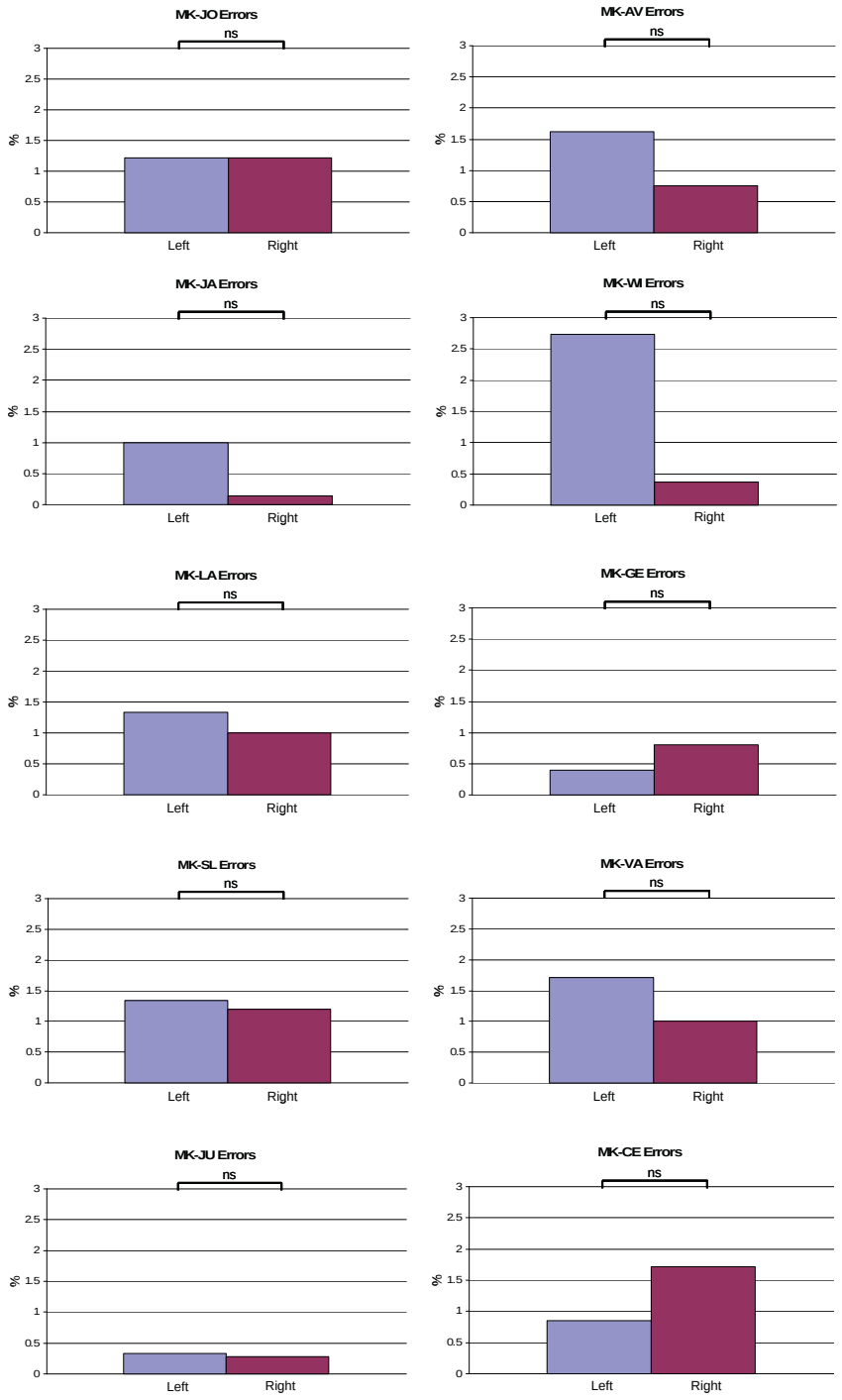


Figure 5.4.3 : Comparisons for each monkey of the right and left hands on the percentage of errors commited when performing the modified Brinkman board task.

No difference was found between left and right hand in any monkey on percentage of errors commited when performing the task (Fig. 5.4.3 and Table 5.4.1). All the monkeys commited very few errors.

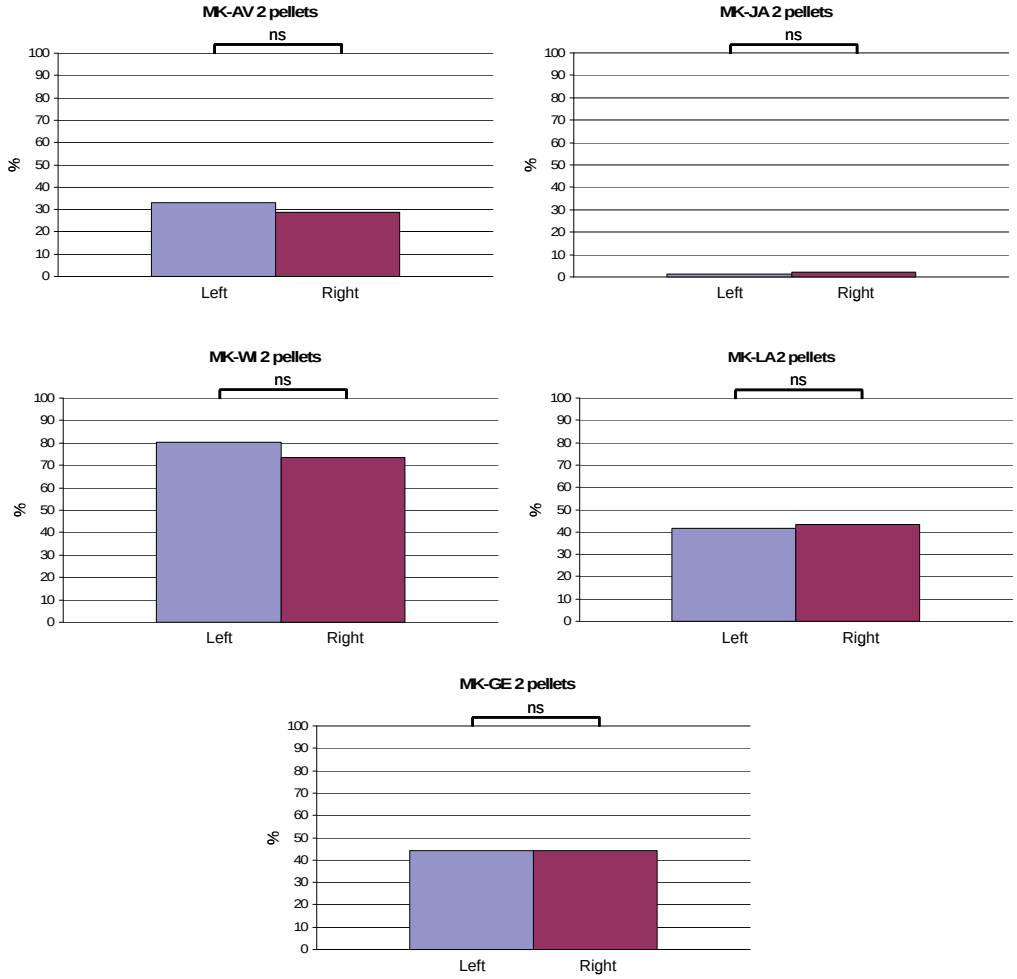


Figure 5.4.4 : Comparisons for each monkey of the right and left hands on the percentage of pellets taken at the same time as another one before bringing it to the mouth.

The strategy consisting of taking two pellets at the same time before bringing them to the mouth was more or less used by the monkeys. Five of them never used it, namely Mk-JO, Mk-SL, Mk-VA, Mk-JU and Mk-CE. On the other five monkeys (Fig. 5.4.4), Mk-AV, Mk-WI, M-LA and Mk-GE adopted much more this way of doing, whereas Mk-JA did it rarely. On these five monkeys, no one employed it more with one hand than the other.

5.4.4 GENERAL CONCLUSION

Table 5.4.1 : Summary of the hand dominance of the ten observed monkeys on the diverse analyzed parameters at the modified Brinkman Board task. “L” and “R” mean left hand dominance and right hand dominance respectively, whereas “No” indicates an absence of manual dominance.

When considering each monkey individually, no real manual dominance was found. Indeed, a manual dominance could be found sometimes in favour of the left and sometimes in favour of the right hand, either for an aspect or for another, but never globally. Considering the score and the contact time, the only trends in favour of a manual dominance were observed in Mk-AV with a better performance with his left hand on four of the six parameters considered, in Mk-JU and Mk-GE showing a right hand dominance on the three contact time parameters and on the score on the vertical wells for Mk-GE, and in Mk-LA having a quicker performance with her right hand on three of the six parameters. Looking at the data on the different aspects separately, no manual dominance could be observed. The only eventually systematic aspect that appeared was on the score for the vertical wells, with a right dominance for five monkeys showing a difference between their two hands. Furthermore, on the other two aspects, namely the errors commited and the strategy consisting in taking two pellets at the same time, no manual dominance was shown. Therefore, overall there is no systematic manual dominance specific to the modified Brinkman board task on any of its analyzed parameters.

Further studies should be conducted on hand preference itself, by letting the monkey use his preferred hand to perform the task, and then on the comparison between hand dominance and hand preference. Along this line, to force the monkey to use his two hands, one after the other, to perform the task could lead to an increased use of the non-preferred hand when the monkey is in a situation of hand preference assessment task. Indeed, Schmitt et al. (2008) suggested a positive feed-forward mechanism where increasing practice with one hand leads to increased use of that hand. It would also be of interest to assess hand dominance and hand preference when the monkeys perform more complex tasks, such as the rotating

Brinkman board task, or bimanual tasks, as well as when they produce daily life movements, such as grooming. Furthermore, Schmitt et al. (2008) showed a significantly more often strong hand preference for older individuals than younger ones in Barbary macaques; such age differences, as well as sex differences, should be assessed in our long-tailed macaques.

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5.5 SOMATOSENSORY EVOKED POTENTIALS

5.5.1 INTRODUCTION

Evoked potentials are electrical signals generated by the nervous system in response to sensory stimuli. Visual, auditory and somatosensory stimuli are frequently used to study evoked potentials. Somatosensory evoked potentials (SSEPs) consist in a series of waves reflecting a sequential activation of neural structures (generators) along somatosensory pathways following a stimulation of peripherical nerves (http:// emedicine.medscape.com/article/1139906-overview). When a stimulus is applied, the afferent sensory volley is transported from periphery to the brain, and responses -extracellular potentials- are generated along the afferent somatosensory pathways. These responses are formed by the synchronized activity in a population of neurons produced by the stimulus (Fig. 5.5.1).

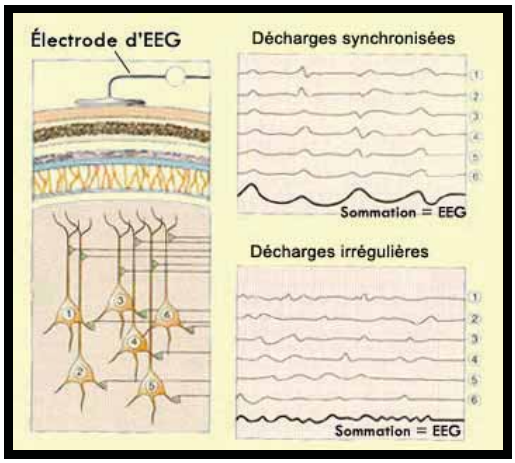


Figure 5.5.1 : The answers are formed by the the synchronized answer of a population of neurons.

The amplitude of the evoked potential, in the μV range, is much weaker than the EEG, ECG or EMG spontaneous activities (Freye, 2005 ; Mauguière and Fischer, 1990). Moreover, the other electrical potentials reflecting spontaneous activity of the nervous system and constituting unwanted noise are mixed with the specific response to the stimulation. Therefore, a signal-averaging of multiple trials is performed. Thus, responses synchronized with stimulation are summed, whereas potentials generated at random are cancelled because of their variable polarity (Desmedt, 1987; Mauguière and Fischer, 1990). The remaining responses thus reflect the potentials evoked by the stimulation. Generally, SSEPs are induced by electric stimulation of the median nerve at the wrist, or of the peroneal nerve at the knee or of the tibial nerve at the ankle, and are recorded by electrodes placed on the scalp, the spine and the peripheric nerves (Fig. 5.5.2). The dorsal column-lemniscal system is the main

anatomical substrate of SSEPs in the CNS (Allison et al., 1991a), which depend on functional integrity of rapid afferent musculary fibers of group IA and of rapid afferent cutaneous fibers of group II (Allison et al., 1991a; Desmedt, 1987).

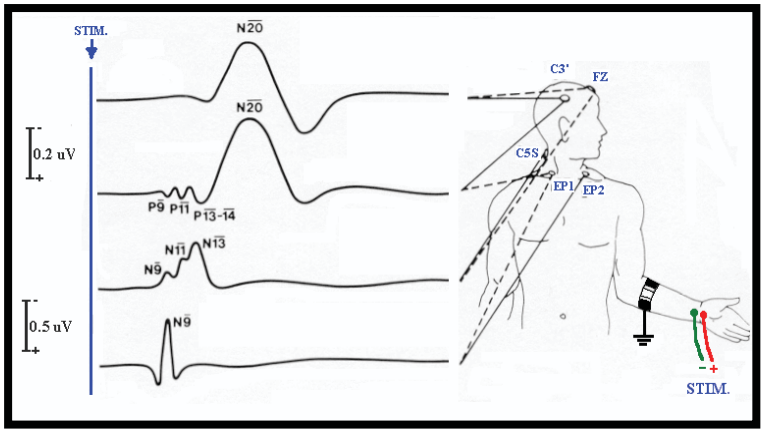


Figure 5.5.2 : SSEPs recorded on the scalp, the neck and the Erb point, during a median nerve stimulation (from http://mfeb.freeservers.com/new_thesis/introduction.htm).

The generated SSEPs propagate in peripherical nerves in direction of the spinal cord and crosses the cellular bodies of the primary sensory neurons in spinal ganglia of ipsilateral dorsal root. A synapse occurs in the dorsal columns nuclei at the level of the lower medulla. Information then circulates in a secondary sensory neuron in the direction of the contralateral ventroposterolateral thalamic nucleus (VPL) by the median lemniscus. In VPL, a new synapse occurs with a tertiary sensory neuron which ends in area 3b of somatosensory cortex (Bear et al., 2001; http:// emedicine.medscape.com/article/1139906-overview). Motor fibers are also activated (Mauguière and Fischer, 1990). Abnormal SSEPs can thus reflect a dysfunction at different levels, such as peripherical nerve, plexus, spinal root, spinal cord, brain stem, thalamocortical projections or primary somatosensory cortex (http:// emedicine.medscape.com/article/1139906-overview).

SSEPs comprise a suite of waves characterized by pics having reproduisible onset latency (in ms) and amplitude (in µV) (Fig. 5.5.3), as well as interpeaks latency. Amplitude reflects the number of activated fibers and their synchronism when discharging (Freye, 2005). SSEPs components are generally named by their polarity and their typical latency at peak in the normal population (Desmedt, 1987; Mauguière and Fischer, 1990). Note that SSEPs measures can differ from an individual to another depending on the body temperature, the blood pressure, the conscious state, the anaesthesia, the administration of drugs, the stature, the age and the sex of the person, the quality of the stimulus, the recording fitting, the filtering and the level stimulated along the peripherical nerve (Desmedt, 1987, Freye, 2005 ; Mauguière et Fischer, 1990 ; http:// emedicine.medscape.com/article/1139906-overview).

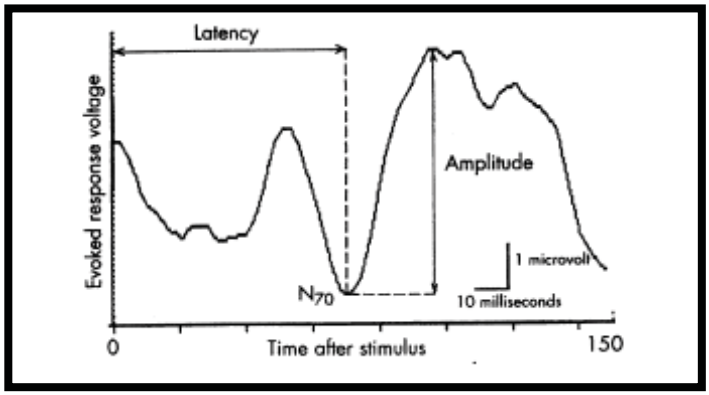


Figure 5.5.3: SSEPs components are characterized by their latency and amplitude.

SSEPs are thus generally classified according to their latency. Considering only electrical stimulation of the median nerve, method used in the present study, short-latency SSEPs components appear at an interval of 20ms to 40ms after stimulation and are generated by subcortical structures, whereas long-latency SSEPs components are recorded from 40ms to 250ms after stimulation and result from cortical generators (Allison et al., 1989a; Allison et al., 1989b; Allison et al., 1991a; Freye, 2005). Another distinction between near-field and far-field potentials is made according to their distance with the recording electrode (Desmedt, 1987; Freye, 2005; Mauguière and Fischer, 1990). Near-field potentials relate to cortical components originating from generators situated in perirolandic cortical areas; these potentials show strong gradients. Far-field potentials relate to subcortical or peripherical generators and show weak gradients.

The typical peaks produced in response to stimulation of the median nerve are N9, P9, N11, P11, N13, P13, P14, N18, N20, P20, P22, P25, P27, N30, P30, N35, P45 (Desmedt, 1987; Mauguière and Fischer, 1990; Allison et al., 1991a). The first peak, N9, is recorded at the Erb point (angle between the clavicular head of the sternocleidomastoid muscle and the clavicule) and reflects activity generated in brachial plexus. P9 is generated in the brachial plexus. These are thus peripherical components. P11, measured at the neck level, and N11 reflect activity in primary afferent somesthesic neuron near the entry of the dorsal root in the spinal cord. N13, measured at the neck level, and P13, measured with an oesophagus electrode, are generated by the activity of post-synaptic neurons of the posterior horn of grey matter in inferior segments of cervical spinal cord. P14 seems to result from the activity of nuclei in the dorsal column or could be due to the activity of the medial caudal lemniscus in lower medulla. It seems that N18 reflects the activity of subcortical structures, among which structures of the brain stem. N20 represents the first cortical negativity; it is recorded as a near-field potential on the parietal area contralateral to the stimulated median nerve. N20

originates principally from the primary somatosensory cortex in the posterior part of the sulcus centralis and thus shows an inversion of polarity across the sulcus centralis in the epidural surface cortical recordings and some recordings on the scalp. This polarity inversion can be used to identify the localization of the sulcus centralis. P22 is produced by a frontal generator localized near the sulcus centralis. The N20-P30 group is recorded principally on the motor cortex and the frontal scalp. The P25-N35 group is recorded near the sulcus centralis and on the central scalp. N20, P20, P25, N30, P30 and N35 result from the activity of neurons in the hand area of primary somatosensory cortex, in the posterior bank of the sulcus centralis contralateral to the stimulation.

In two studies, Arezzo et al. (1979 and 1981) showed in rhesus macaque (Macaca Mulatta) that early components (less than 15 ms in human), generated from peripherical nerve until thalamocortical radiations, and cortical components look in their configuration and topography like human components (Fig. 5.5.4). The latencies of monkeys SSEPs are however about 10 ms shorter than those of humans (Allison et al., 1991a ; Desmedt, 1987). The different cortical components in macaque and in old-world monkeys, P10-N20, N10-20 and P12-N25, correspond to human potentials P20-N30, N20-P30 and P25-N35 (Allison et al., 1989a ; Allison et al., 1991a,b; McCarthy et al., 1991). The localization of SSEPs generators in monkey and human, performed by numerical cartography of evoked potentials fields, provoked a lot of debates (Allison et al., 1989a ; Allison et al., 1991a ; Arezzo et al., 1981, Desmedt, 1987 ; McCarthy et al., 1991). Nevertheless, it has been proposed that the SSEPs groups P10-N20 and N10-20 in anaesthetized monkey and P20-N30 and N20-P30 in human are produced by a tangential generator situated in the posterior bank of the sulcus centralis, that is in area 3b of contralateral primary somatosensory cortex. This generator produces potentials of which the amplitude diminishes with the recording's distance from the sulcus centralis (Allison et al., 1991a). It seems that there is no generator in M1. The groups P12-N25 in anaesthetized monkey and P25-N35 in human are produced a few milliseconds later by a radially oriented generator in cortical areas 1 and 2 of contralateral S1. These potentials, recorded on the surface of the cortex, show a polarity inversion when recorded into the white matter (Allison et al., 1991a). Allison et al. (1989b) showed long-latency potentials in human (from 40ms to 250ms), which are also generated in area 3b and 1 of S1, but also in other areas of S1 and S2. Potentials can also be generated ipsilaterally to the stimulation.

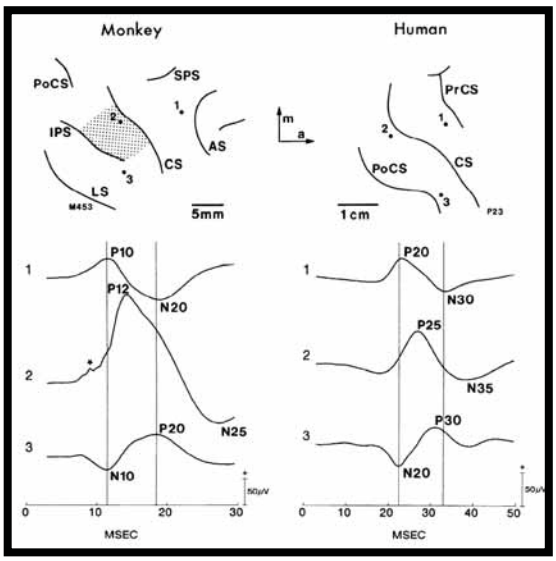


Figure 5.5.4 : Comparison of short-latency SSEPs induced by contralateral median nerve stimulation in human and monkey. Intracortical recordings were derived from the right sensorimotor cortex in anaesthetized human and monkey. Grey zone of monkey's cortex represents S1 hand region. AS: Arcuate sulcus, CS: Centralis sulcus, IPS: Intraparietal sulcus, LS: Lateral sulcus, PrCS: Precentral sulcus, PoCS: Postcentral sulcus, SPS: Superior precentral sulcus. Stimulus was delivered at 0 ms (McCarthy et al., 1991).

The responses to a stimulation of the median nerve can be recorded in a non-invasive way with electrodes placed on the scalp, along the vertebral column, or near the stimulated peripherical nerve, that is on the Erb point (Freye, 2005). As this electrical activity is transmitted from cortical layer through the cerebrospinal fluid, the meninx, the bone of the skull and the skin of the scalp, the signal spreads, looses amplitude and its distribution is not always clear (Freye, 2005). The responses can also be recorded in an invasive way. During neurosurgical or diagnostical interventions, SSEPs can be recorded intracortically in human. This method offers a better spatial resolution than recordings on the scalp surface, as intracortical amplitudes are greater (Allison et al., 1991a).

To perform the recordings, it is necessary to place a reference electrode, of which potential is substracted to the one measured with the other electrodes. Therefore, its potential should be constant. The ideal would be to put it at an isoelectrical body part; however, there is no such part, as the heart generates electrical potentials in the whole body. This reference electrode is thus placed in a body part where potentials gradients associated to intracerebral generators activated by the sensory stimulation in question are almost negligeable. Furthermore, a ground is used to isolate the circuit and to guarantee an equipotentiality as it allows the electrical charges to pass by.

Evoked potentials are clinically useful as they are the unique non-invasive method allowing to diagnose neurological disorders by testing, as already mentioned, the integrity of the somatosensory function along peripherical nerves, plexus, dorsal roots of spinal nerves, spinal cord, brain stem, thalamus and finally sensory cortex (Freye, 2005 ; Mauguière et

Fischer, 1990). They give informations about localization of trauma in spinal cord, of pathologies, of lesions, of degenerescence or demyelination such as multiple sclerosis, in peripherical and central nervous system (Freye, 2005 ; Mauguière et Fischer, 1990). SSEPs also allow to calculate afferent conduction velocities, and thus to detect conduction problems and to evaluate sensitive axons regeneration (Desmedt, 1987 ; Freye, 2005 ; Mauguière et Fischer, 1990). The most important criteria of SSEPs abnormalities are the absence of an obligatory peak or the abnormal prolongation of an interpeak interval. It could be however that a patient suffering from a neurological disorder doesn't show any alteration of SSEPs due to the fact that many parallel ways exist for the information processing. During orthopedic or neurologic surgical interventions, recorded SSEPs are also used to localize somatosensory cortex, to control anaesthesia or an eventual anoxia or ischemia of somatosensory pathways (Freye, 2005). They also allow to assess the state of a coma patient (Mauguière et Fischer, 1990). Furthermore, it has been shown that SSEPs allow to predict the degree of recovery of a human patient having undergone an important cerebral damage (Carter et Butt, 2005). As a function of the different generators' positions, short-latency potentials are used to monitor nerves, spinal cord and brain stem. Long-latency potentials are useful to study cerebral cortex.

The aim of the present investigation was to analyze whether SSEPs measurements could be a predictor to determine the beginning of the plateau phase of manual dexterity of the monkeys. It has been demonstrated in human that SSEPs could predict the level of recovery after a cerebral lesion (Carter and Butt, 2005).

5.5.2 METHODS

In the present study, six monkeys (Mk-JO, Mk-JA, Mk-WI, Mk-AV, Mk-SL and Mk-MO) were subjected to SSEPs recordings. As it was a new technique in our laboratory, imported by external collegues, the recordings presented here served as tests to design a protocol. Thus, the recordings performed on two monkeys (Mk-JO and Mk-AV) were not usable and therefore not considered further. The recordings performed on the four other monkeys were relevant, at least for a part. For SSEPs recordings in the operating room, monkeys were anaesthetized with 0.3 ml of domitor (medetomidin chlorhydrate: 1 volume) and ketalar (ketamin chlorhydrate: 2 volumes) mixture.

To note that SSEPs can change with anaesthetics. In general, the larger the latency of the component and the higher is the number of synapses between the site of stimulation and the neural generator of the component, the greater is the effect of anaesthetic agents on the components of the SSEPs (Hayton et al., 1999). In order to test this variability in our protocol,

a short study was led on the effect of the domitor/ketalar mixture on the SSEPs; the obtained results are presented below.

The monkey was then placed into a Faraday cage to avoid interferences with other electrical fields, positioned on the stomach with a tissue under his thorax to facilitate his respiration (Fig. 5.5.5). A rectangular grid of 16 electrodes was put into the recording chamber on the dura for electrocorticography. These electrodes (impedance under 5kOhms) were oriented vertically with an interdistance of 4.5mm along the medio-lateral axis and 6 mm along the rostro-caudal axis. The electric stimulation at the right median nerve consisted in 200μs current impulses at 6mA intensity and around 5Hz frequencies and activated a thumb contraction. Different reference electrodes were used: wrist, second cervical (C2), frontal or averaging (average of all the electrodes). For all monkeys, the ground was the coupled ears. A filtering was used at 1Hz for the low frequencies and at 4000Hz for the high frequencies. The number of trials used to average the responses was either five hundred (EP® program; see below) or 2 minutes recording (EEG® program; see below). During the whole recording session, rectal temperature of the monkey was controlled. After completion of the recordings, a 0.1ml Antisedan® injection was made for the monkey to wake up well and rapidly.

Electrophysiological activities were acquired with the SystemPlus® program, with a 0.0061μV/digit resolution. This program allowed to directly visualize the average responses. For two monkeys (Mk-WI and Mk-MO) the program's format used was EP®, which allowed 32'000Hz sampling rate, but only from four electrodes; in this case, we chose the four extremes electrodes of the grid. The data were then exported on Excel to perform graphics. For the other monkeys, the program's format used was EEG®, which allowed 1024Hz sampling rate, but was not exportable on Excel. Therefore, analyses were performed on the program EEGLab®.

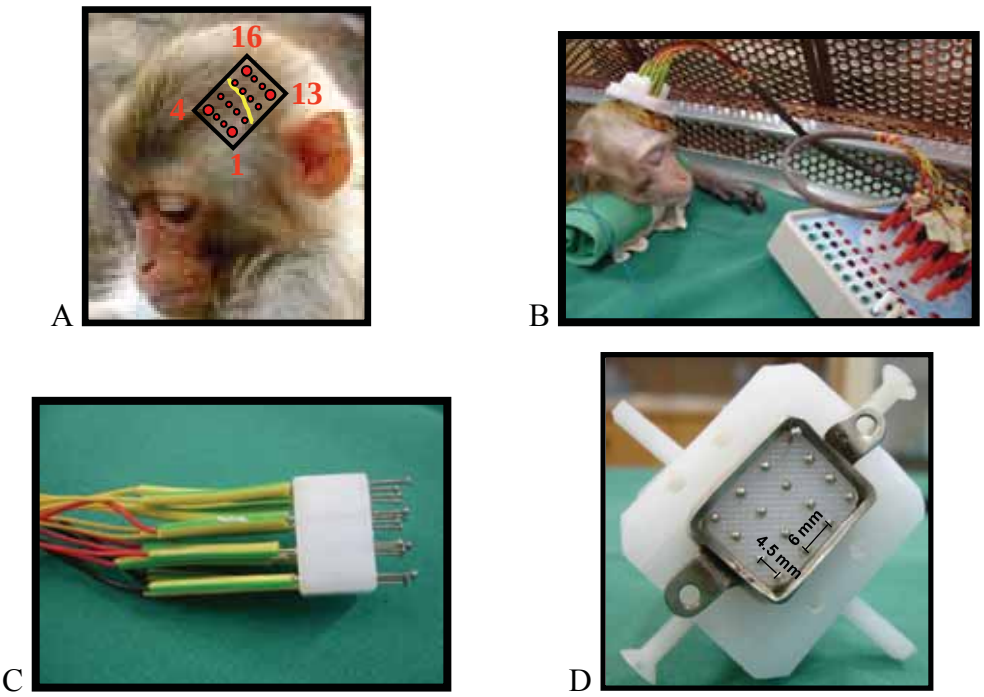


Figure 5.5.5 : A. Location of the recording chamber (black) on the left hemisphere, with the central sulcus (yellow) and the numerated electrodes (red) at the four extremities of the chamber. Electrode 1 is situated on the M1 hand area, electrode 4 on the M1 leg area, electrode 13 on the S1 hand area and electrode 16 on the S1 leg area. (Modified from http:/(/www.futura-sciences.com/galerie_photos/showphoto.php/photo/2003). B. The monkey was placed into a Faraday cage to avoid interferences with other electrical fields and positioned on the stomach with a tissue under his thorax to facilitate his respiration. A rectangulary grid of 16 electrodes (C and D) was put into the recording chamber on the dura.

The traces before stimulation were constant and formed a baseline amplitude. The stimulation was followed by a stimulation artefact and the amplitude diminished then to attain a second baseline amplitude which could be different to the one before stimulation. In this study, we considered this post-stimulation amplitude as a reference to calculate the amplitude of the SSEPs components. The considered corrected amplitudes were obtained by calculating the absolute value of the difference between the amplitude of the peaks and the reference amplitude. In this work, analyses were carried on the latencies and amplitudes of the first and second cortical components (Fig. 5.5.6), measured about twenty minutes after the beginning of the anaesthesia on the most lateral electrodes (supposed placed on the hand representation's area), one on the most rostral (M1) and the other one on the most caudal (S1) part.

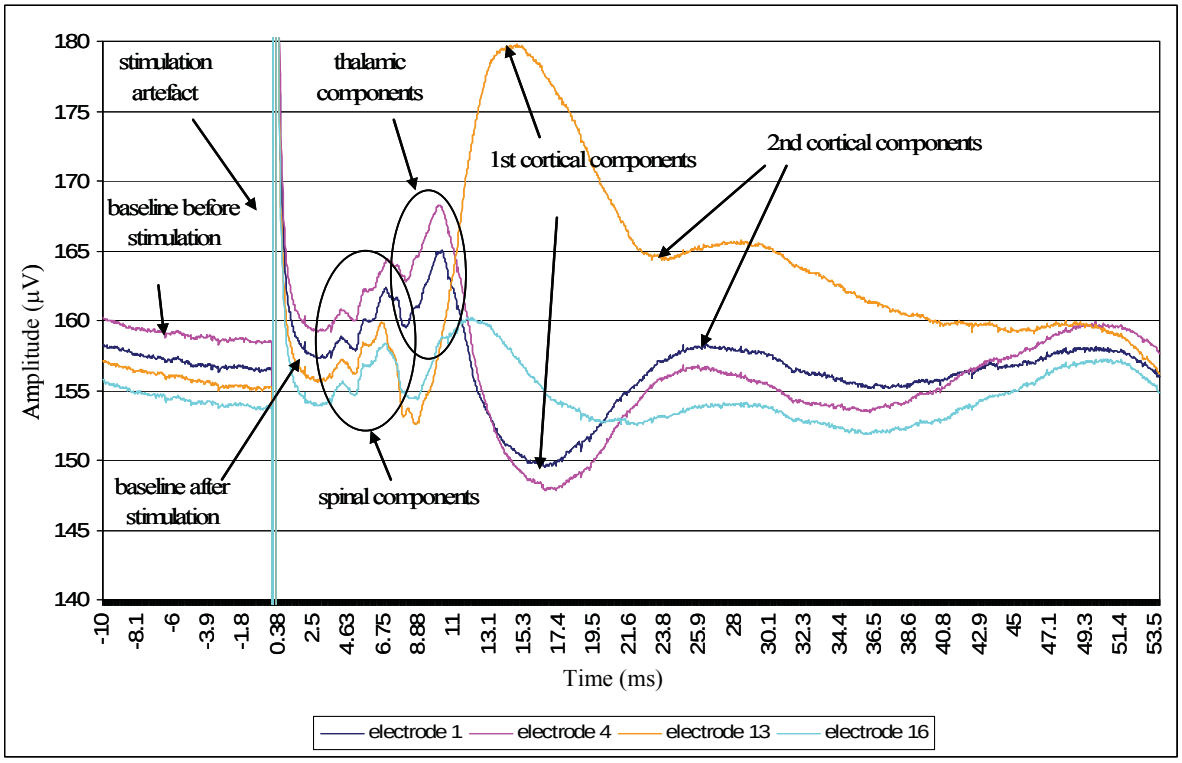


Figure 5.5.6: Different components one can observe on SSEPs recordings performed on the dura. In this work, we analyzed the latencies and amplitudes of the first and second cortical components on the most lateral electrodes, one on the most rostral (M1; blue line) and the other one on the most caudal (S1; orange line) part.

Recordings were performed from day 108 pre-lesion until day 99 post-lesion for Mk-MO, from day 1 pre-lesion until day 84 post-lesion (first) for Mk-JA, from day 18 pre-lesion until day 184 post-lesion for Mk-SL and from about day 80 post-lesion (last) for Mk-WI.

5.5.3 RESULTS

5.5.3.1 Effects of the anaesthesia on SSEPs components

By comparing recordings obtained at regular time intervals during the whole course of the anaesthesia, it appeared that SSEPs responses changed over time.

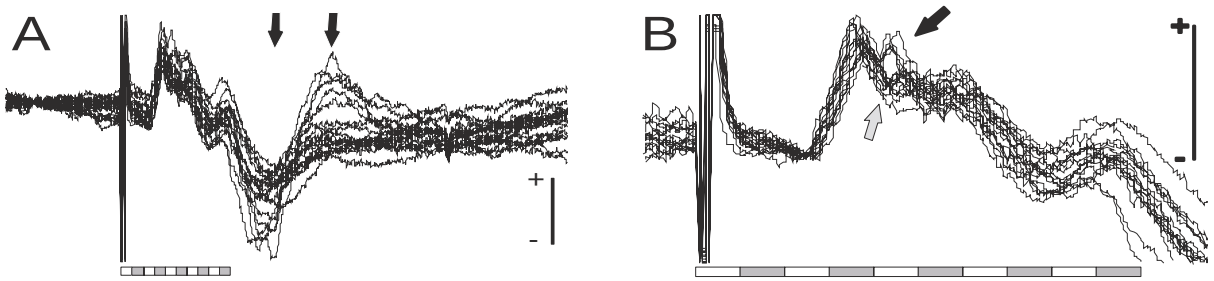


Figure 5.5.7: SSEPs components vary during the time course of the anaesthesia. A: Superposition of SSEPs obtained from one animal at regular time points after the initiation of the anaesthesia. Notice the strong variability observed in late components (black arrows). B: Magnification of the early SSEPs components. For better visual discrimination, all recordings have been aligned on the initiation of the first depolarisation. Notice that the P5.3 wave is well defined in some recordings (black arrow) but absent from others (grey arrow). Scale bars: 10 ms and 5 μV.

Figure 5.5.7A shows a superposition of 15 single SEPs obtained from one scalp electrode in one animal at intervals of 5 min. The signals with a latency superior to 10 ms (i.e. responses of cortical origin) present a strong variability in amplitude (Fig. 5.5.7A, black arrows). Such changes have not only been observed for the late potentials, but also for the early components of subcortical origin. Figure 5.5.7B shows the same superposition of single SEPs, but this time focusing on the early components. To better compare the shape of the signals, each recording was aligned on the start of the response. In this case, the P4.5 wave appears well recognisable in a subset of recordings (Fig. 5.5.7B, black arrow) and absent from the others (Fig. 5.5.7B, grey arrow), indicating that the anaesthesia not only affects cortical components, but that it can also impact on components that reflect subcortical processes.

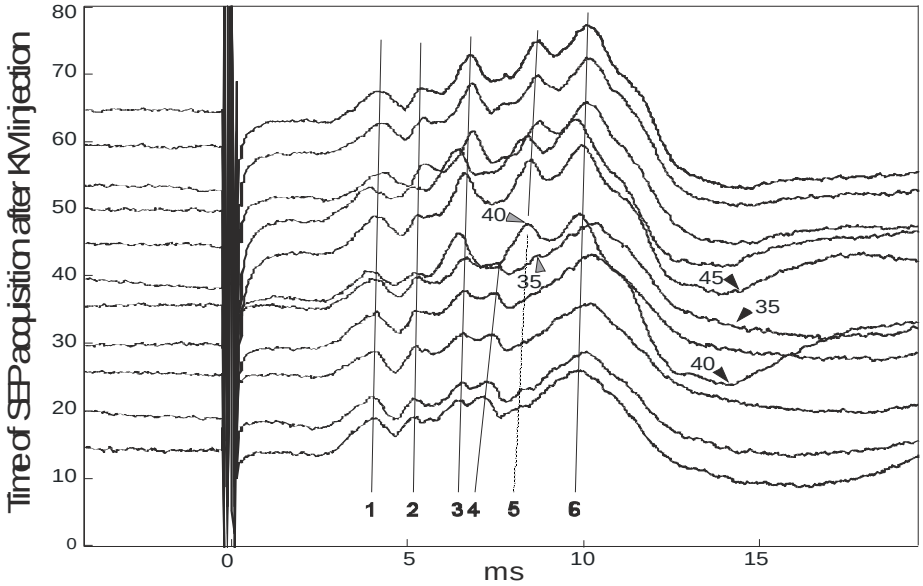


Figure 5.5.8: SSEPs recorded at regular time intervals in one animal during the time course of a ketamine/domitor anaesthesia. Notice that the amplitude of individual components can vary considerably during the course of the anaesthesia.

The question then arises as to whether these amplitude changes were occurring gradually over the whole duration of the anaesthesia or rapidly at certain stages of the anaesthesia. Figure 5.5.8 depicts a waterfall plot of all SSEPs recorded during a single experiment. The first recording, obtained 15 min after the injection of the ketamine/domitor mixture, presents a first, well defined, peak after 4 ms (Fig. 5.5.8, line 1). It is followed by three peaks at 5.2, 6.5 and 7.1 ms (Fig. 5.5.8, lines 2, 3, 4), and by a last large peak at 9.9 ms (Fig. 5.5.8, line 6). The recordings obtained during the 35 first minutes of the anaesthesia were rather similar to the first recording. There was nevertheless a conspicuous increase in the latency of the 7.1 ms peak associated with a decrease of its amplitude (Fig. 5.5.8, line 4). The next recording, taken 5 min later, i.e. 40 min after the ketamine/domitor mixture injection, presented clear changes with regard of the previous ones. While peaks at 3.9, 5.2 and 9.9 are present and similar to those present in the preceding recordings, a peak at 6.4 ms seems to be related to the 6.5 ms peak present in the first recording but with a suddenly large amplitude. The signal previously present at 7.1 ms seems to disappear, while a large peak now arises at 8.4 ms (Fig. 5.5.8, black arrowhead). This shape of the early SSEP remains quite stable for the next 30 min., with small changes in latencies and amplitudes of the components. Similar observations could be made for later components. From the recording at 40 min on to the end of the experiment, the repolarisation starting after the P10.0 is steeper and of larger amplitude than in the first recordings. In this case, the animal emerged from anaesthesia 70 min after the ketamine/domitor mixture injection, and the acquisition of SSEPs was stopped. This data indicates that slow gradual changes occur for some signals, but that rapid changes can also occur over a very short time scale.

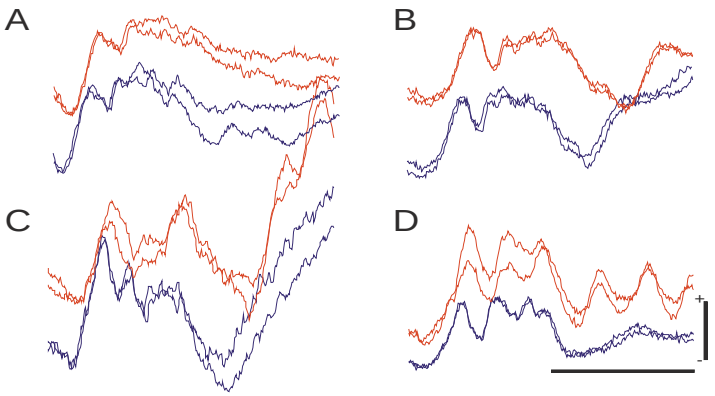


Figure 5.5.9: Comparing SSEPs obtained few minutes after the ketamine/domitor mixture injection with SSEPs obtained at awakening time. A-B: Data from two animals showing moderate early SSEPs characteristics changes between the early and late recordings. C-D: SSEPs from two animals which present changes in the P6.2 and subsequent components between the early and late recordings. Early recordings in blue, late recordings in red. Scale bars: 5 ms and 2 μV.

The difference between the recordings obtained 15-20 min after the initiation of the anaesthesia and those obtained just before the animals awake vary among animals. Figure 5.5.9A, B presents two examples where the differences between the SSEPs shape of the firsts and of the lasts recordings are limited, while differences are conspicuous in recordings obtained from two other experiments (Fig. 5.5.9C, D).

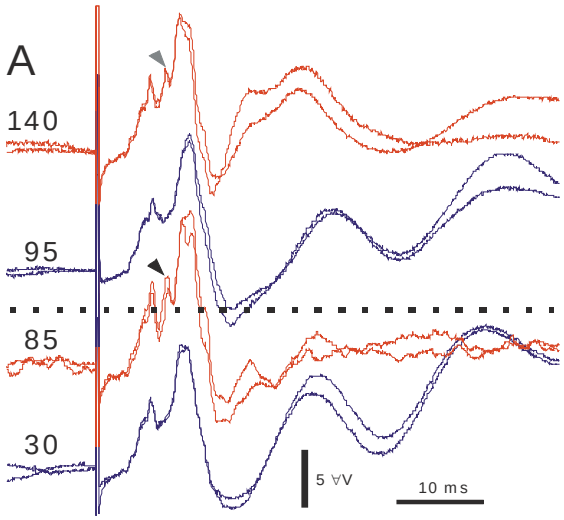


Figure 5.5.10: A: Specific SSEPs components are inhibited during the early phases of the ketamine/domitor anaesthesia. The recordings in the lower half of the figure were obtained about 30 (blue) and 85 (red) minutes after a first injection of the ketamine/domitor mixture. Notice the emergence of a well-build peak (black arrowhead) with a latency of ca 8 ms during the near awakening phase. A second injection of the ketamine/domitor mixture was done at this stage. In the recordings taken rapidly after the second injection (upper half, blue) the newly formed peak disappears and the SSEP returns to a shape corresponding to that observed in the firsts minutes after the first injection. When the awakening stage is reached for the second time, the peak reappears (grey arrowhead) in the SSEP recording (upper half, red). These recordings were obtained from an epidural electrode, and the reference electrode was set at a cervical level. Recordings early after a ketamine/domitor mixture injection in blue, recordings at awakening time in red. Scale bars: 10 ms and 5 μV.

To ascertain that the changes were related to the ketamine/domitor mixture, in some experiments, a second dose of anaesthetics was injected when the animals approached arousal (eyelid movements, EMG signals in the EEG). Figure 5.5.10 shows data from one such experiment. In this experiment, epidural electrodes were used and the reference electrode was placed over the cervical spinal cord on the basis of the cranium. In recordings obtained 30 min after the initial injection of the ketamine/domitor mixture, no peak is discernable at a latency of 8.3 ms, whereas a peak at such a latency is conspicuous 85 min after the injection (Fig. 5.5.10, black arrowhead). A new dose of the ketamine/domitor mixture was added at that moment, and in recordings obtained ten minutes later, this component had disappeared. Later, when the anaesthesia weakened and signs of arousal returned, the signal at 8.3 ms reappeared (Fig. 5.5.10, grey arrowhead).

Experiment	P3.8	P4.5	P6.2	P10.0	N15.0
1	3.06 ± 0.21	3.16 ± 0.23	3.49 ± 0.26	2.77 ± 0.48	-3.34 ± 0.84
2	3.32 ± 0.33	3.11 ± 0.45	--	1.54 ± 0.51	-2.81 ± 1.45
3	3.01 ± 0.50	3.46 ± 0.40	2.89 ± 0.52	1.58 ± 0.48	-3.86 ± 1.09
4	3.17 ± 0.20	3.98 ± 0.18	3.73 ± 0.32	--	0.73 ± 0.76
5	4.49 ± 0.50	3.47 ± 0.87	3.31 ± 0.37	1.84 ± 0.60	-3.48 ± 1.15
6	4.71 ± 0.39	4.01 ± 0.31	3.96 ± 0.39	2.28 ± 0.52	-2.68 ± 0.75

Table 5.5.1: Mean amplitude (±std) for five specific SSEP components obtained from data of six experiments. The median nerve stimulation for contralateral to the recording site over the parietal cortex.

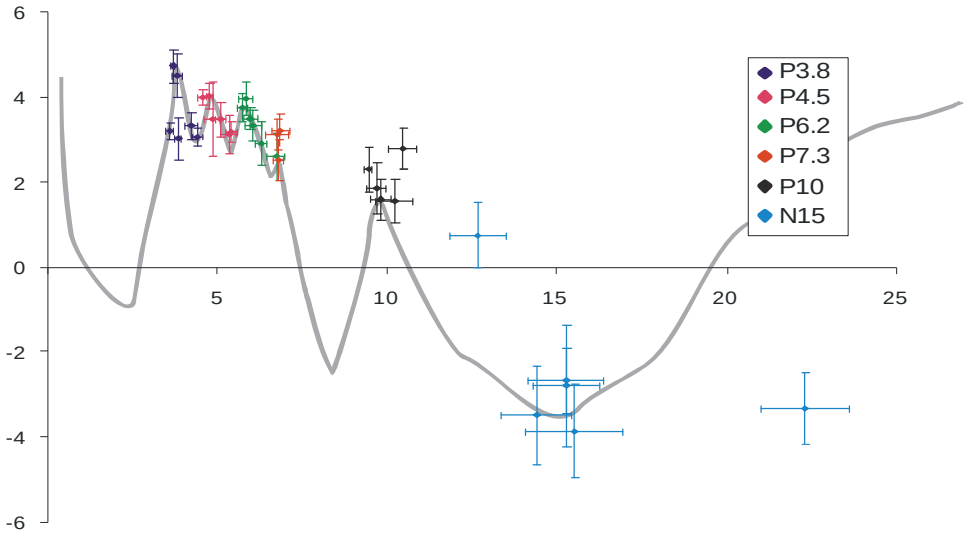


Figure 5.5.11: Variability of the amplitude and latency of SSEP components. Each point corresponds to the mean amplitude and mean latency of one SSEP component measured every 5 min during the course of the anaesthesia during one experiment with scalp recordings. The standard deviation of latency and amplitude is plotted for each point. A schematic SSEP curve (grey) as been plotted on the graph to show the components investigated.

Table 5.5.1 and figure 5.5.11 show the variability of the amplitude of SSEPs components during the course of anaesthesia.

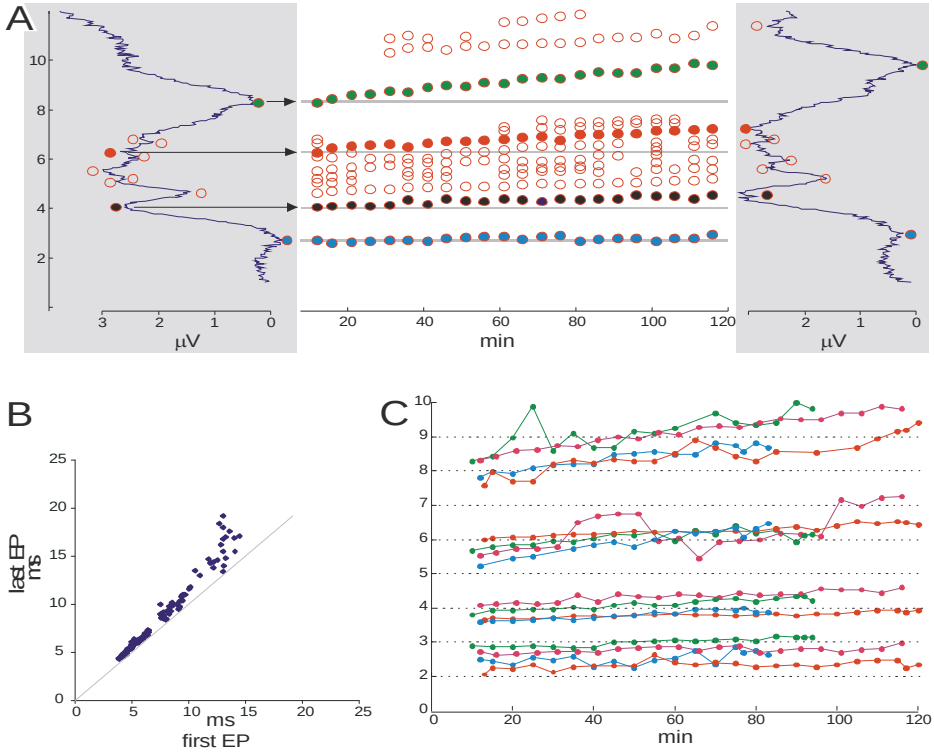


Figure 5.5.12: The latency of SSEP components tends to increase during the course of the experiment. A: The left and the right panels show respectively an early and a late SSEP recording with diverse components labeled with a circle. The middle panel shows the latency of individual components regularly increasing over the whole duration of the anaesthesia. Notice that the latency for the initiation of the SSEP (blue circles) does not change much during the whole experiment. For the first peak (P3.8, black circles), the latency regularly increases during the course of the anaesthesia. Later components (P6.2, red circles; N8, green circles) behave similarly, with a larger latency increase for later components. B: Relation between the latency of individual components in the first and last SSEP recording of one session. Notice that during the course of the ketamine/domitor anaesthesia the latency of all individual components increases. C: Progression of latencies over the duration of the anaesthesia for experiments on four different animals. Notice that the latency increases similarly for all animals.

Not only does the amplitude of the signals but also their latency changes during the course of a ketamine/domitor anaesthesia. Figure 5.5.12A shows an example, where the latencies of each component has been measured individually for different recordings. The left and the right panels show respectively the first and last recordings of the experiment, with different components marked by circles. The middle panel plots the latency of different components as a function of the time elapsed since the injection of the ketamine/domitor mixture. The first component (Fig. 5.5.12A, blue circles), which corresponds to the time between the moment of the stimulation and the appearance of the first response, remains rather constant for the two hours of the recording period. The following component, corresponding to the peak of the P3.8 wave (Fig. 5.5.12A, black circles) shows a slight but distinct increase of latency, which is particularly clear towards the end of the experiment. This increase in latency is also found in subsequent components (Fig. 5.5.12A, red and green circles), with the rule that the later the components in the first SSEP response, the largest the

latency difference observed for this same component when measured in the last SSEP recording of the session.

This increase in latency stays in a linear relationship with the delay of the signal as measured in the first recording (Fig. 5.5.12B). In this figure, the latency of each component measured in the first recording of a session was plotted against the latency of the same component measured in the last recording of the session. All points are positioned above the line where points would lie if the latency would not change, thus indicating that, the latency of all waves was larger in the last recording when compared to the first recording. The increase of latency was larger for late components, as indicated by the increased distance to the equality line for the later signals (Fig. 5.5.12B).

This tendency was fairly constant across sessions, as shown in figure 5.5.12C. In this figure, the latencies of four SSEPs landmarks are shown for four different experiments. Here too, the time of initiation of the SSEPs did not change with a clear trend over the duration of the experiments. Subsequent components however demonstrate a clear tendency to become slower as the time of anaesthesia increases (Fig. 5.5.12C).

Overall, the present data demonstrate that in the macaque, the use of a combination of ketamine hydrochloride (ketamine) and of medetomidine hydrochloride (domitor) alters the characteristics of SSEPs components of both cortical and subcortical origin. Acquisition of SSEPs at regular intervals during the course of the anaesthesia indicate that between the time of the ketamine/domitor mixture injection to the moment where the animal begins to move, the latency of the subcortical and cortical components increases regularly and that this increase does not reflect changes in body temperature (data not shown). For recordings obtained at similar times after the initiation of the anaesthesia, there was also intra-individual and inter-individual variability.

In the following part assessing changes in SSEPs components after motor cortex lesion, the anaesthetics were given in the same amounts and the data acquisition was made at comparable delays after the injection to the same anaesthesia level. Nevertheless, these anaesthesia effects should be taken into account for the following part, and only changes that would be of greater importance than those observed here to be due to anaesthesia could probably be attributed to the motor cortex lesion and the recovery processes.

5.5.3.2 SSEPs cortical components parallel the functional recovery of manual dexterity after lesion of motor cortex

As SSEPs were recorded in Mk-MO with a wrist reference and in Mk-JA and Mk-SL with a C2 reference, a comparison was performed between SSEPs measured with both references in Mk-WI, a monkey with SSEPs measured with C2, wrist and frontal references. To note that these measures on Mk-WI were performed after bilateral cortical lesions and with electrodes under the skin and not with electrodes placed on the dura. Nevertheless, these data having been recorded 90-150 days after lesion, they could allow us to observe if there is a covariation or not between measures with C2 reference and with wrist reference.

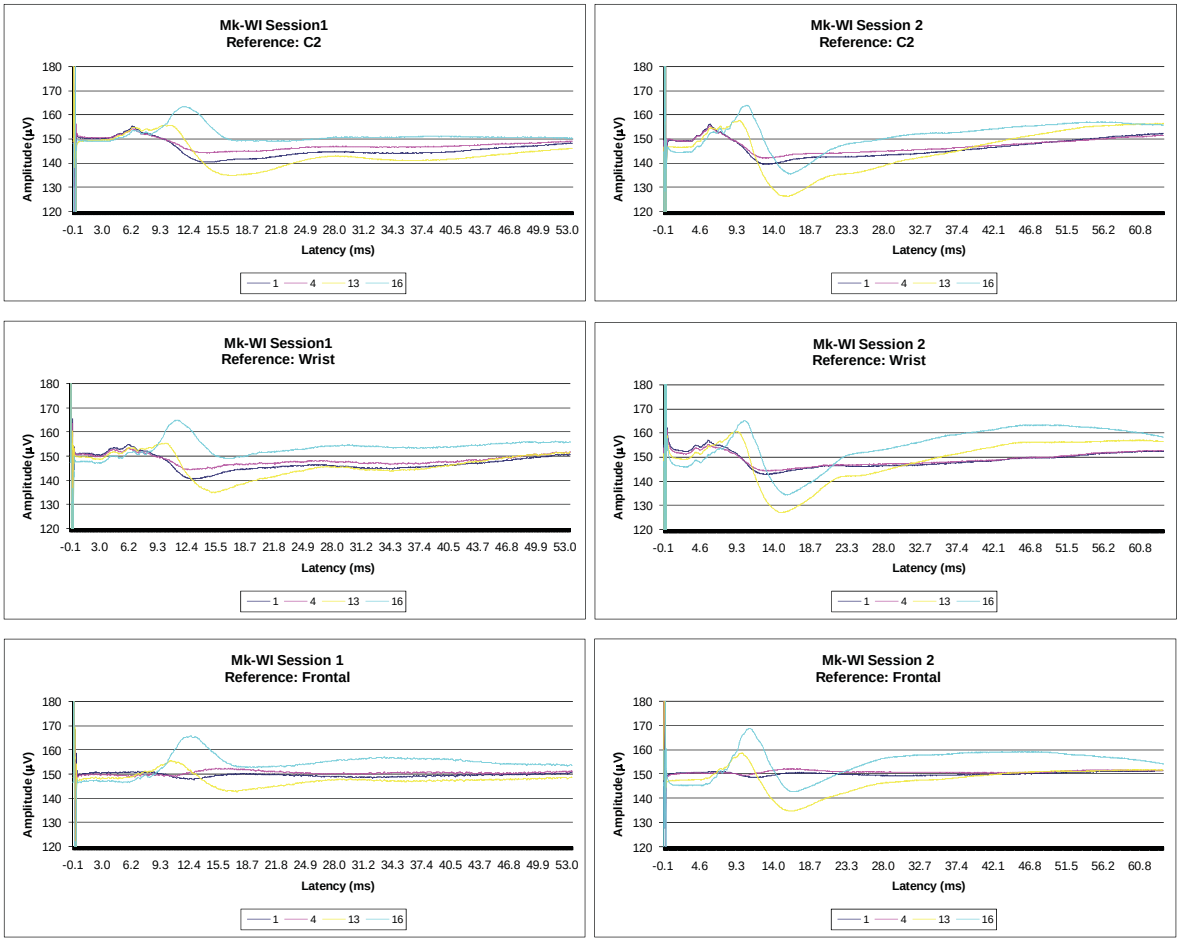
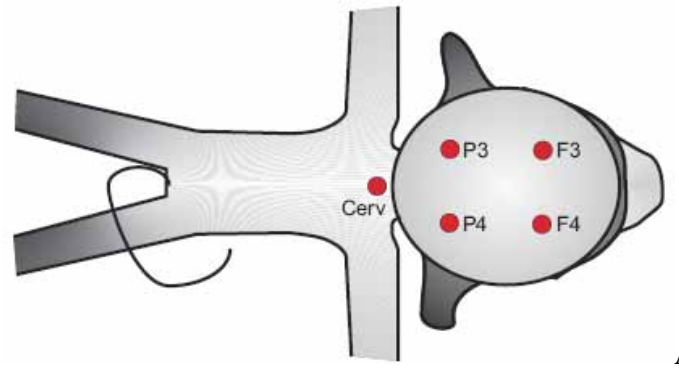


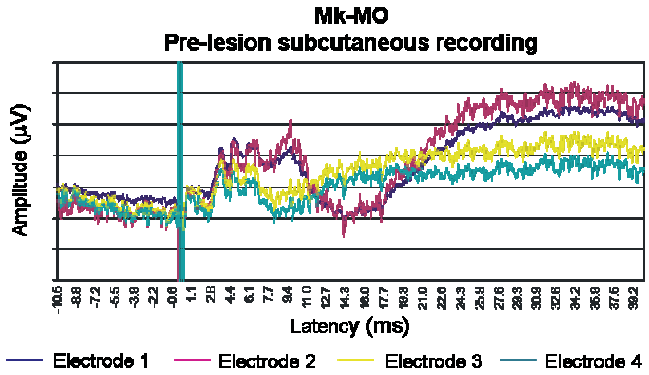
Figure 5.5.13 : SSEPs obtained with a C2 reference, a wrist reference and a frontal reference in Mk-WI on two different sessions.

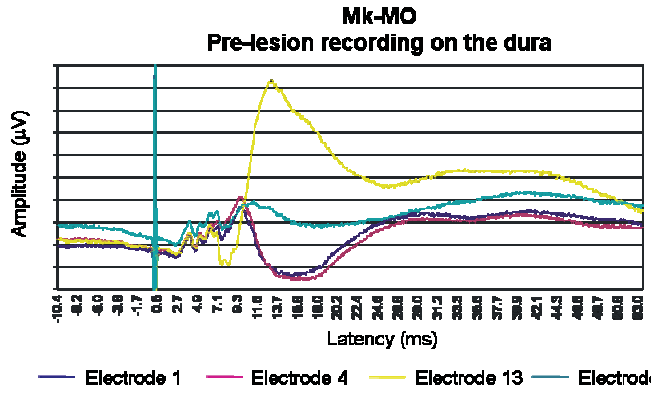
Figure 5.5.13 shows that the morphology of the curves was not affected by the difference of reference. Amplitudes could vary, but the measures obtained were similar, at least for the main cortical components with the two references of interest for the present study, namely C2 and wrist. Therefore, one could conclude that although the recordings in the three monkeys of the study have been realized with different references, the same cortical components should appear in both cases.

An important problem met was the lack of data, due in part to the fact that two types of measurements were performed, namely with the above described grid placed on the surface of the dura and subcutaneously, with four needle electrodes (MicroMed) placed under the skin over the frontal and parietal cortex at points corresponding to the F3, P3, F4 and P4 positions in a standard 10-20 configuration (Fig. 5.5.14A).



A





B

Figure 5.5.14 : A. Position of the four needle electrodes used subcutaneously to obtain SSEPs. B. SSEPs recordings obtained with electrodes placed subcutaneously (top) and placed on the dura (bottom).

These two options did not lead to the same results (Fig. 5.5.14B). One could expect weaker amplitudes when recordings were performed subcutaneously, due to the greater distance between the recording electrode and the generator than when electrodes are placed directly on the surface of the dura; but no reason could justify the greater difference of

latencies observed between the two types of recordings, leading to ask if the measured components are the same. Therefore, in the part treated here, only the measures performed with the grid placed on the dura were considered.

An important remark to note is the lack of data, especially during the pre-lesion period. Consequently, this lack of normative data leads to be very cautious when interpreting the results. Nevertheless, diverse observations could be done (Fig. 5.5.15). Mainly, the amplitude of the first cortical component as measured on the electrode 13 was observed in the three monkeys to vary the most after the lesion and to covary with the manual recovery. The other parameters could also vary after the lesion, more or less in covariation with the hand performance and according to the monkey, but to a lesser extent.

Indeed, immediately after the lesion, Mk-SL showed a total loss of manual dexterity during five days. Then, a progressive recovery took place, reaching a behavioural plateau on the total, vertical and horizontal score about 52 days after the lesion, with a light pejoration from day 135 post-lesion. For the contact time, the recovery plateau appeared after 80 days for the vertical wells, whereas it did not appear clearly for the horizontal wells. During the recovery and plateau phases, an important dispersion in the contact time could be observed, either for the vertical or the horizontal wells, reflecting an irregular performance. The latency of the first cortical component showed an enhancement after the lesion on electrode 1, stabilizing at day 43 post-lesion, just before the behavioural plateau for the score. For the latency of the second cortical component measured on electrode 1, two time points stood out at days 43 and 59 post-lesion, corresponding to the period of stabilization of the manual score. On electrode 13, the data on the latencies were less informative, due to the great pre-lesion variation. The amplitude of the first cortical component measured on electrode 13 seemed to reflect the effect of the lesion, as it enhanced considerably during the acute phase, returning progressively to its pre-lesion value at day 59, which was the same period as the beginning of the plateau for the manual score (day 52). On electrode 1, the same pattern, although not present for the first component, appeared for the second component, with a return to the pre-lesion values at day 31 post-lesion. On electrode 13, the only change on the amplitude of the second cortical component was a peak appearing at day 13.

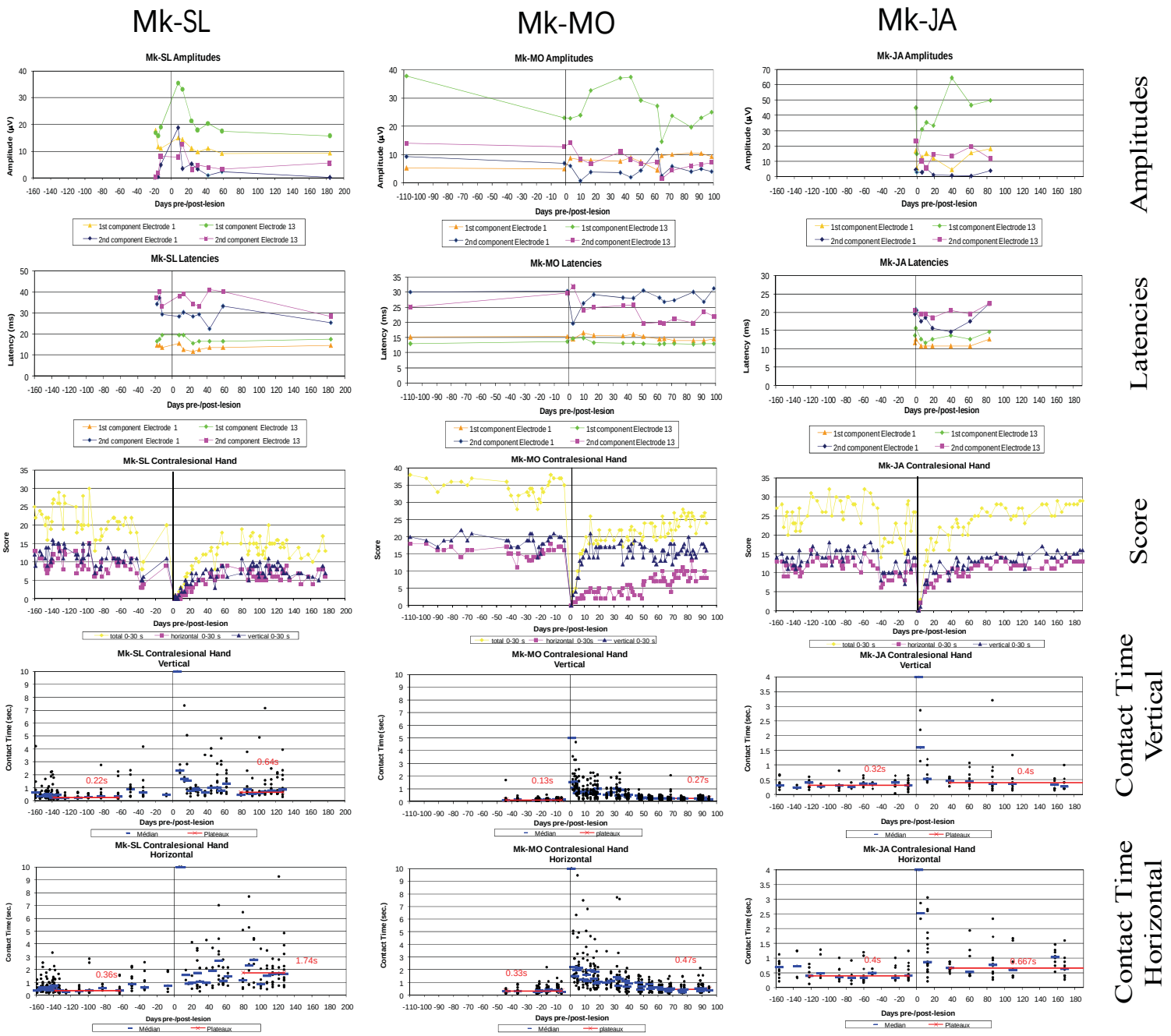


Figure 5.5.15: Evolution of the various parameters in Mk-SL, Mk-MO and Mk-JA, from left to right. The first two graphics illustrate the evolution of the amplitudes and the latencies of the first and second SSEPs cortical components recorded on electrode 1 (orange and blue, respectively) and electrode 13 (green and pink, respectively). On the third graphic is represented the evolution of the score (total score is in yellow, vertical score in blue and horizontal score in pink). The last two graphics illustrate the evolution of the contact time for the vertical and the horizontal wells, respectively.

Concerning Mk-MO, the same complete loss of manual dexterity occurred after the lesion. For the score, the monkey recovered progressively to reach a first behavioural plateau after about 15 days. Then, a transition phase took place from day 49 to day 63, reaching a second plateau about 72 days after the lesion. In parallel, for the contact time, high times with an important dispersion could be observed after the lesion, diminishing progressively to reach a plateau 48 days post-lesion for the vertical wells and 55 days post-lesion for the horizontal wells. On the two electrodes, the latency of the first cortical component enhanced lightly after the lesion and returned to its pre-lesion value 17 days after the lesion. This time point corresponded to the first behavioural plateau for the score. The same pattern could be observed for the latency of the second cortical component on both electrodes. For this latency, on electrode 13, a second plateau took place after about 51 days, corresponding to the plateau for the contact time and to an improvement phase for the score. The amplitude of the first cortical component measured on electrode 1 enhanced after the lesion, remaining stable until about day 60 post-lesion and re-enhancing to stabilize from day 65 post-lesion, a little bit after the plateau for the contact time and before the plateau for the score. Although the value of this amplitude was very different between the two pre-lesion measures on electrode 13, it appeared that it enhanced importantly from day 10 until day 50 post-lesion, corresponding to the first behavioural plateau period for the score. Then, it diminished from day 51 to day 65 in parallel to the transition phase between the two behavioural plateaux for the score, reaching finally a stable plateau from day 72, which corresponded to the beginning of the second plateau for the score. For the amplitude of the second cortical component, after a light enhancement on electrode 13 and a light decrease on electrode 1, a first stabilization took place from day 17 to day 62, respectively 51, corresponding to the first manual recovery plateau for the score. Then, on both electrodes, a transition phase occurred in parallel to the increase of the performance for the score, reaching a stable plateau 72 days post-lesion, similarly to the score.

After his complete loss of manual dexterity following the lesion, Mk-JA recovered progressively until reaching a stable behavioural plateau for the score about 80 days after the lesion. For the contact time, there was an enhancement of the times associated to an important dispersion following the lesion, with a stabilization about 38 days post-lesion. On both electrodes, the latency of the first cortical component, after a decrease immediately after the lesion, seemed to remain more or less stable from day 6 post-lesion, just before the beginning of the recovery for the score, and enhanced at day 84 post-lesion, corresponding to the beginning of the behavioural plateau for the score. Later data that could eventually have shown a plateau are missing. The latency of the second cortical component on electrode 1

reflected the behavioural recovery phase. Indeed, it evolved in a curve diminishing and re-enhancing until day 84 post-lesion. Again, this time point would correspond to the beginning of the behavioural plateau for the score if supplementary data were avalaible to confirm it. On electrode 13, this latency was not informative. The amplitude of the first cortical component on electrode 1 showed an inconstant variation along the behavioural recovery phase, decreasing immediately after the lesion, enhancing at day 6 and re-decreasing until day 40, re-enhancing finally until day 84 post-lesion. This amplitude on electrode 13 reflected well the behavioural recovery phase, first decreasing strongly immediately after the lesion and then progressively but importantly enhancing until day 40 post-lesion, decreasing finally until day 84 post-lesion to reach its pre-lesion value. The amplitude of the second cortical component on electrode 1 reflected mainly the acute phase post-lesion with a light enhancement. On electrode 13, a decrease followed immediately the lesion, with an enhancement stabilized from day 19 post-lesion, which could correspond to the progressive stabilization of the manual dexterity on the contact time.

5.5.4 DISCUSSION

First of all, as already mentioned above, there was an important lack of data, especially during the pre-lesion period. Therefore, these results should be carefully considered. Particularly, as the baseline was incomplete, the post-lesion variations were difficult to interpret. Then, some pre-lesion variations inter- and intra-individually on the pre-lesion phase could be observed in terms of latencies and amplitudes, which could be due to the material used to record the SSEPs, to the size difference between the monkeys, to the effect of the anaesthesia, to the body temperature, to the position of the electrodes on the median nerve, or to the position of the recording chamber.

Globally, the latency of the first cortical component was informative only for the acute phase on both electrodes, as it was little subjected to variations except immediately after the lesion. The latency of the second cortical component was mainly informative for Mk-MO, indicating the acute effect of the lesion and the recovery phase, especially on electrode 13, and for Mk-JA on electrode 1, showing the post-lesion variability during the recovery phase. The amplitude of the first component measured on electrode 1 seemed to reflect partly the behavioural performance on the acute phase and during the recovery. On electrode 13, this amplitude varied strongly in the three monkeys, first enhancing and then decreasing along the time course of the recovery phase. The amplitude of the second component on electrode 1 and on electrode 13 were mainly informative in Mk-MO, as they covaried with the manual score, partly also in Mk-SL, and in Mk-JA for the acute post-lesion phase.

In summary, the diverse analyzed parameters could more or less covariate with the manual performance of one or another monkey, thus representing sometimes information sources. In particular, the amplitude of the first cortical component measured on electrode 13 seemed to represent the most informative parameter, undergoing systematically the stronger variations after the lesion and during the recovery, and evolving in covariation with the manual performance measured by the score, thus possibly reflecting a cortical reorganization through activation changes. For example, a stronger amplitude after the lesion could reflect a higher neuronal recruitement to carry out the task. Therefore, this amplitude of the first cortical component, measured by electrode 13, which is placed above the potentials generator in S1 (vs M1 for electrode 1 and 4) and situated above the hand representation's area (vs leg for electrodes 4 and 16), could represent a good predictor of recovery.

However, as already mentioned, these preliminary data served mainly with the aim of establishing a recording protocol. Therefore, a regularity of recording, the use of the same reference and position of the electrode (subcutaneously or on the dura) were lacking. Consequently, it would be optimal for the future experiments to have a larger amount of pre- and post-lesion data recorded with the same reference for all the monkeys. As the anaesthesia had an influence on the SSEPs, it would be important to systematize as well as possible the time of the recordings in relation to the beginning of the anaesthesia; therefore, the observed differences would not be imputed to the effect of the anaesthetic agent. To note also that the number of subjects studied here was very low.

Although this method was shown here to be quite complex, the SSEPs recordings turned out to be relevant for the diagnosis, especially after a stroke (Carter and Butt, 2005), for the multiple sclerosis and for the monitoring during surgical interventions (Desmedt, 1987 ; Mauguière et Fischer, 1990). The results obtained here confirm the utility of SSEPs that allowed to bring to light the cortical reorganization after an unilateral M1 lesion, mainly by the effect of the lesion and the recovery on the amplitude of the first cortical component on the electrode placed above the hand region in S1 in the three monkeys.

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5.6 DOES THE BEHAVIOURAL PERFORMANCE VARY ALONG A DAILY SESSION AS ASSESSED BY THE CONTACT TIME?

5.6.1 PURPOSE

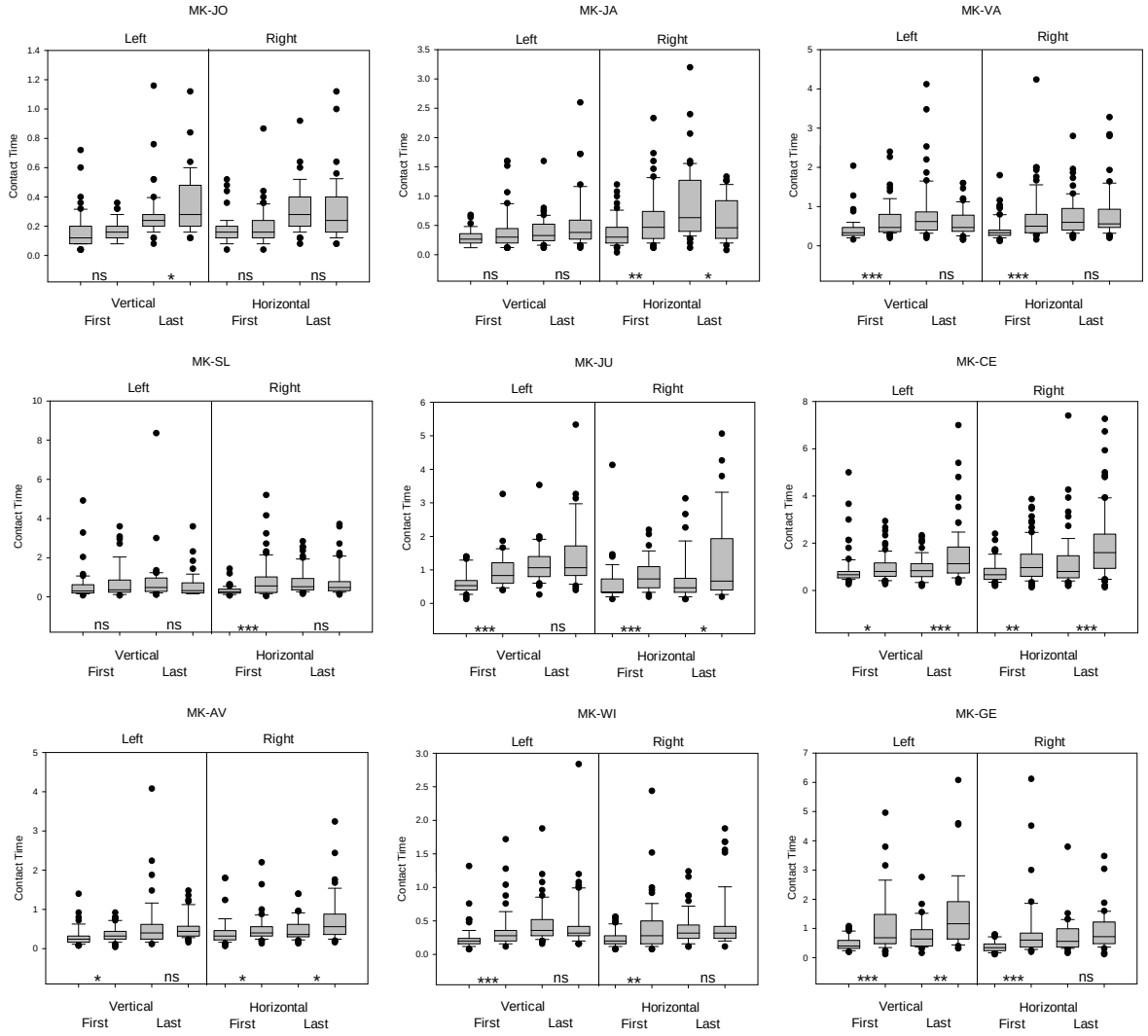
The aim of the present analyses was to assess if the monkeys performed the modified Brinkman board task with a constant efficiency before the lesion, as well as after the lesion. Indeed, the monkeys could be less motivated at the end of the task, particularly after the lesion, or on the contrary quicker on the final straight, especially before the lesion.

5.6.2 METHODS

The performance of nine monkeys was analyzed, for each hand separately, by measuring the time needed to grasp a reward (food pellet) in a well (contact time) when performing the modified Brinkman board. This parameter was observed on the first five vertical, the first five horizontal, the last five vertical and the last five horizontal wells. Data of many training sessions were cumulated and comparisons were made between the first five and last five retrieved pellets, either on the vertical, the horizontal or the total (vertical plus horizontal, meaning ten first and ten last) wells, with the Mann-Whitney non-parametrical statistical test. The observations were carried first on the pre-lesional plateau data for both hands, and then on the post-lesional plateau as compared to the pre-lesional plateau, for the contralesional hand only.

5.6.3 RESULTS

A



B

	Left Hand Contact Time Vertical	Left Hand Contact Time Horizontal	Right Hand Contact Time Vertical	Right Hand Contact Time Horizontal
Mk-JO	ns	*	ns	ns
Mk-JA	ns	ns	**	*
Mk-VA	***	ns	***	ns
Mk-SL	ns	ns	***	ns
Mk-JU	***	ns	***	*
Mk-CE	*	***	**	***
Mk-AV	*	ns	*	*
Mk-WI	***	ns	**	ns
Mk-GE	***	**	***	ns

Figure 5.6.1 : A. Boxplots representing the pre-lesional distribution of the contact time for each monkey and each hand separately. Comparisons were carried on the first five versus last five pellets. B. Recapitulating table of the pre-lesional performances comparisons between pellets grasped at the beginning versus at the end of the task, measured by the contact time.

Pre-lesionally (Fig. 5.6.1), Mk-JO showed a constant performance with both hands between the beginning and the end of the task. Indeed, no difference was found on the different wells, except on the horizontal wells with his left hand. Along this line, Mk-JA, Mk-VA, Mk-SL and Mk-WI had a similar pattern for their left hand only, except on the vertical wells for Mk-VA and Mk-WI in favour of a better performance at the beginning of the task. On the contrary, a quasi-systematic worse performance at the end of the task for both hands was observed in Mk-JU, Mk-CE, Mk-AV and Mk-GE, again with one exception on the horizontal wells with the left hand for Mk-JU and Mk-AV and with the right hand for Mk-GE. With their right hand, Mk-JA, Mk-VA, Mk-SL and Mk-WI showed a comparable better performance at the beginning of the task, except on the horizontally oriented wells for Mk-VA, Mk-SL and Mk-WI.

Thus, on the two main tendencies that could be distinguished, namely a better performance at the beginning of the task and a constant efficiency along the task, the monkeys were distributed in three groups : one showing a quasi-systematic better performance at the beginning (Mk-JU, Mk-CE, Mk-AV and Mk-GE), one being quasi-totally constant (Mk-JO), and one having either one or the other tendency according to the hand (Mk-JA, Mk-VA, Mk-SL and Mk-WI). To note that in this last group, the tendency was always a trend towards a stable performance with the left hand and to a better performance at the beginning with the right hand.

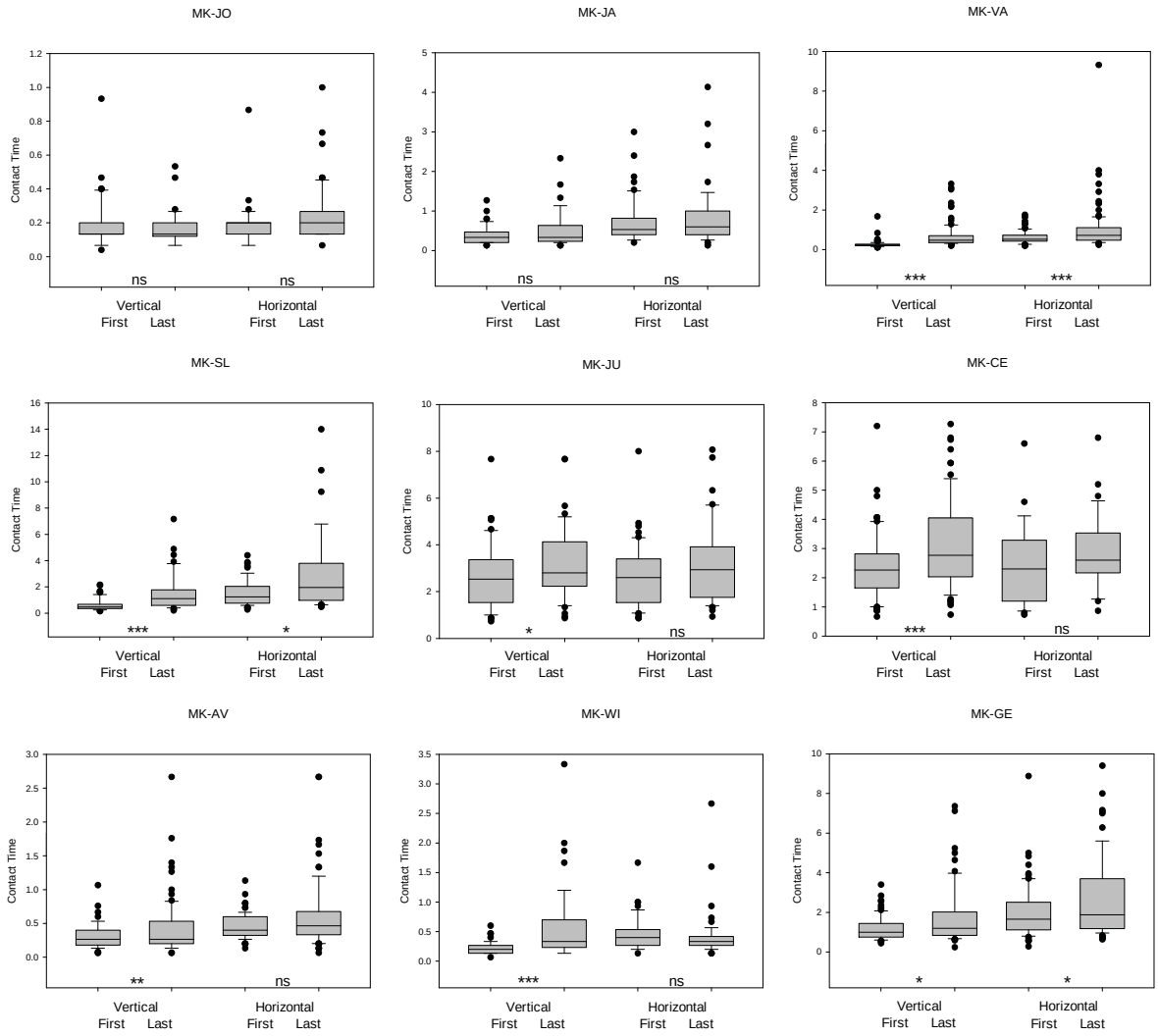


Figure 5.6.2: Boxplots representing the post-lesional distribution of the contact time for each monkey for the contralesional hand (left for Mk-JU and right for all the other monkeys). Comparisons were performed between first five and last five pellets.

When looking at the post-lesional plateau data (Fig. 5.6.2) compared to the pre-lesional plateau data, diverse patterns appeared (Table 5.6.1), unlinked to the delivery of treatment or not, nor to the pre-lesional main tendency. Two monkeys (Mk-JO and Mk-CE) showed a discrepancy on the two parameters (vertical and horizontal wells), but in an inverse direction, that is from a pre-lesion constant performance to a better performance at the beginning of the task post-lesion (Mk-JO) and from a better performance at the beginning of the task pre-lesion to a constant performance post-lesion (Mk-CE). One monkey (Mk-WI) remained stable in his tendency towards a better performance at the beginning. All the other monkeys had changes for some parameters and stability for the others. Mk-JA and Mk-GE changed on the vertical and horizontal wells, in the direction of a stability along the task for Mk-JA and Mk-GE on the vertical wells, and a better performance at the beginning for Mk-GE on the horizontal wells. The post-lesional tendency to perform better at the beginning for

the vertical wells in Mk-VA, Mk-SL and Mk-AV was similar to the pre-lesional data, whereas a change occured for the horizontal wells, sometimes in one direction (Mk-VA and Mk-SL) and sometimes in the other (Mk-AV). Finally, Mk-JU showed the same stability as pre-lesionally for the horizontal wells and a change for the vertical wells, in the direction of a more constant performance.

	Contralesional Hand Contact Time Vertical	Contralesional Hand Contact Time Horizontal	% recovery (total score)
TREATED			
Mk-JO Pre	ns	ns	
Mk-JO Post	**	*	58.82
Mk-JA Pre	**	*	
Mk-JA Post	ns	ns	100
Mk-VA Pre	***	ns	
Mk-VA Post	***	***	88.46
Mk-SL Pre	***	ns	
Mk-SL Post	***	*	78.95
CONTROL			
Mk-JU Pre	***	ns	
Mk-JU Post	ns	ns	39.13
Mk-CE Pre	**	***	
Mk-CE Post	ns	ns	41.66
Mk-AV Pre	*	*	
Mk-AV Post	**	ns	103.85
Mk-WI Pre	**	ns	
Mk-WI Post	***	ns	94.74
Mk-GE Pre	***	ns	
Mk-GE Post	ns	**	37.5

Table 5.6.1 : Comparisons of the contact time between pellets grasped at the beginning versus at the end of the task with the contralesional hand, performed on the pre-lesional and post-lesional plateaux. The pre-lesion comparisons are the same as in Figure 5.6.1 (panel B) for the right hand in all monkeys, except that it is the left hand for Mk-JU. The last column serves as an indicator of the general manual recovery, expressed by the percentage of total score recovery.

5.6.4 GENERAL CONCLUSION

No general trend could be inferred from the present data, both before and after the motor cortex lesion. Therefore, one cannot argue that an eventual baseline motivation, in the sense of an intrinsic trait that could be measured by the stability of the performance versus a worse performance at the end of the task for individuals more subjected to demotivation, could influence the data. Furthermore, no decline of motivation due to the lesion and the capacity of recovery themselves seemed to have intervened too. Indeed, there is no evidence

of a link between the level of recovery, indicated here by the percentage of recovery on the total score, and such demotivation, measured potentially by a worse performance at the end of the task. In the same line, a greater fatiguability that could have been derived from to the lesion can also be ruled out here.

5.7 DOES THE NUMBER OF PELLETS CONSIDERED FOR THE ANALYSIS OF CONTACT TIME INFLUENCE THE BEHAVIOURAL RESULTS?

5.7.1 PURPOSE

The aim of the present analyses was to assess if the behavioural results on the contact time parameter in the modified Brinkman board task differed when considering various number of retrieved pellets. Indeed, one could intuitively argue that the more the sample size is important, the more the results will be valid. Nevertheless, the balance cost/profit should be taken into account; indeed, the analyses of the contact time take a long time. The present investigation will try to answer the question of the pertinence to consider many pellets, such as performed in the various parts considering behaviour in the present work, versus the first pellet taken in each well orientation, such as performed by Freund et al. (2009).

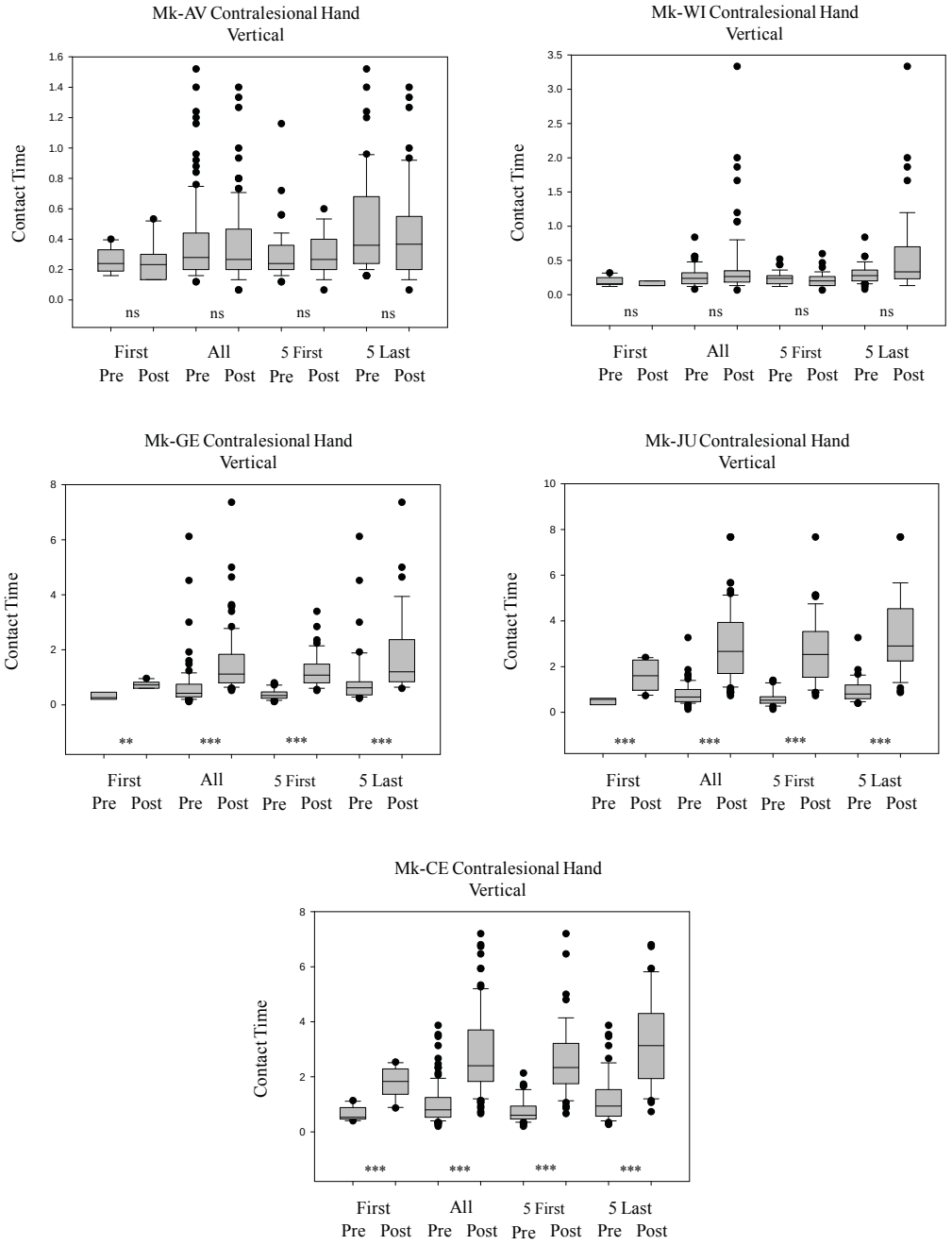
5.7.2 METHODS

Analyses were conducted on nine monkeys, subjected to an unilateral lesion of the motor cortex and trained to perform the modified Brinkman board task. Comparisons were performed between pre-lesion and post-recovery plateaux on the contact time to grasp the pellets, either on the vertical, on the horizontal or on the total (vertical and horizontal pooled) wells. Four different types of comparisons were made for the contralesional hand, on the vertical and the horizontal wells separately: first, only the first pellet grasped was considered; second, the first five and last five pellets were taken into account (referred to below as “All”); third, the first five pellets were used; fourth, the last five pellets were retained. The statistical test used was the Mann-Whitney non-parametrical test.

5.7.3 RESULTS

5.7.3.1 Vertical wells

Control monkeys



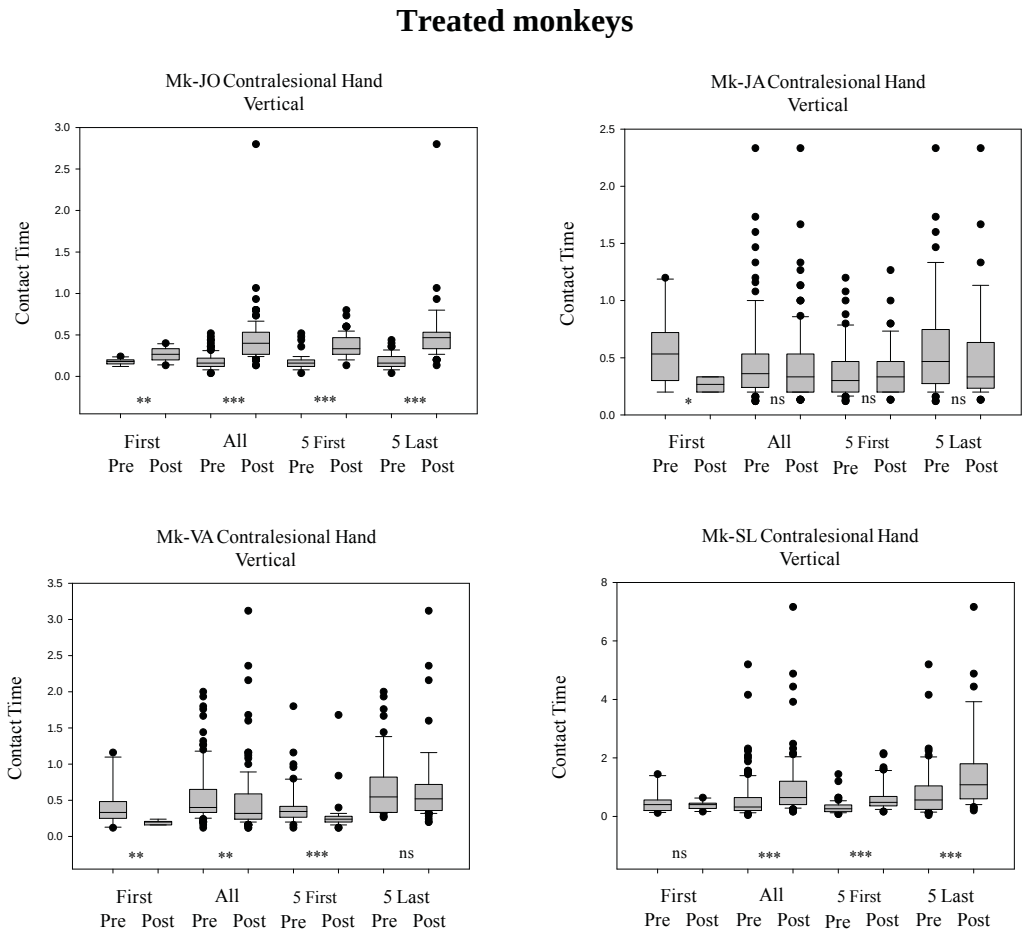
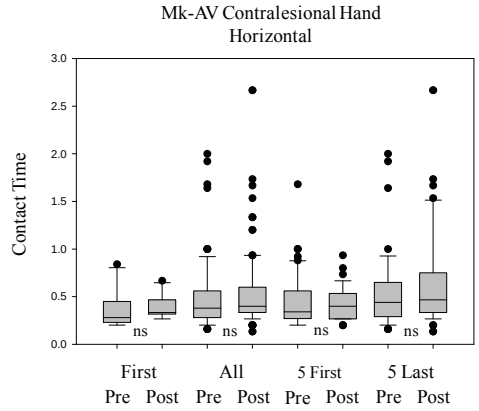


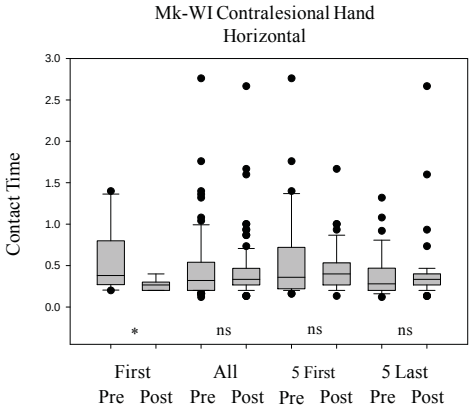
Figure 5.7.1 : Boxplots representing the distribution of the contact time in the pre-lesional plateau compared to the post-lesional plateau for the diverse vertical wells considered: first pellets (n=1 per session), all pellets (n=10 per session), 5 first pellets (n=5 per session) and 5 last pellets (n=5 per session).

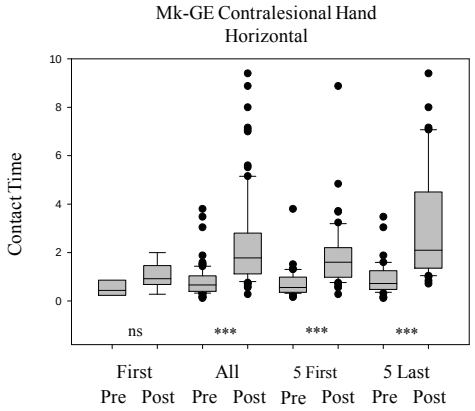
When looking at the vertical wells, no difference was found between the four different types of comparison for the control monkeys, although the median values and the distributions could vary a lot (Fig. 5.7.1A). Nevertheless, when looking at the treated monkeys (Fig. 5.7.1B), a bias toward a better recovery when considering only the first pellet could be observed in the monkeys Mk-JA and Mk-SL. Indeed, a better performance post-recovery was found in Mk-JA when considering the first pellet, whereas a post-recovery level similar to the pre-lesion one was observed when considering more pellets, whether it was the first five, the last five or all pellets. In Mk-SL, when retaining only the first pellet, the results would lead to conclude that the monkey recovered totally, whereas the three other analysis options would demonstrate the contrary, that is a remaining deficit of manual dexterity. This bias was not observed in Mk-JO, who did not show any difference according to the number of pellets taken into account. In Mk-VA, the results obtained corresponded to those of Mk-JO, except for the last five pellets, showing a less good recovery. To note that, as for the control monkeys, the median and distribution values could differ across the different analysis options.

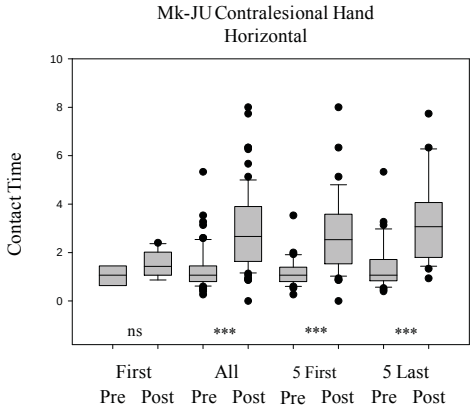
5.7.3.2 Horizontal wells

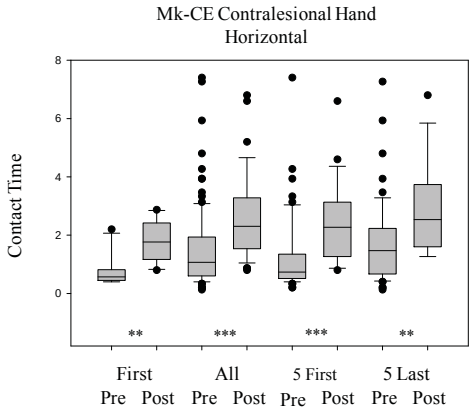
Control monkeys











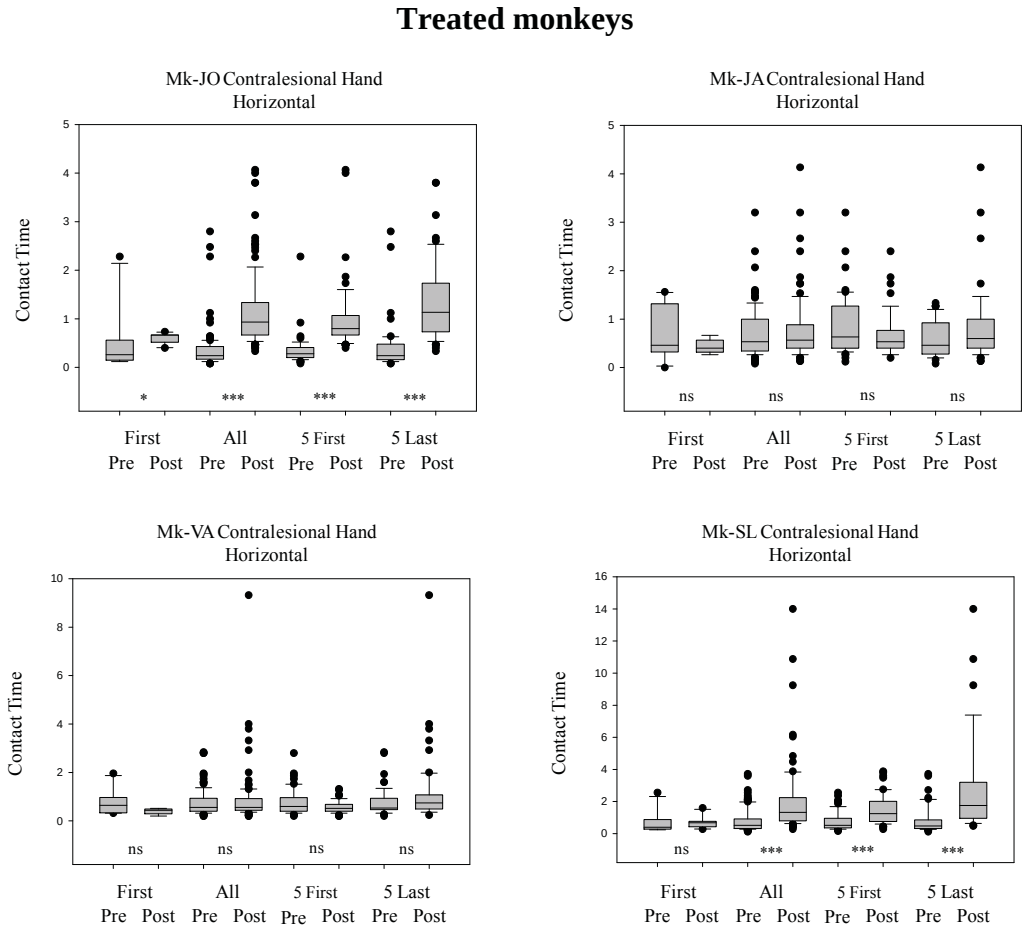


Figure 5.7.2 : Boxplots representing the distribution of the contact time in the pre-lesional plateau compared to the post-lesional plateau for the diverse horizontal wells considered: first pellets (n=1 per session), all pellets (n=10 per session), 5 first pellets (n=5 per session) and 5 last pellets (n=5 per session).

Considering the horizontal wells, the same bias towards a better recovery when analyzing the first pellet uniquely appeared in the treated monkey Mk-SL, whereas it did not appear in the three other treated monkeys (Fig. 5.7.2B). However, in this orientation, a similar bias occurred in three of the five control monkeys, namely Mk-WI, Mk-GE and Mk-JU (Fig. 5.7.2A).

5.7.4 GENERAL CONCLUSION

Overall, these results indicate that some differences of variable extent might appear depending on the number of pellets taken into account to perform the analyses. Mainly, a bias toward a better recovery when considering only the first vertical and the first horizontal pellets occurred. In a strange way, this effect was observed mainly in the treated monkeys when looking at the vertical wells, whereas when analyzing the horizontal wells, it was present mainly in the control monkeys. The motivational argument in favour of taking only

the first vertical and the first horizontal wells into account does not apply, as this bias in favour of a better performance when considering the first pellets, if being motivational, should also appear when considering the first five pellets, on the contrary to the last five pellets of the fifty wells task, which is not the case. Therefore, it seems probable that taking only the first pellet into account does not constitute a representative sample and could sometimes lead to erroneous conclusions. The whole data suggest that performing the analyses at least on the first five vertical and the first five horizontal wells represents a reasonable compromise to not measure all wells but have enough wells to obtain an adequate representation of the manual performance.

5.7.5 REFERENCES

Freund P, Schmidlin E, Wannier T, Bloch J, Mir A, Schwab ME, Rouiller EM (2009) Anti-Nogo-A antibody treatment promotes recovery of manual dexterity after unilateral cervical lesion in adult primates--re-examination and extension of behavioral data. Eur J Neurosci 29:983-996.

5.8 EVALUATION OF THE MOTOR PERFORMANCE OF THE MONKEYS ON MULTIPLE MANUAL PREHENSION TASKS.

5.8.1 PURPOSE

In our laboratory, the monkeys were trained to perform various manual prehension tasks (see "General Material and Methods" for a detailed description), that allow to assess the manual dexterity of the monkeys over time and after they were subjected to an unilateral lesion of the primary motor cortex. As indicated by their names, the modified Brinkman board task tests the grasping of static pellets, the rotating Brinkman board task of pellets moving on a rotating board, and, finally the hidden Brinkman board task of static pellets but without visual feedback. These tasks differ thus from one to another and potentially highlight different components of the manual prehension. The aim here is to assess the differences of hand recovery from motor cortex lesion between these different tasks, as well as to compare the manual performances between grasping pellets in the vertical and in the horizontal wells, before and after the lesion.

5.8.2 METHODS

5.8.2.1 Subjects and Behavioural Tasks

For the present investigation, data were collected in 4 male long-tailed macaques (*Macaca Fascicularis*), weighting between 3 and 4 kg and ranging from 2.5 to 3.5 years old at the time of initiation of motor training sessions. All the behavioural and surgical procedures were approved by the local ethical committee, in accordance with the Guidelines for the Care and Use of Laboratory Animals and approved by Swiss veterinary authorities.

These monkeys were trained as described in the "General Material and Methods". For the present study, analyses carried on their contralesional hand performance at the modified Brinkman board task, the rotating Brinkman board task and the hidden Brinkman board task. The work sessions were analyzed with a video recorder allowing frame per frame analysis, with a resolution of 25 frames per second. The following parameters, already described in the "General Material and Methods" chapter, were analyzed. The number of pellets successfully grasped in 30 seconds (score) was counted, in the modified Brinkman board task only. The "contact time", which is the time the monkey took to retrieve each food pellet from the well. For the modified Brinkman board task, this parameter was analyzed on the 5 first and 5 last vertical and horizontal visited wells (20 wells in total), respectively. For the rotating

Brinkman board task, this parameter was analyzed on all wells (n=32). To note here that the analyses of the contact time in the rotating Brinkman board task differed slightly in terms of temporal resolution between two monkeys (Mk-JO and Mk-AV) and the two other monkeys (Mk-JA and Mk-WI), as the performances of the first two monkeys were analyzed in a first type of protocol allowing a resolution of 0.25s, whereas for the two other monkeys, a new protocol was established, which allowed a resolution of 0.04s. Nevertheless, as the post-lesion performance of each monkey was compared to his own pre-lesion performance, this reduced the possible erroneous conclusions, although caution had to be kept. For the hidden Brinkman board task, the contact time was analyzed on all the pellets retrieved by the monkey (n=20 maximum). Finally, the mean percentage of errors committed when performing the task, mainly the loss of food pellets, was counted for the three tasks. These data were analyzed and coded on an analysis protocol sheet and a database was then created on Excel. Graphics, panels and statistical analyses were performed using the softwares Excel, and SigmaPlot/SigmaStat. Statistical comparisons between the pre-lesion and the post-lesion plateaux were performed by using the non-parametrical Mann-Withney test.

5.8.2.2 Surgical Procedures

All surgical procedures (ethical and veterinarian authorizations, preparation, sterile conditions, anaesthesia, medication, cortical chamber implant and motor cortex lesion) are described in the "General Material and Methods" part. The surgery took place once the monkey reached a behavioural plateau.

5.8.3 RESULTS

5.8.3.1 Treated monkeys

5.8.3.1.1 Mk-JO

5.8.3.1.1.1 Modified Brinkman board task

5.8.3.1.1.1.1 Score

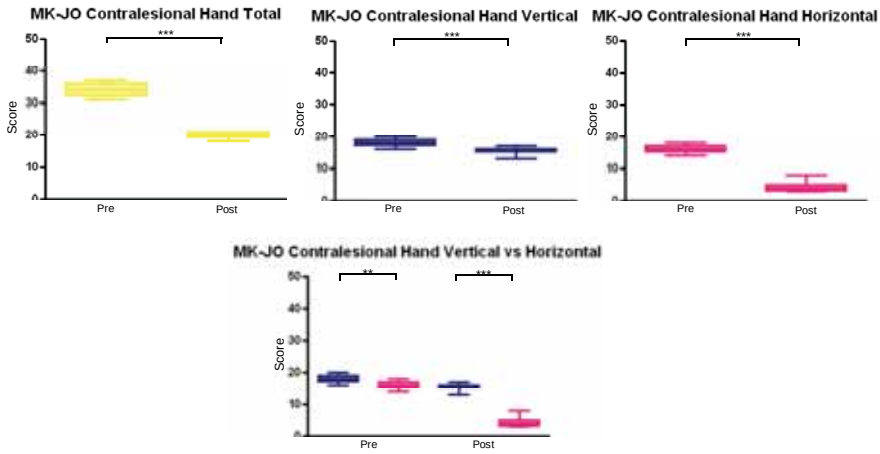


Figure 5.8.1: Comparisons of the distributions of the scores (number of pellets retrieved in 30 seconds) in Mk-JO with his contralesional hand between pre- and post-lesion plateaux for the total (yellow), the vertical (blue) and the horizontal (pink) wells, as well as between vertical and horizontal wells, represented here by boxplots.

In terms of score (number of pellets retrieved in 30 seconds; fig. 5.8.1) for the modified Brinkman board task, before the lesion, the performance of Mk-JO was better for vertical than for horizontal wells ($p=0.0011$). The "final" recovery plateau, although still incomplete, it represented 59% of the pre-lesion total score ($p<0.0001$), 88% of the pre-lesion vertical score ($p=0.0010$) and 29% of the pre-lesion horizontal score ($p<0.0001$), with again a better score for the vertical than the horizontal wells ($p<0.0001$).

5.8.3.1.1.1.2 Contact Time

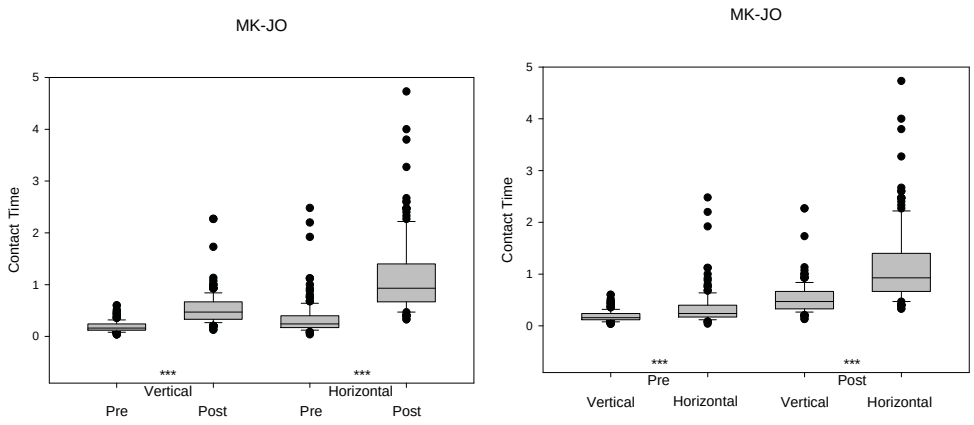


Figure 5.8.2: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JO with his contralesional hand in the modified Brinkman board task, between pre- and post-lesion plateaux for the vertical (n=10 per session) and the horizontal (n=10 per session) wells (left panel), and between vertical and horizontal wells before and after the lesion (right panel), represented here by boxplots.

When looking at the contact time (in seconds; fig. 5.8.2) in Mk-JO, as for the score, the pre-lesion performance was better (shorter contact time) for the vertical than for the horizontal wells ($p=0.0001$). The "final" recovery plateau of contact time was 2.94 times higher for the vertical wells ($p<0.0001$) and 3.88 times higher for the horizontal wells ($p<0.0001$), with again a better (shorter) contact time on the vertical than the horizontal wells ($p<0.0001$).

5.8.3.1.1.1.3 Errors

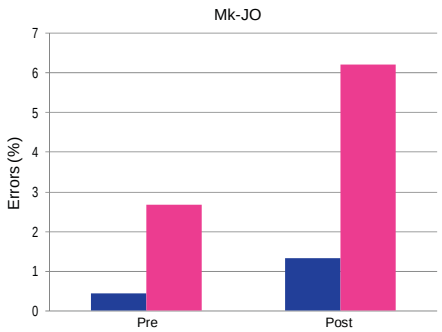


Figure 5.8.3: Percentage of errors commited by Mk-JO with his contralesional hand in the modified Brinkman board task. Comparisons were performed between pre- and post-lesion plateaux, as well as between vertical (blue; n=25 per session) and horizontal (pink; n=25 per session) wells before and after the lesion.

Before the lesion, Mk-JO commited on average 0.44% errors on the vertical wells and 2.67% errors on the horizontal wells (fig. 5.8.3). There was no statistically significant difference between errors on the vertical wells and errors on the horizontal wells ($p=0.7703$).

The rate of errors in the "final" plateau phase post-lesion was 3 times higher for the vertical wells (p=0.7261) and 2.33 times higher for the horizontal wells (p=0.6002). Neither of these differences nor the difference between errors on the vertical wells and errors on the horizontal wells (p=0.6414) were statistically significant.

5.8.3.1.1.2 Rotating Brinkman board task

5.8.3.1.1.2.1 Contact Time

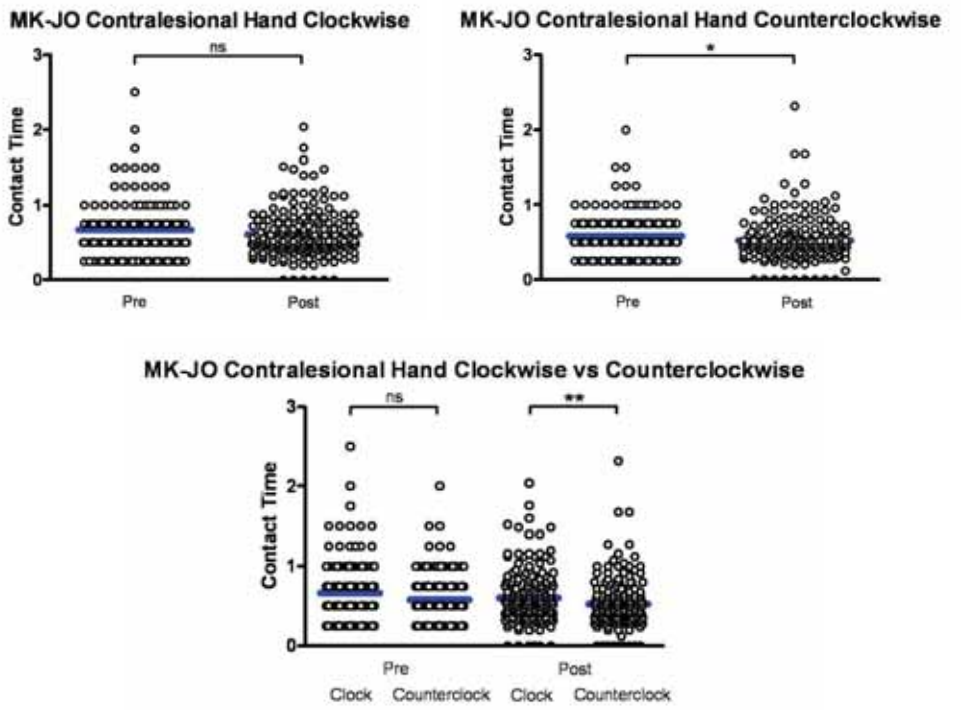


Figure 5.8.4: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JO with his contralesional hand in the rotating Brinkman board task, between pre- and post-lesion plateaux for the clockwise (n=32 per session) and contraclockwise (n=32 per session) rotations, as well as between the two rotation directions before and after the lesion (bottom), represented by boxplots.

In the rotating Brinkman board task, before the lesion, the median contact time value (fig. 5.8.4) was 0.50s for both rotation orientations. The "final" recovered contact time median value was 0.52s (1.04 times the pre-lesion value; p=0.2257) when the board rotated clockwise, and 0.48s (0.96 times the pre-lesion value; p=0.0127) when it rotated contraclockwise. Thus, Mk-JO recovered totally his manual dexterity at this task for the contact time parameter. Contrarily to before the M1 lesion, the contact time when the board rotated clockwise was significantly higher than when the board rotated contraclockwise (p=0.0041) after the lesion.

5.8.3.1.1.2.2 Errors

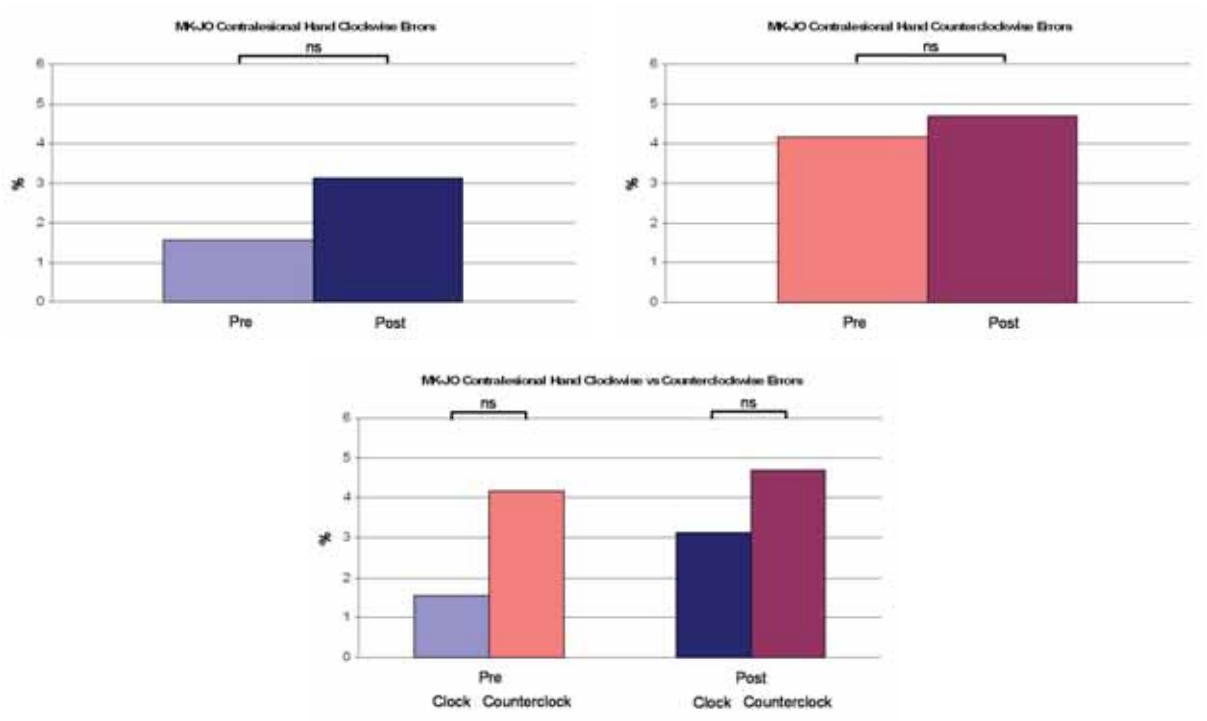


Figure 5.8.5: Statistical comparisons were performed on the percentage of errors commited by Mk-JO with his contralesional hand in the rotating Brinkman board task, between the pre- and the post-lesion plateaux for the clockwise rotation (blue; n=32 per session), the contraclockwise rotation (pink; n=32 per session), and between the two rotation orientations before and after the lesion.

In terms of errors, before the lesion, Mk-JO commited on average 1.56% of errors when the board rotated clockwise and 4.17% of errors when the board rotated contraclockwise (fig. 5.8.5). The rate of errors at the "final" post-lesion plateau was 3.13% (2.01 times more than before the lesion; p=0.2866) and 4.69% (1.12 times the pre-lesion value; p=0.2287) when the board rotated clockwise and contraclockwise respectively.

Both before and after the lesion, the errors percentage when the board rotated clockwise did not significantly differ from those committed when the board rotated contraclockwise (p=0.7782 and p=0.6766, respectively).

5.8.3.1.1.3 Hidden Brinkman board task

5.8.3.1.1.3.1 Contact Time With Visual Feedback

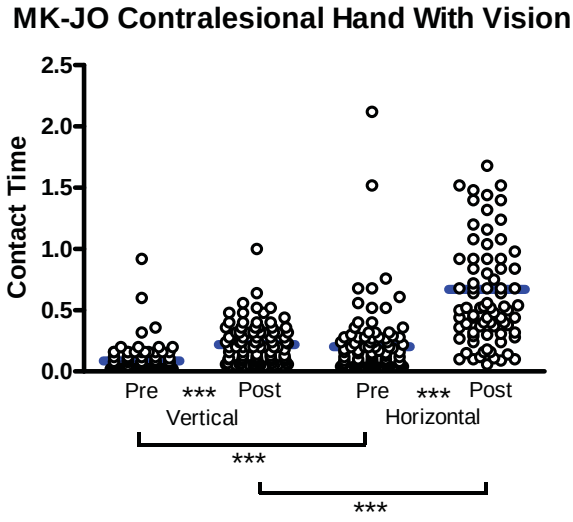


Figure 5.8.6: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JO with his contralesional hand in the hidden Brinkman board task with visual feedback, between pre- and post-lesion plateaux for the vertical (n=10 max. per session) and the horizontal (n=10 max. per session) wells, as wells as between vertical and horizontal wells before and after the lesion.

In the hidden Brinkman board task (fig. 5.8.6), when having a visual feedback, Mk-JO showed a pre-lesion contact time median value of 0.04s for the vertical wells and 0.12s for the horizontal wells, with a better score for the vertical than the horizontal wells ($p<0.0001$). After recovering from the M1 lesion, Mk-JO did not recover totally, as the "final" plateau of recovery was 0.20s for the vertical wells (5 times the pre-lesional value, $p<0.0001$) and 0.52s for the horizontal wells (4.33 times the pre-lesional value, $p<0.0001$), with significantly shorter contact times for the vertical than the horizontal wells ($p<0.0001$).

5.8.3.1.1.3.2 Errors With Visual Feedback

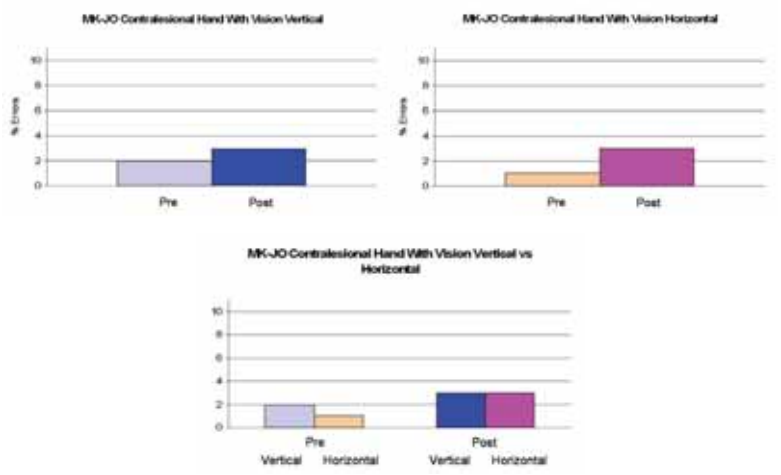


Figure 5.8.7: Statistical comparisons were performed on the errors commited by Mk-JO with his contralesional hand in the hidden Brinkman board task when having a visual feedback, between the pre- and the post-lesion plateaux for the vertical (blue; n=10 max. per session) and the horizontal (pink; n=10 max. per session) wells, as well as between vertical and horizontal wells before and after the lesion.

Concerning the errors when performing the hidden Brinkman board task with visual feedback (fig. 5.8.7), before the lesion, Mk-JO commited on average 2% of errors on the vertical wells and 1% of errors on the horizontal wells, with a statistically identical rate of errors in the vertical an in the horizontal wells ($p=0.8973$). In the "final" recovery plateau, the percentage of errors was higher, but not significantly, than before the lesion, as it was 3% for the vertical wells (1.5 times more than before lesion; $p=0.8976$) and 3% for the horizontal wells (3 times more than before lesion; $p=0.7957$). As before the lesion, there was no statistically significant difference between percentages of errors committed in the vertical and the horizontal wells ($p=0.9990$).

5.8.3.1.1.3.3 Contact Time Without Visual Feedback

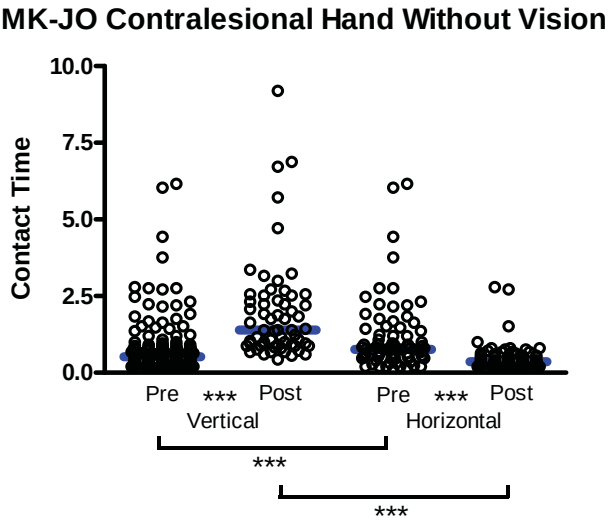


Figure 5.8.8: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JO with his contralesional hand in the hidden Brinkman board task without visual feedback, between pre- and post-lesion plateaux for the vertical (n=10 max. per session) and the horizontal (n=10 max. per session) wells, as wells as between vertical and horizontal wells before and after the lesion.

Without visual feedback (fig. 5.8.8), the contact time of Mk-JO was 2.69 times longer for the vertical wells (1.40s. and 0.52s., respectively; $p<0.0001$), and 0.47 times the pre-lesion contact time for the horizontal wells (0.36s. and 0.76s., respectively; $p<0.0001$). Both before and after the lesion, the contact times of the vertical wells differed significantly to those of the horizontal wells ($p<0.0001$ in the two cases, but in favour of the vertical wells before the lesion and of the horizontal wells after the lesion). Here, Mk-JO showed thus a partial recovery on the total and the vertical wells, and a complete, even enhanced recovered performance in the horizontal wells. This point will be discussed in the "Discussion" part.

5.8.3.1.1.3.4 Errors Without Visual Feedback

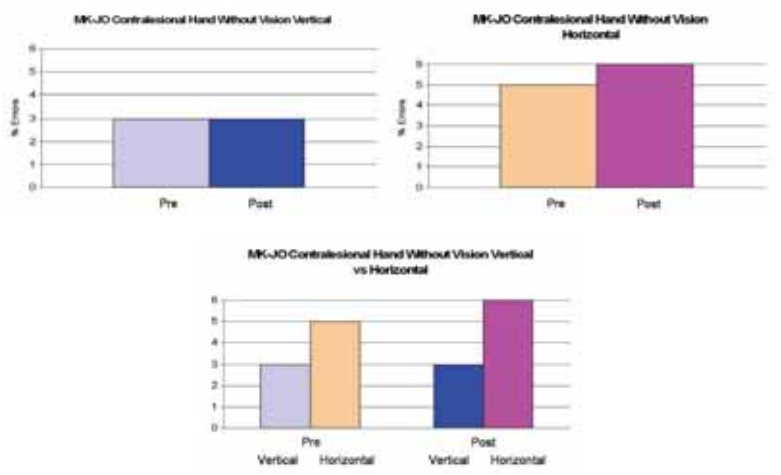


Figure 5.8.9: Statistical comparisons were performed on the errors commited by Mk-JO with his contralesional hand in the hidden Brinkman board task without having a visual feedback, between the pre- and the post-lesion plateaux for the vertical (blue; n=10 max. per session) and the horizontal (pink; n=10 max. per session) wells, as well as between vertical and horizontal wells before and after the lesion.

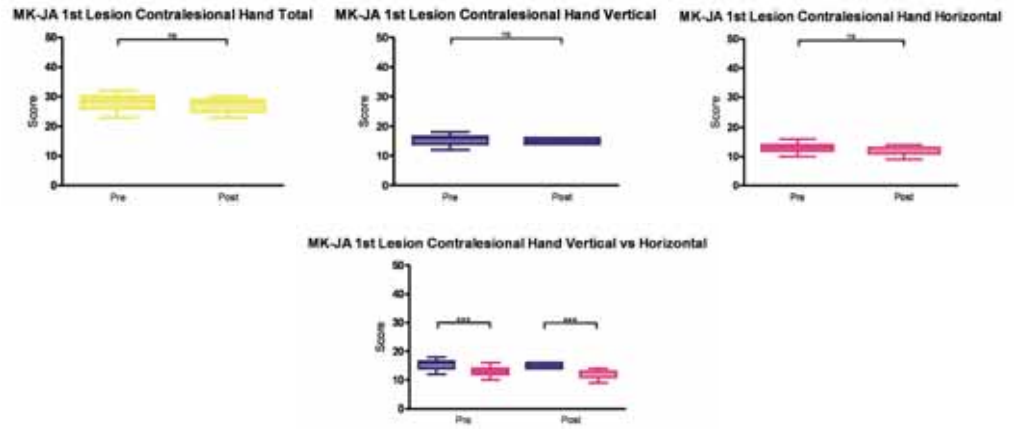
After the lesion, the errors committed in the situation without visual feedback (fig. 5.8.9) were identical for the vertical wells and 1.20 times higher for the horizontal wells ($p=0.9705$). The errors committed in the two well orientations did not differ, both pre- and post-lesion ($p=0.6305$ for both).

5.8.3.1.2 Mk-JA

5.8.3.1.2.1 Modified Brinkman board task

5.8.3.1.2.1.1 Score

A



B

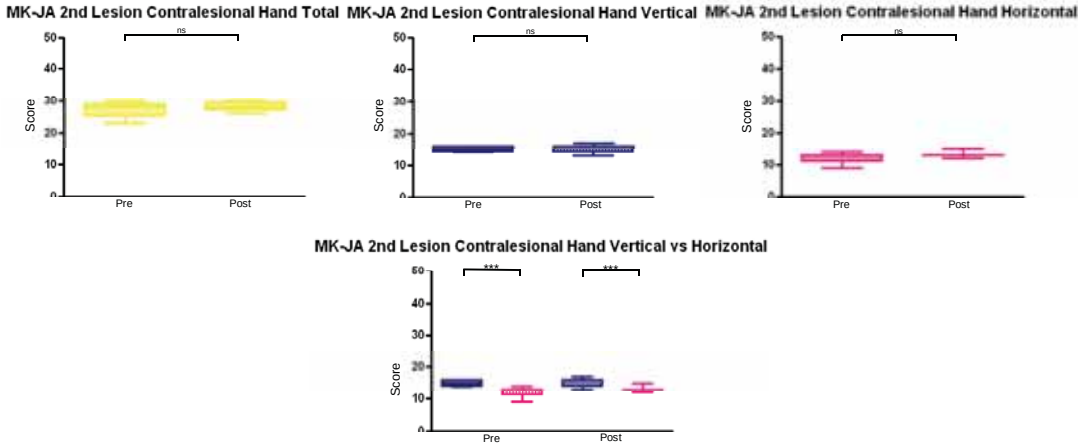


Figure 5.8.10: Comparisons of the distributions of the scores (number of pellets retrieved in 30 seconds) in Mk-JA with his contralesional hand between pre- and post-first (A) and second (B) lesion plateaux for the total (yellow), the vertical (blue) and the horizontal (pink) wells, as well as between vertical and horizontal wells, represented here by boxplots.

As Mk-JA suffered epileptic seizures following the first lesion he was subjected to, he received an antiepileptic drug (Luminal), which we suspected later to have a counteracting effect to the ibotenic acid excitotoxic effect. Thus, after the first motor cortex lesion he was subjected to, Mk-JA recovered unusually quick and good. Later, he was subjected to a second M1 lesion (see "Time course of experimental protocol, ICMS, Lesion and Cell reimplantation" in the "Annexes" part). To note here that the same phenomenon occurred in Mk-WI (see farther), who was trained in parallel to Mk-JA.

After the first lesion, Mk-JA recovered 95.98% for the total score (p=0.1328), 95.69% for the vertical score (p=0.2477) and 96.34% for the horizontal score (p=0.2914), with a better score for the vertical than the horizontal wells (p<0.0001) (fig. 5.8.10A). To note that no treatment was delivered after this first lesion.

This behavioural recovery plateau was considered as the pre-second lesion plateau. The "final" recovery plateau (fig. 5.8.10B) was 104 % of the pre-lesion score for the total wells (p=0.1545), 103% for the vertical wells (p=0.2717) and 105% for the horizontal wells (p=0.2587), with a better score on the vertical than the horizontal wells (p<0.0001). Mk-JA recovered thus totally from the second M1 lesion he was subjected to.

5.8.3.1.2.1.2 Contact Time

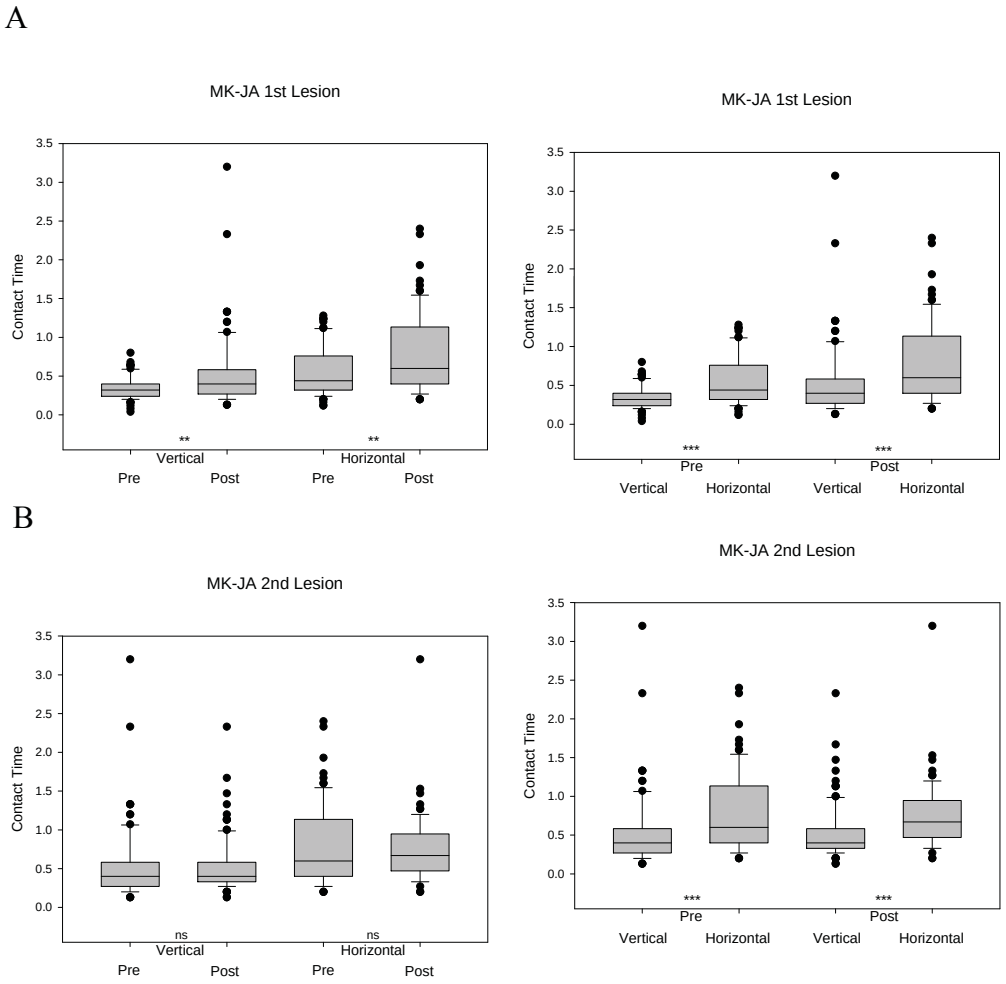


Figure 5.8.11: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JA with his contralesional hand in the modified Brinkman board task, between pre- and post-first (A) and second (B) lesion plateaux for the vertical (n=10 per session) and the horizontal (n=10 per session) wells (left panel), and between vertical and horizontal wells before and after the lesion (right panel), represented here by boxplots.

In terms of contact time, Mk-JA's performance at the pre-first lesion plateau was better for the vertical than for the horizontal wells ($p<0.0001$). The recovery from the first lesion (fig. 5.8.11A) was 1.25 times the pre-lesional value for the vertical wells ($p=0.0031$) and 1.25 times the pre-lesional value for the horizontal wells ($p=0.0035$), with a better score on the vertical than the horizontal wells ($p=0.0001$). Therefore, contrarily to the score parameter, Mk-JA did not recover totally after this first lesion on the contact time parameter.

As for the score, this recovery plateau was considered as the pre-second lesion plateau. The "final" recovery contact time value (fig. 5.8.11B) was identical for the vertical wells (0.40s.; $p=0.6888$) and 1.12 times the pre-lesional value for the horizontal wells (0.67s. vs 0.60s.; $p=0.7942$), with a better score on the vertical than the horizontal wells ($p<0.0001$). Mk-JA recovered thus totally from this second lesion on the contact time parameter.

5.8.3.1.2.1.3 Errors

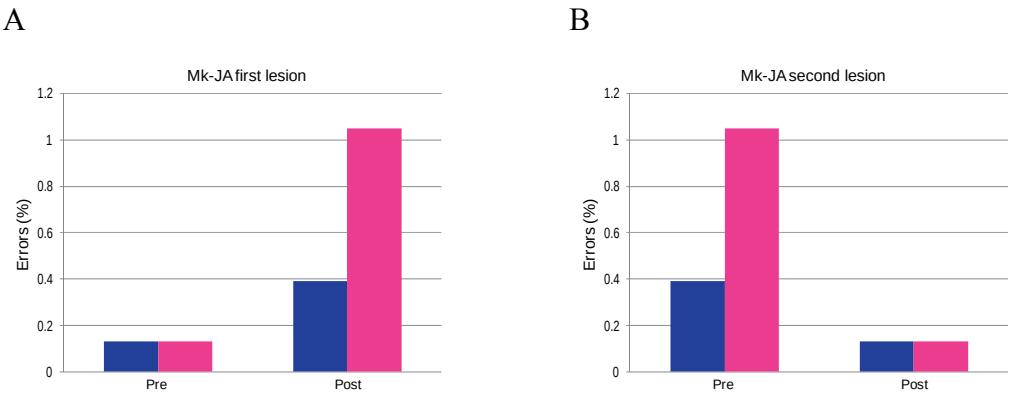


Figure 5.8.12: Percentage of errors commited by Mk-JA with his contralesional hand in the modified Brinkman board task. Comparisons were performed between pre- and post-first (A) and second (B) lesion plateaux, as well as between vertical (blue; n=25 per session) and horizontal (pink; n=25 per session) wells before and after the lesion.

Before the first lesion, Mk-JA commited on average 0.13% errors on the vertical and the horizontal wells at this modified Brinkman board task, with the same errors' rate in the vertical and in the horizontal wells ($p=0.9999$). In the recovery plateau following the first lesion, the errors rate was 3 times higher for the vertical wells ($p=0.9070$) and 8.08 times higher for the horizontal wells ($p=0.8152$). Although more errors were committed, these differences were not statistically significant, as well as the difference between errors on the vertical (0.38%) and on the horizontal (1.05%) wells ($p=0.9071$) (fig. 5.8.12A).

Again, this recovery plateau was considered as the pre-second lesion plateau. In the "final" recovery plateau, the errors rate was 0.34 times the pre-lesional plateau for the vertical

wells (0.13%; p=0.9070) and 0.12 times the pre-lesional plateau for the horizontal wells (0.13%; p=0.8151), with the same rate of errors in the two well orientations (p= 0.9999). To note that these errors rates corresponded to those committed before the first lesion (fig. 5.8.12B).

5.8.3.1.2.2 Rotating Brinkman board task

5.8.3.1.2.2.1 Contact Time

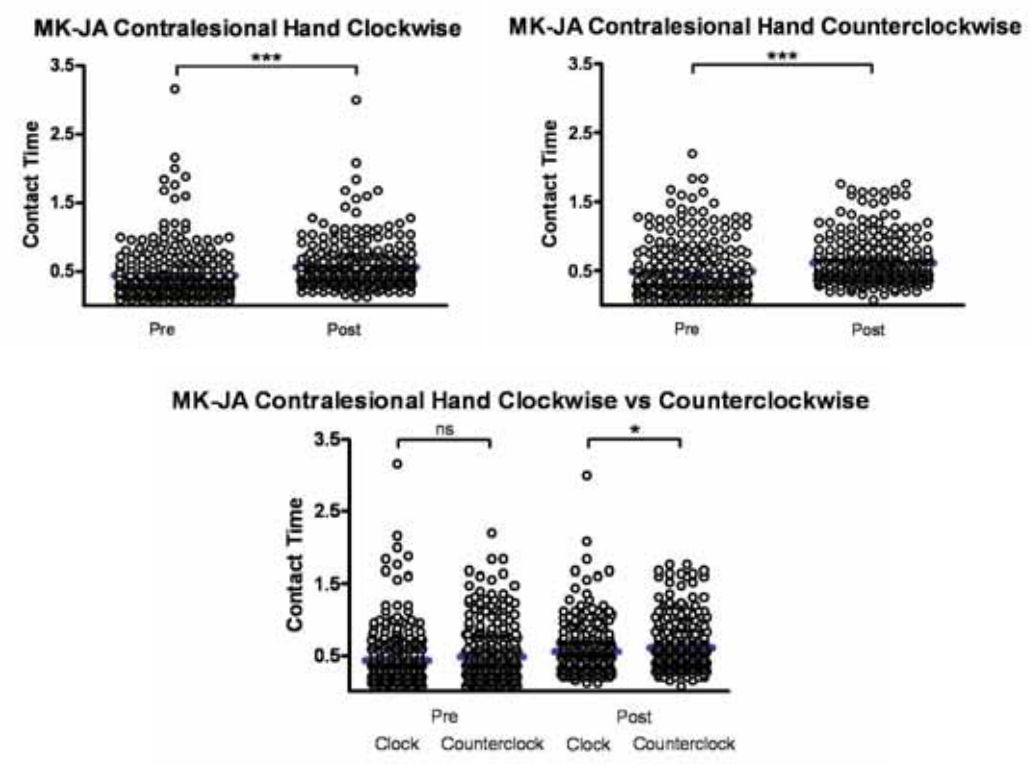


Figure 5.8.13: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JA with his contralesional hand in the rotating Brinkman board task, between pre- and post-lesion plateaux for the clockwise (n=32 per session) and contraclockwise (n=32 per session) rotations, as well as between the two rotation directions before and after the lesion (bottom), represented by boxplots.

In the rotating Brinkman board task, the data around the first lesion were not analyzed. Only the comparisons between pre- and post-second lesion plateaux were performed.

After recovering from the second M1 lesion, Mk-JA reached a behavioural plateau with contact times that were significantly higher than before the lesion. Indeed, the contact time median values were 1.5 times higher (0.48s. vs 0.32s.; $p<0.0001$) when the board rotated clockwise and 1.44 times higher (0.52s. vs 0.36s.; $p<0.0001$) when the board rotated contraclockwise (fig. 5.8.13). When comparing the values between the two rotation orientations, no significant difference was found before the lesion ($p=0.1341$), whereas after the lesion, the contact time when the board rotated contraclockwise was significantly higher than when it rotated clockwise ($p=0.0221$).

Contrarily to what was observed in the modified Brinkman board task, Mk-JA did not recover totally his manual dexterity when performing the rotating Brinkman board task, at least when considering the contact time.

5.8.3.1.2.2.2 Errors

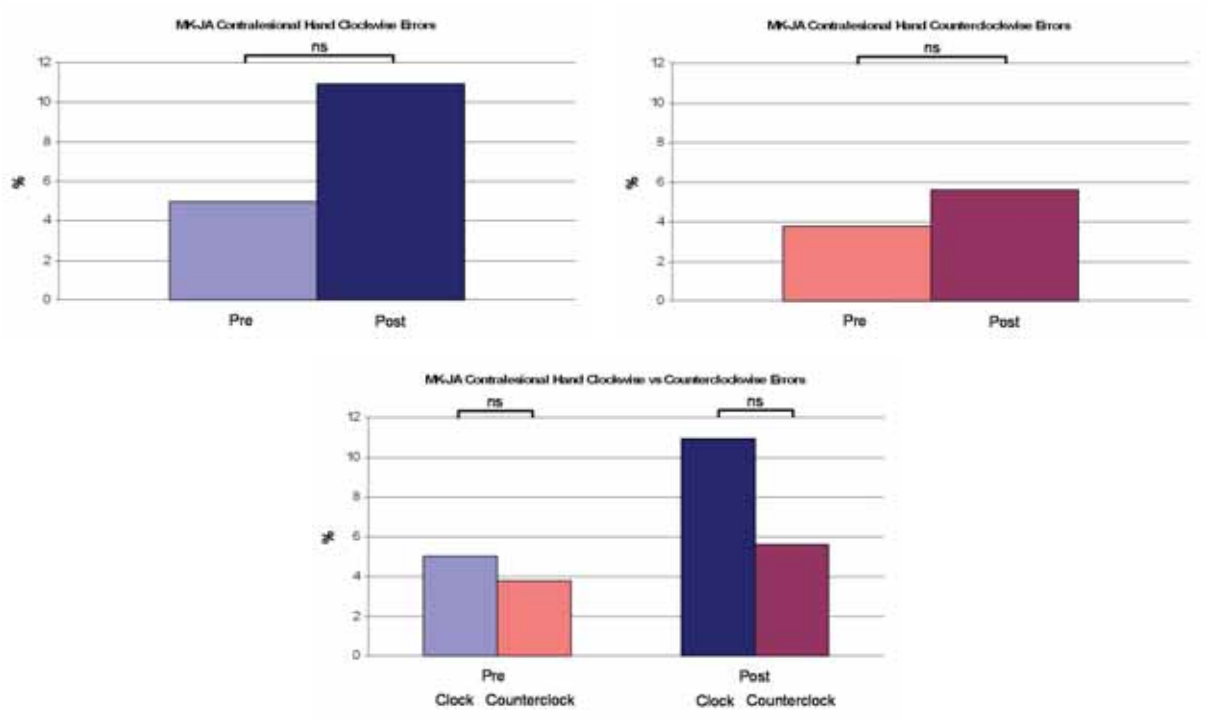


Figure 5.8.14: Statistical comparisons were performed on the percentage of errors commited by Mk-JA with his contralesional hand in the rotating Brinkman board task,.between the pre- and the post-lesion plateaux for the clockwise rotation (blue; n=32 per session), the contraclockwise rotation (pink; n=32 per session), and between the two rotation orientations before and after the lesion.

When comparing the errors committed in the pre-lesion behavioural plateau to those committed in the post-lesion one (fig. 5.8.14), it appeared that Mk-JA did not commit significantly more errors after the lesion, even the rate of errors was 2.19 times higher when the board rotated clockwise (10.94% vs 5% of errors; p=0.1325), and 1.50 times higher when it rotated contraclockwise (5.63% vs 3.75% of errors; p=0.5525).

The errors rate when the board rotated clockwise was statistically similar to that committed when the board rotated contraclockwise, both before and after the lesion (p=0.7771 and p=0.2191, respectively).

5.8.3.1.2.3 Hidden Brinkman board task

5.8.3.1.2.3.1 Contact Time With Visual Feedback

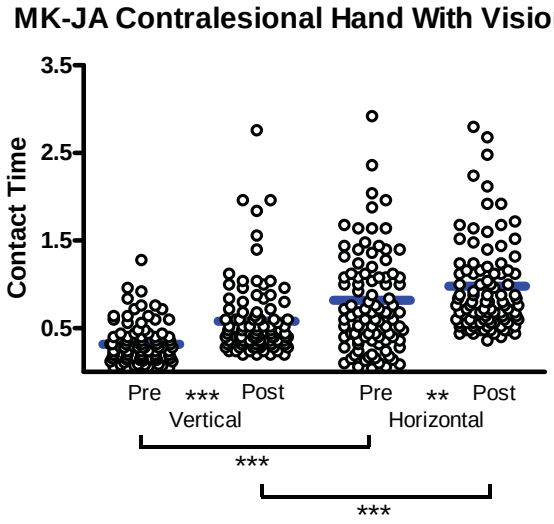


Figure 5.8.15: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JA with his contralesional hand in the hidden Brinkman board task with visual feedback, between pre- and post-second lesion plateaux for the vertical (n=10 max. per session) and the horizontal (n=10 max. per session) wells, as wells as between vertical and horizontal wells before and after the lesion.

As for the rotating Brinkman board task, in the hidden Brinkman board task, both with and without vision, the data around the first lesion were not analyzed. Only the comparisons between pre- and post-second lesion plateaux were performed.

When looking at the contact time when having a visual feedback (fig. 5.8.15), the post-lesion behavioural plateau contact time values were 1.63 times and 1.24 times longer than the pre-lesion values for the vertical (0.43s vs 0.27s; $p<0.0001$) and the horizontal (0.82s vs 0.66s; $p=0.0086$) wells, respectively, with a better performance for the vertical than for the horizontal wells, both before and after the lesion ($p<0.0001$).

5.8.3.1.2.3.2 Errors With Visual Feedback

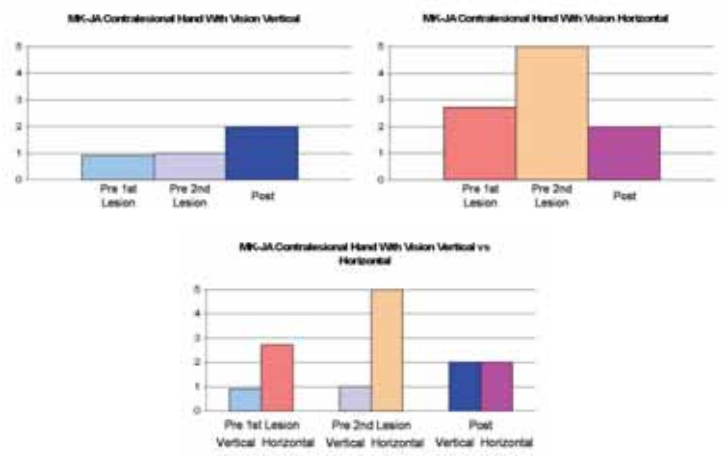


Figure 5.8.16: Statistical comparisons were performed on the errors commited by Mk-JA with his contralesional hand in the hidden Brinkman board task when having a visual feedback, between the pre- and the post-fisrt and second lesion plateaux for the vertical (blue; n=10 max. per session) and the horizontal (pink; n=10 max. per session) wells, as well as between vertical and horizontal wells before and after the lesions.

Before the second lesion, in the behavioural plateau, MK-JA commited on average 1% of errors on the vertical wells and 5% of errors on the horizontal wells, and there was no statistically significant difference between errors on the vertical and the horizontal wells ($p=0.6046$) (fig. 5.8.16). The post-lesion rate of errors was 2% on the vertical wells (2 times the pre-lesional score; $p=0.8973$) and 2% on the horizontal wells (0.4 times the pre-lesional score; $p=0.6976$). Although the monkey showed for horizontal wells more errors committed after the first lesion and then a return to the pre-all lesions errors rate when having recovered from the second lesion, whereas for the vertical wells, more errors were committed after the second lesion than before and after the first one (identical errors rate), none of these differences was significant.

5.8.3.1.2.3.3 Contact Time Without Visual Feedback

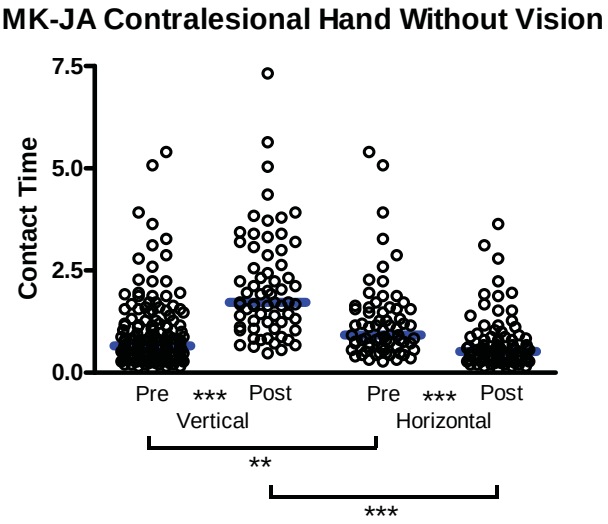


Figure 5.8.17: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JA with his contralesional hand in the hidden Brinkman board task without visual feedback, between pre- and post-second lesion plateaux for the vertical (n=10 max. per session) and the horizontal (n=10 max. per session) wells, as wells as between vertical and horizontal wells before and after the lesion.

Without visual feedback (fig. 5.8.17), the contact time median value was 2.61 times longer than before the lesion for the vertical wells (1.72s. vs 0.66s.; $p<0.0001$), and 0.57 times shorter than the pre-lesion value for the horizontal wells (0.52s. vs 0.92s.; $p<0.0001$). Both before and after the lesion, the contact times in the vertical and the horizontal wells differed significantly, in favour of the vertical wells pre-lesion ($p=0.0011$) and of the horizontal wells post-lesion ($p<0.0001$).

5.8.3.1.2.3.4 Errors Without Visual Feedback

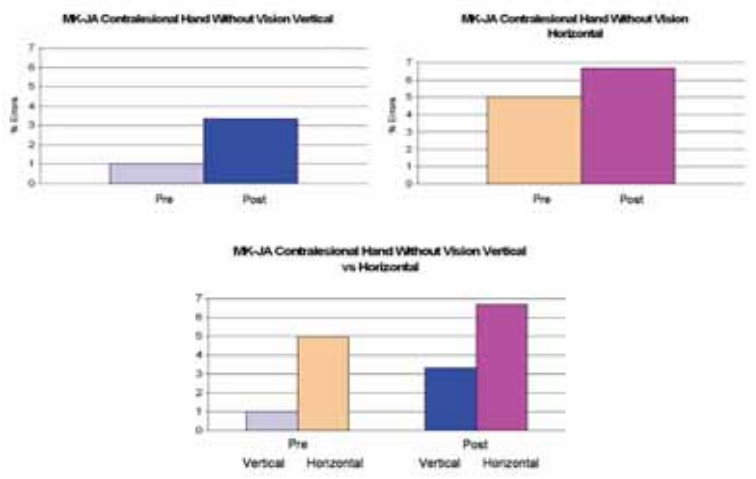


Figure 5.8.18: Statistical comparisons were performed on the errors commited by Mk-JA with his contralesional hand in the hidden Brinkman board task without having a visual feedback, between the pre- and the post-lesion plateaux for the vertical (blue; n=10 max. per session) and the horizontal (pink; n=10 max. per session) wells, as well as between vertical and horizontal wells before and after the lesion.

In terms of errors, Mk-JA committed more errors after than before the lesion, although not significantly (fig. 5.8.18). Indeed, the rate of errors was 3.33 times higher for the vertical wells (3.33% vs 1%; p=0.6607) and 1.33 times higher for the horizontal wells (6.67% vs 5%; p=1.0000). The monkey committed statistically as many errors in the vertical as in the horizontal wells, both before and after the lesion (p=0.1431 and p=0.4894, respectively).

5.8.3.2 Control monkeys

5.8.3.2.1 Mk-AV

5.8.3.2.1.1 Modified Brinkman board task

5.8.3.2.1.1.1 Score

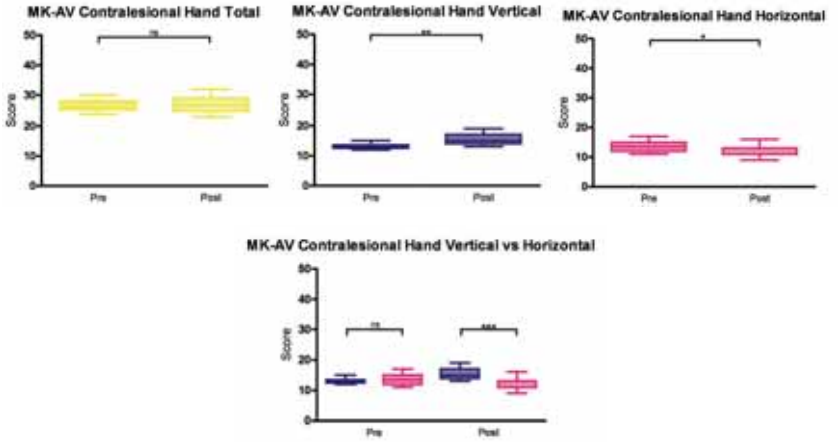


Figure 5.8.19: Comparisons of the distributions of the scores (number of pellets retrieved in 30 seconds) in Mk-AV with his contralesional hand between pre- and post-lesion plateaux for the total (yellow), the vertical (blue) and the horizontal (pink) wells, as well as between vertical and horizontal wells, represented here by boxplots.

Considering the number of pellets retrieved in 30 seconds in the modified Brinkman board task (fig. 5.8.19), Mk-AV reached a behavioural plateau about 150 days before the lesion, which unfortunately was situated more in the premotor cortex (PM) than in M1. After the lesion, the "final" stable recovery plateau amounted to 102% for the total score ($p=0.5586$), 115% for the vertical score ($p=0.0026$) and 81% for the horizontal score ($p= 0.0357$). Mk-AV recovered thus well from the lesion, but only partially for the horizontal wells, whereas his recovery was complete for the total wells and even enhanced compared to his pre-lesion score for the vertical wells. His manual performance was identical for the vertical and the horizontal wells before the lesion ($p= 0.7209$), whereas after the lesion, it was better for the vertical than for the horizontal wells ($p<0.0001$).

5.8.3.2.1.1.2 Contact Time

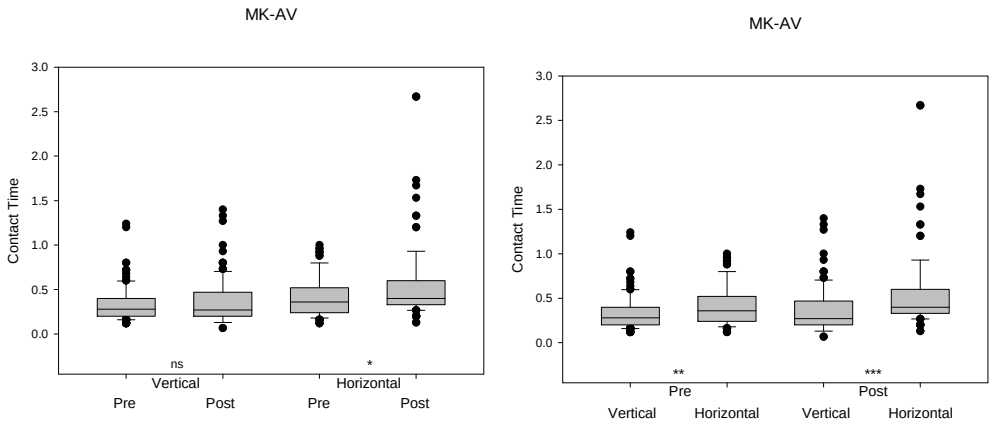


Figure 5.8.20: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-AV with his contralesional hand in the modified Brinkman board task, between pre- and post-lesion plateaux for the vertical (n=10 per session) and the horizontal (n=10 per session) wells (left panel), and between vertical and horizontal wells before and after the lesion (right panel), represented here by boxplots.

In this modified Brinkman board task, MK-AV showed a "final" recovered contact time (fig. 5.8.20) that was 0.96 times the pre-lesional score for the vertical wells (0.27s vs 0.28s.; p=0.646) and 1.1 times the pre-lesional score for the horizontal wells (0.40s vs 0.36s.; p=0.034). Thus, Mk-AV did not recover totally for the horizontal wells on this contact time parameter. Both before and after the lesion, the contact time was shorter for the vertical than for the horizontal wells (p=0.0072 and p<0.0001, respectively).

5.8.3.2.1.1.3 Errors

Figure 5.8.21: Percentage of errors commited by Mk-AV with his contralesional hand in the modified Brinkman board task. Comparisons were performed between pre- and post-lesion plateaux, as well as between vertical (blue; n=25 per session) and horizontal (pink; n=25 per session) wells before and after the lesion.

Concerning the errors (fig. 5.8.21), before the lesion, MK-AV commited on average 0.2% of errors on the vertical wells and 0.2% of errors on the horizontal wells. In the "final"

recovery plateau, Mk-AV recovered totally, as he committed exactly the same rate of errors than before the lesion, for the vertical (p= 0.9534) and the horizontal (p=0.9999) wells. Furthermore, the same errors rate was found in the two well orientations, both before and after the lesion (p=0.9999 and p=0.9534, respectively).

5.8.3.2.1.2 Rotating Brinkman board task

5.8.3.2.1.2.1 Contact Time

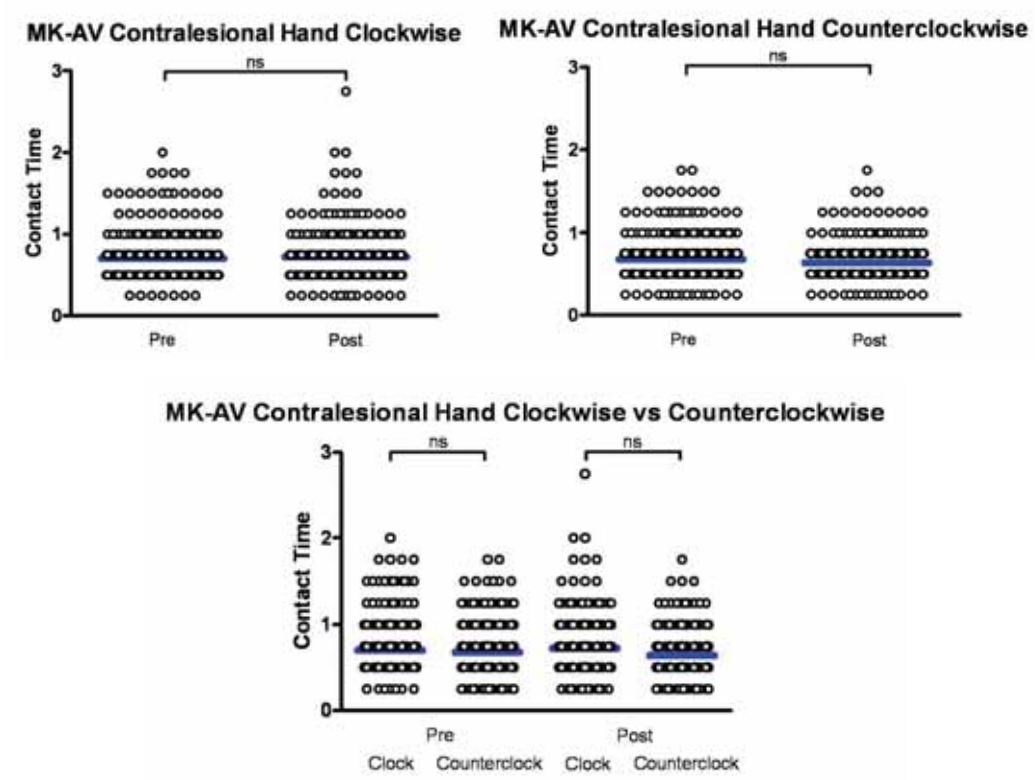


Figure 5.8.22: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-AV with his contralesional hand in the rotating Brinkman board task, between pre- and post-lesion plateaux for the clockwise (n=32 per session) and contraclockwise (n=32 per session) rotations, as well as between the two rotation directions before and after the lesion (bottom), represented by boxplots.

In the rotating Brinkman board task, after the PM lesion, Mk-AV recovered totally in terms of contact time (fig. 5.8.22), as the median values in this recovery plateau were identical to those of the pre-lesion plateau (0.50s. for both rotation orientations; p=0.9525 and p=0.0868 for the clockwise and the contraclockwise rotation orientations, respectively). To note that the contact times of the two rotation orientations were thus similar, both before and after the lesion.

5.8.3.2.1.2.2 Errors

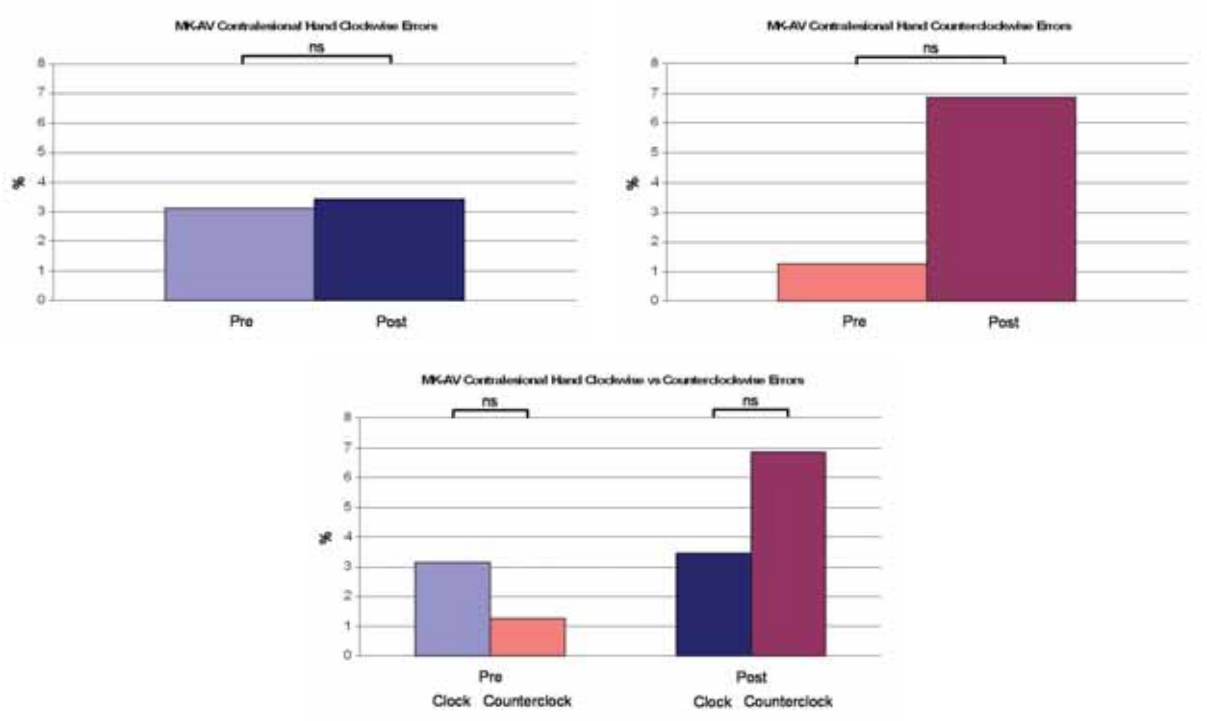


Figure 5.8.23: Statistical comparisons were performed on the percentage of errors commited by Mk-AV with his contralesional hand in the rotating Brinkman board task,.between the pre- and the post-lesion plateaux for the clockwise rotation (blue; n=32 per session), the contraclockwise rotation (pink; n=32 per session), and between the two rotation orientations before and after the lesion.

Before the lesion, Mk-AV committed on average 3.13% of errors when the board rotated clockwise and 1.25% of errors when it rotated contraclockwise (fig. 5.8.23). After the PM lesion, as in the modified Brinkman board task, he committed more errors, progressively decreasing from day 12 post-lesion, reaching a plateau about 54 days after the lesion. The rates of errors were then 3.44% (1.1 times the pre-lesion median value; p=0.8917) and 6.88% (5.5 times the pre-lesion median value; p=0.2883), respectively. Although it seemed that the monkey committed more errors in the horizontal wells, these errors rates did not differ significantly from the pre-lesion ones. Furthermore, before and after the lesion, the errors committed when the board rotated clockwise and contraclockwise were statistically identical (p=0.6722 and p=0.4376).

5.8.3.2.1.3 Hidden Brinkman board task

5.8.3.2.1.3.1 Contact Time With Visual Feedback

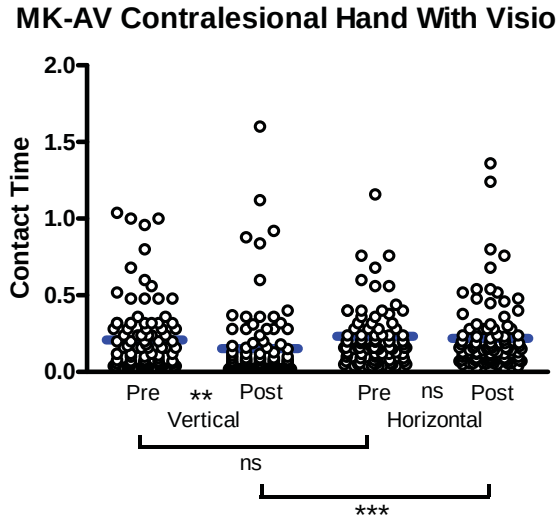


Figure 5.8.24: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-AV with his contralesional hand in the hidden Brinkman board task with visual feedback, between pre- and post-lesion plateaux for the vertical (n=10 max. per session) and the horizontal (n=10 max. per session) wells, as wells as between vertical and horizontal wells before and after the lesion.

In the hidden Brinkman board task, when having a visual feedback (fig. 5.8.24), MK-AV reached a pre-lesion behavioural plateau of 0.12s for the vertical wells and 0.16s for the horizontal wells. The higher value for the horizontal wells was not statistically significant ($p=0.1824$). After the mainly in PM lesion, the "final" recovery was 0.5 times the pre-lesional score for the vertical wells (0.06s; $p=0.0013$) and 0.94 times the pre-lesional score for the horizontal wells (0.15s; $p=0.6962$), with a better performance for the vertical than the horizontal wells ($p<0.0001$). Mk-AV recovered thus totally his manual dexterity in terms of contact time in this task; his performance was even enhanced for the vertical wells.

5.8.3.2.1.3.2

5.8.3.2.1.3.2 Errors With Visual Feedback

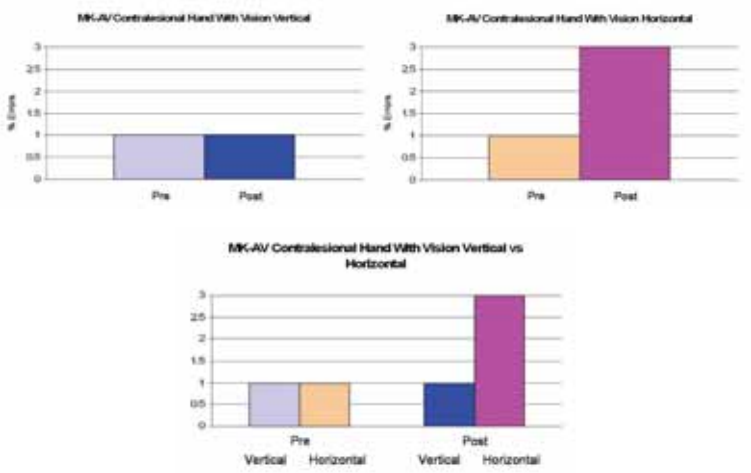


Figure 5.8.25: Statistical comparisons were performed on the errors commited by Mk-AV with his contralesional hand in the hidden Brinkman board task when having a visual feedback, between the pre- and the post-lesion plateaux for the vertical (blue; n=10 max. per session) and the horizontal (pink; n=10 max. per session) wells, as well as between vertical and horizontal wells before and after the lesion.

Concerning the errors (fig. 5.8.25), before the lesion, MK-AV commited on average 1% of errors for the vertical and the horizontal wells. In the "final" recovery plateau, 3 times more errors, although not statistically significant, were committed for the horizontal wells (3%; p=0.7957), whereas the same rate of errors was committed for the vertical wells (1%; p=0.9990). As before the lesion, there was no significant difference between errors in the vertical wells and errors in the horizontal wells (p=0.7957).

5.8.3.2.1.3.3 Contact Time Without Visual Feedback

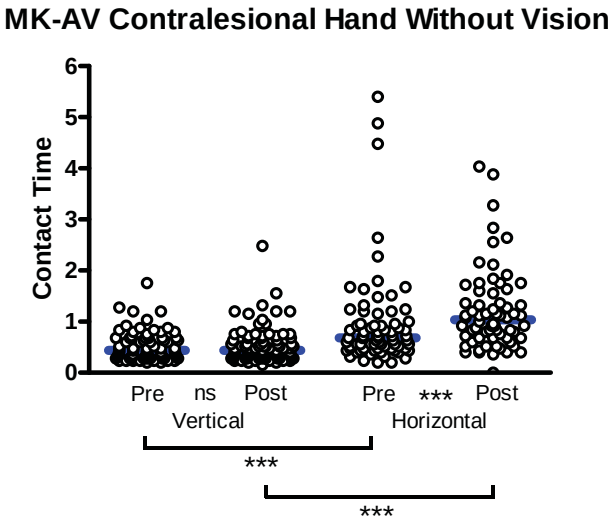


Figure 5.8.26: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-AV with his contralesional hand in the hidden Brinkman board task without visual feedback, between pre- and post-lesion plateaux for the vertical (n=10 max. per session) and the horizontal (n=10 max. per session) wells, as wells as between vertical and horizontal wells before and after the lesion.

Without visual feedback (fig. 5.8.26), after having recovered, the monkey showed a median contact time 1.53 times significantly higher than before the lesion for the horizontal wells (1.04s vs 0.68s; $p<0.001$), whereas his recovery was complete for the vertical (0.44s pre- and post-lesion; $p=0.8834$) wells. Both before and after the lesion, the contact times for the vertical wells were significantly lower than for the horizontal wells ($p<0.0001$).

5.8.3.2.1.3.4 Errors Without Visual Feedback

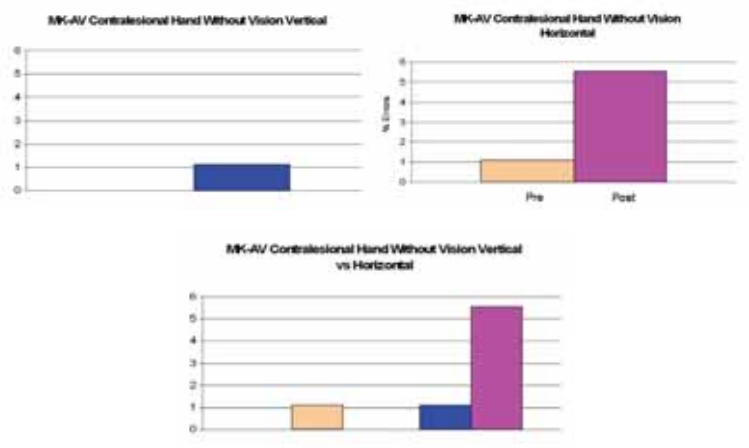


Figure 5.8.27: Statistical comparisons were performed on the errors commited by Mk-AV with his contralesional hand in the hidden Brinkman board task without having a visual feedback, between the pre- and the post-lesion plateaux for the vertical (blue; n=10 max. per session) and the horizontal (pink; n=10 max. per session) wells, as well as between vertical and horizontal wells before and after the lesion.

When looking at the errors committed when there was no visual feedback (fig. 5.8.27), it appeared that Mk-AV did not commit any errors for the vertical wells before the lesion. For the horizontal wells, he committed on average 1.11% of errors. After the lesion, in the "final" recovery plateau, he committed more errors, although not significantly, in all the wells. Indeed, the rate of errors was 5.01 times higher for the horizontal wells ($p=0.4470$), and he committed 1.11% of errors for the vertical wells ($p=1.0000$). The higher errors rate in the horizontal than in the vertical wells was not significant, both before and after the lesion ($p=1.0000$ and $p=0.4359$).

5.8.3.2.2 Mk-WI

5.8.3.2.2.1 Modified Brinkman board task

5.8.3.2.2.1.1 Score

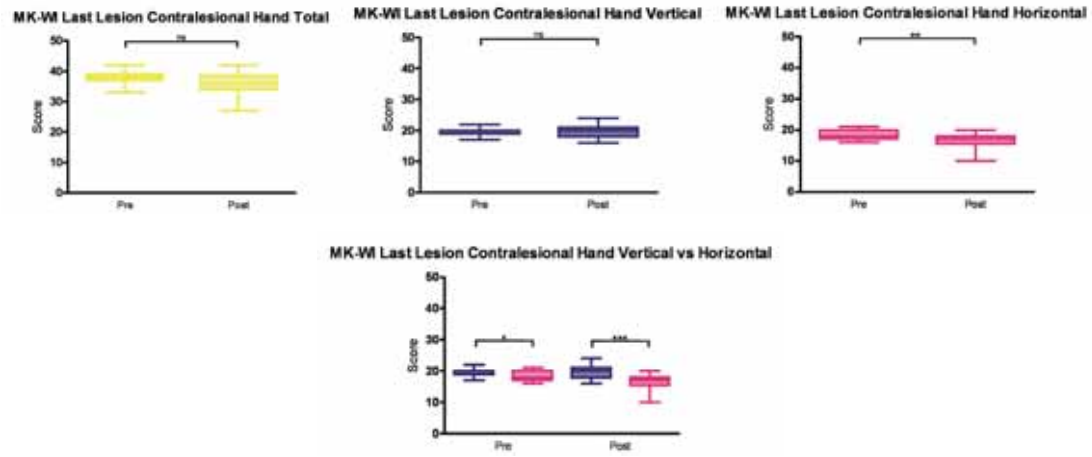


Figure 5.8.28: Comparisons of the distributions of the scores (number of pellets retrieved in 30 seconds) in Mk-WI with his contralesional hand between pre- and post-last lesion plateaux for the total (yellow), the vertical (blue) and the horizontal (pink) wells, as well as between vertical and horizontal wells, represented here by boxplots.

As Mk-JA, Mk-WI was treated with an antiepileptic drug (Luminal). After having tried several times to obtain the expected effects after a motor cortex lesion in Mk-WI, both in the left and the right hemisphere, we suspected the Luminal to have a counteracting effect to the ibotenic acid excitotoxic effect. Mk-WI was thus subjected to a last M1 lesion (left side) about one year after the previous lesion he was subjected to (see "Time course of experimental protocol, ICMS, Lesion and Cell reimplantation" in the "Annexes" part), without receiving any antiepileptic treatment. The following analyses were carried on the pre- and post-lesion plateau with respect to this last lesion, for the modified Brinkman board task uniquely.

The "final" recovery plateau reached 95% for the total score ($p=0.0769$), 100% for the vertical score ($p=1.0$) and 90% for the horizontal score ($p=0.0053$) (fig. 5.8.28). Thus, Mk-WI recovered totally for the total and the vertical wells, whereas his manual dexterity recovery was incomplete for the horizontal wells. To note that the score was better for the vertical wells than for the horizontal ones, both before ($p=0.0394$) and after ($p<0.0001$) the lesion.

5.8.3.2.2.1.2 Contact Time

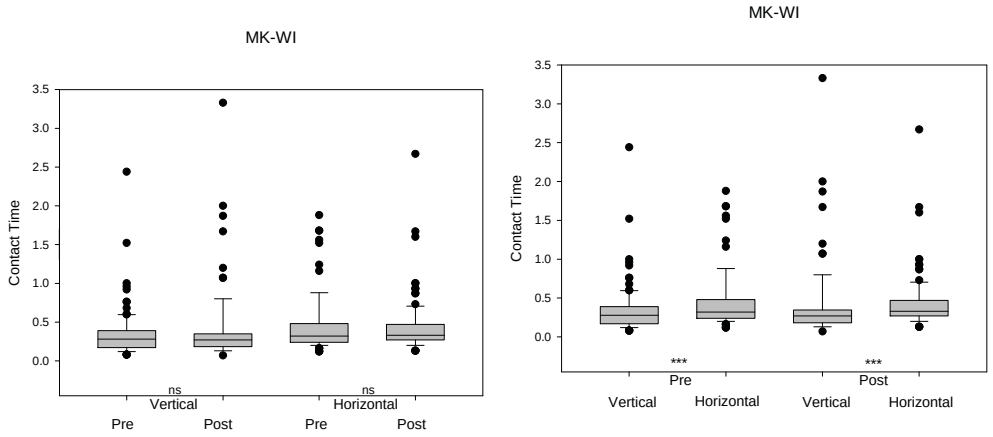


Figure 5.8.29: Comparisons of the distributions of the contact times spent in the wells to retrieve the pellets by Mk-JO with his contralesional hand in the modified Brinkman board task, between pre- and post-lesion plateaux for the vertical (n=10 per session) and the horizontal (n=10 per session) wells (left panel), and between vertical and horizontal wells before and after the lesion (right panel), represented here by boxplots.

Before this fifth lesion, Mk-WI showed contact time median values of 0.26s for the vertical wells and 0.34s for the horizontal wells (fig. 5.8.29). After the M1 lesion, Mk-WI recovered totally his manual dexterity, as this recovery was 0.98 times the pre-lesional score for the vertical wells (0.25s; $p=0.7611$) and identical to the pre-lesional score for the horizontal wells (0.34s $p=0.9352$), with a better score for the vertical than for the horizontal wells ($p<0.0001$), as it was the case before the lesion ($p<0.0001$).

5.8.3.2.2.1.3 Errors

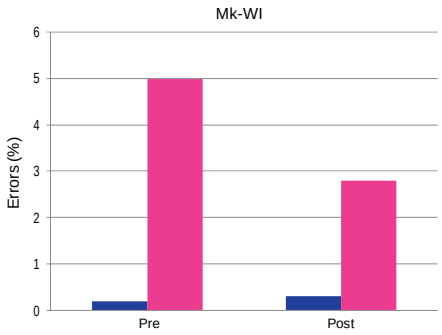


Figure 5.8.30: Percentage of errors commited by Mk-WI with his contralesional hand in the modified Brinkman board task. Comparisons were performed between pre- and post-last lesion plateaux, as well as between vertical (n=25 per session) and horizontal (n=25 per session) wells before and after the lesion.

In terms of errors (fig. 5.8.30), before this fifth lesion, MK-WI commited on average 0.19% of errors for the vertical wells and 5% of errors for the horizontal wells. The number of errors committed in the horizontal wells was not significantly higher than those in the vertical wells (p=0.4845). The errors rate in the "final" recovery plateau was statistically identical to that in the pre-lesion plateau. It was on average 1.60 times higher for the vertical wells (0.30%; p=0.9999) and 0.56 times the pre-lesion plateau errors rate for the horizontal wells (2.80%; p=0.6410). As before the lesion, there was no significant difference between the errors committed in the vertical wells and those committed in the horizontal wells (p=0.8154). To note that despite this total recovery, the post-recovery errors rate never returned to the errors rate of the plateau preceeding all surgeries.

	CONTACT TIMES	TREATED		CONTROL	
		Mk-JO	Mk-JA	Mk-AV	Mk-WI
Modified Brinkman board task	Contact time Vertical Pre	0.16	0.40	0.28	0.26
	Contact time Vertical Post	0.47	0.40	0.27	0.25
	Contact time Horizontal Pre	0.24	0.60	0.36	0.34
	Contact time Horizontal Post	0.93	0.67	0.40	0.34
Rotating Brinkman board task	Contact Time Clockwise Pre	0.50	0.32	0.50	no
	Contact Time Clockwise Post	0.52	0.48	0.50	no
	Contact Time Contraclockwise Pre	0.50	0.36	0.50	no
	Contact Time Contraclockwise Post	0.48	0.52	0.50	no
Hidden Brinkman board task With Vision	Contact Time Vertical Pre	0.04	0.27	0.12	no
	Contact Time Vertical Post	0.20	0.43	0.06	no
	Contact Time Horizontal Pre	0.12	0.66	0.16	no
	Contact Time Horizontal Post	0.52	0.82	0.15	no
Hidden Brinkman board task Without Vision	Contact Time Vertical Pre	0.52	0.66	0.44	no
	Contact Time Vertical Post	1.40	1.72	0.44	no
	Contact Time Horizontal Pre	0.76	0.92	0.68	no
	Contact Time Horizontal Post	0.36	0.52	1.04	no

Table 5.8.1 : Summary table of the contact time median values obtained by the two treated and the two control monkeys in the different manual dexterity tasks, before and after the motor cortex lesion.

	ERRORS	TREATED		CONTROL	
		Mk-JO	Mk-JA	Mk-AV	Mk-WI
Modified Brinkman board task	Errors Vertical Pre	0.44 %	0.38%	0.2%	0.19%
	Errors Vertical Post	1.32 %	0.13%	0.2%	0.30%
	Errors Horizontal Pre	2.67 %	1.05%	0.2%	5%
	Errors Horizontal Post	6.22 %	0.13%	0.2%	2.80%
Rotating Brinkman board task	Errors Clockwise Pre	1.56%	5%	3.13%	no
	Errors Clockwise Post	3.13%	10.94%	3.44%	no
	Errors Contraclockwise Pre	4.17%	3.75%	1.25%	no
	Errors Contraclockwise Post	4.69%	5.63%	6.88%	no
Hidden Brinkman board task With Vision	Errors Vertical Pre	2%	1%	1%	no
	Errors Vertical Post	3%	2%	3%	no
	Errors Horizontal Pre	1%	5%	1%	no
	Errors Horizontal Post	3%	2%	1%	no
Hidden Brinkman board task Without Vision	Errors Vertical Pre	3 %	1%	0%	no
	Errors Vertical Post	3 %	3.33%	1.11%	no
	Errors Horizontal Pre	5 %	5%	1.11%	no
	Errors Horizontal Post	6 %	6.67%	5.56%	no

Table 5.8.2 : Summary table of the rate of errors committed by the two treated and the two control monkeys in the different manual dexterity tasks, before and after the motor cortex lesion.

		TREATED		CONTROL	
	COMPARISON PRE- VS POST-	Mk-JO	Mk-JA	Mk-AV	Mk-WI
Modified Brinkman board task	Score Total	59%***	104% (ns)	102% (ns)	95% (ns)
	Score Vertical	88%**	103% (ns)	115%**	100% (ns)
	Score Horizontal	29%***	105% (ns)	81%*	90%**
	Contact time Vertical	x2.94***	x1.00 (ns)	x0.96 (ns)	x0.98 (ns)
	Contact time Horizontal	x3.88***	x1.12 (ns)	x1.10*	x1.00 (ns)
	Errors Vertical	x3.00 (ns)	x2.26 (ns)/0.34 (ns)	x1.00 (ns)	x1.60 (ns)
	Errors Horizontal	x2.33 (ns)	x3.20 (ns)/0.21 (ns)	x1.00 (ns)	x0.56 (ns)
Rotating Brinkman board task	Contact Time Clockwise	x1.04 (ns)	x1.50***	x1.00 (ns)	no
	Contact Time Contraclockwise	x0.96*	x1.44***	x1.00 (ns)	no
	Errors Clockwise	x2.01 (ns)	x2.19 (ns)	x1.10 (ns)	no
	Errors Contraclockwise	x1.12 (ns)	x1.50 (ns)	x5.50 (ns)	no
Hidden Brinkman board task With Vision	Contact Time Vertical	x5.00***	x1.63***	x0.50**	no
	Contact Time Horizontal	x4.33***	x1.24**	x0.94 (ns)	no
	Errors Vertical	x1.50 (ns)	x2.00 (ns)	x1.00 (ns)	no
	Errors Horizontal	x3.00 (ns)	x0.40 (ns)	x3.00 (ns)	no
Hidden Brinkman board task Without Vision	Contact Time Vertical	x2.69***	x2.61***	x1.00 (ns)	no
	Contact Time Horizontal	x0.47***	x0.57***	x1.53***	no
	Errors Vertical	x1.00 (ns)	x3.33 (ns)	+1.11 (ns)	no
	Errors Horizontal	x1.20 (ns)	x1.33 (ns)	x5.01 (ns)	no

Table 5.8.3 : Summary table of the comparison between pre- and post- motor cortex lesion plateaux. Significances in red express an incomplete recovery, whereas those in green represent a total or enhanced recovery.

COMPARISON VERTICAL VS HORIZONTAL, CLOCKWISE VS CONTRACLOCKWISE		TREATED		CONTROL	
		Mk-JO	Mk-JA	Mk-AV	Mk-WI
Modified Brinkman board task	Score Pre-lesion	V > H	V > H	V = H	V > H
	Score Post-lesion	V > H	V > H	V > H	V > H
	Contact Time Pre-lesion	V > H	V > H	V > H	V > H
	Contact Time Post-lesion	V > H	V > H	V > H	V > H
	Errors Pre-lesion	V = H	V = H	V = H	V = H
	Errors Post-lesion	V = H	V = H	V = H	V = H
Rotating Brinkman board task	Contact Time Pre-lesion	C = CC	C = CC	C = CC	no
	Contact Time Post-lesion	CC > C	C > CC	C = CC	no
	Errors Pre-lesion	C = CC	C = CC	C = CC	no
	Errors Post-lesion	C = CC	C = CC	C = CC	no
Hidden Brinkman board task With Vision	Contact Time Pre-lesion	V > H	V > H	V = H	no
	Contact Time Post-lesion	V > H	V > H	V > H	no
	Errors Pre-lesion	V = H	V = H	V = H	no
	Errors Post-lesion	V = H	V = H	V = H	no
Hidden Brinkman board task Without Vision	Contact Time Pre-lesion	V > H	V > H	V > H	no
	Contact Time Post-lesion	H > V	H > V	V > H	no
	Errors Pre-lesion	V = H	V = H	V = H	no
	Errors Post-lesion	V = H	V = H	V = H	no

Table 5.8.4 : Summary table of the comparison between manual performances in the vertical and the horizontal wells for the modified and the hidden Brinkman board tasks, and between clockwise and contraclockwise rotations for the rotating Brinkman board task well, performed before and after the lesion.

5.8.4 GENERAL CONCLUSION

When looking at the manual performance of the two treated (Mk-JO and Mk-JA) and one control (Mk-AV) monkeys at the different tasks before the lesion -as data were analyzed on the modified Brinkman board task uniquely for the other control monkey (Mk-WI)-, and if considering that a higher contact time and more numerous errors would reflect a greater difficulty for the monkey to retrieve the pellets, various differences appeared among the monkeys. First, on the contact time parameter, Mk-JO was globally better than the two other monkeys, with some identical performances as Mk-AV (rotating and hidden without vision Brinkman board tasks), and Mk-JA showed worse performances than the two other monkeys, except at the rotating Brinkman board task, but the temporal resolution of the analyses at this task being better for this monkey could explain it. Second, from one monkey to another, the greater difficulties are not always in the same tasks. But a general trend could be inferred, both wells orientations taken into account: there were particularly greater contact times at the hidden Brinkman board task wihtout vision for all three monkeys, and a better performance at the hidden Brinkman board task with vision followed by the modified and finally the rotating Brinkman board task for Mk-JO and Mk-AV, whereas Mk-JA had a better performance at the rotating Brinkman board task, followed by the the hidden Brinkman board task with vision and then by the modified Brinkman board task. The higher contact times at the hidden Brinkman board task without vision can easily be explained by the difficulty of the task, as the monkey has to grope and proceed by touch uniquely to retreive the pellets. Concerning the difference of difficulty degree of the rotating Brinkman board task between Mk-JA and the two other monkeys, when looking at the contact time values, it seems due to the difference of temporal resolution in the analyses. Finally the lower contact times for the hidden Brinkman board task with vision than for the modified Brinkman board task can first be explained by the less numerous wells. Indeed, it has been shown (see chapter 5.6 “Does the behavioural performance vary along a daily session as assessed by the contact time”) that the monkey could be less efficient at the end of the task than at the beginning. Therefore, as the modified Brinkman board task contains more pellets, one can imagine that the last pellets increased the median contact time compared to the hidden Brinkman board task. Second, the fact that the hand of the monkey is more confined in the hidden Brinkman board task represents perhaps contra-intuitively an advantage to retrieve the pellets. Third, one cannot exclude that an appetency aspect played a role here. Indeed, the monkeys performed the tasks always in the same order in a given training session. First they began with the hidden Brinkman board task

without vision, which is the less motivating for the monkeys, as they have to proceed by groping. Then, the same task with visual feedback was performed; here it is possible that additionally to the aspect of beginning of training session and thus of eating, the monkeys showed a particularly high velocity to perform this task, as it followed immediately the frustration of having been slown down to pick the pellets by the hidden aspect of the previous task. Then the monkeys performed the modified Brinkman board task, ending finally with the rotating Brinkman board task.

A question arising from these differences is whether there is a link between the difficulty of the task and the degree of recovery after the motor cortex lesion. Indeed, did the monkeys recover better at the tasks at which they had better contact times, and inversely recover worse at those at which they had worse performances before the lesion? The answer to this question is yes and no. Yes, as the recovery for the hidden Brinkman board task without vision, which was the most difficult before the lesion, was generally bad; no, as it could be observed in the three monkeys that complete recoveries occurred at tasks that were not the easiest, and incomplete recoveries were found in the tasks that had the lowest contact times.

Another point to mention here is that the errors committed were not more numerous in the tasks that had the higher contact times. The only general trend concerning the errors rate was a tendency to commit fewer errors at the modified Brinkman board task. Otherwise, the errors did not seem to follow any trend such as that observed for the contact times. When comparing the errors committed between nonkeys, Mk-AV committed fewer errors than the two other monkeys, among which Mk-JO committed more often more errors than Mk-JA.

After the lesion, the rank of the tasks in terms of difficulty, as defined by their contact time values, was quite comparable to what was observed before the lesion, except in Mk-JO for the horizontal wells at the modified and the hidden Brinkman board task with vision, which had a higher difficulty degree than before the lesion. This seems to be related to the worse manual recovery of Mk-JO, particularly to retrieve pellets that were placed in the horizontal wells. Another exception appeared in Mk-JA, who showed a higher difficulty degree in the hidden Brinkman board task with vision than before the lesion. The distribution of the errors rate in the different tasks was similar to before the lesion, with again a general tendency to commit fewer errors at the modified Brinkman board task. A general remark concerning the errors is that although there was never any statistical difference, the rate of errors was often higher after the recovery, even when the recovery for the contact time or the score was complete.

When comparing the performances in the vertical and in the horizontal wells, it appeared that the performance in the vertical wells was almost always better than in the horizontal wells, both before and after the lesion. Sometimes, the performances in the two types of well were identical; this was always the case on the errors parameter, probably due to the very little rate of errors committed, even after recovering from the lesion. It seems thus that retrieving the pellets in the horizontal wells represents a greater difficulty than in the vertical ones, even before the lesion. To note that although nothing significant appeared on the errors, there were often more errors committed in the horizontal wells, with some exceptions.

Concerning the comparison between the performances in the clockwise and the contraclockwise rotations, they were comparable before the lesion, both for the contact time and the errors. After the lesion, different patterns were observed according to the monkey. Indeed, for the contact times, Mk-JO had better performances when the board rotated contraclockwise, Mk-JA when it rotated clockwise, whereas Mk-AV showed the same identical performances than before the lesion. The errors rates were statistically identical for the two rotation orientations.

When looking at the recoveries in the diverse tasks, differences were observed. Mk-JO recovered only partially his manual dexterity for the score and the contact time at the modified Brinkman board task, and the contact time at the hidden Brinkman board task with and without vision, except for the horizontal wells when having no visual feedback. The contact times were proportionally higher at the hidden Brinkman board task with vision, then at the modified Brinkman board task, and finally at the hidden Brinkman board task without vision. Interestingly, before the lesion, he showed lower contact times at the hidden Brinkman board task with visual feedback and higher contact times when having no visual feedback. Surprisingly, Mk-JO recovered totally at the rotating Brinkman board task, as well as for the horizontal wells in the hidden Brinkman board task without vision. One can imagine that the rotating aspect could have helped him to retrieve the pellets in an optimal position for him. Another explanation would be the less temporal resolution of the analyses, which decreased the probability to observe a difference.

To note here the importance to follow the experiment on a long time period; indeed, for example Mk-JO recovered completely his manual dexterity at the rotating Brinkman board task only about 120 days after the lesion. This recovery would not have been observed if the experiment had been stopped earlier. Along this line, the effects shown in the chapter treating of the ipsilesional hand and its relationship with the recovery of the contralesional hand (see chapter 5.1 in the “Supplemental analyses”) reflects also the time necessary to follow the

evolution of the behaviour after the lesion, as well as before it, as a learning phase is needed by the monkey to reach his maximal capacity.

The other treated monkey, Mk-JA, showed a total recovery of his manual dexterity in the modified Brinkman board task. As Mk-JO, for the horizontal wells at the hidden Brinkman board task without vision, the manual recovery was complete. On the contrary, the recovery of Mk-JA was incomplete for all the other tasks, namely the rotating and the hidden Brinkman board task, both with and without visual feedback, with a propotionnally greater difficulty for the vertical wells at the hidden Brinkman board task without vision. Again, where the monkey was the best before the lesion, namely the rotating Brinkman board task, he did not recover totally. He recovered however totally in the modified Brinkman board task, a task for which he was better than the hidden Brinkman board task without vision. One could have expected the same complete recovery for the hidden Brinkman board task with vision, as his manual performance in terms of contact time was comparable for these two tasks before the lesion, but the recovery at this task was partial.

It seems thus that after the lesion, contrarily to Mk-JO, the moving aspect of the rotating Brinkman board task was a constraint for Mk-JA. Furthermore, the confinement in the hidden Brinkman board task, and thus the less space avalaible to move the hand constituted probably a deleterious element for these two monkeys after the lesion. In the hidden Brinkman board task without vision, Mk-JO and Mk-JA showed a better performance after the lesion for the horizontal wells. This phenomenon could be due to the fact that fewer pellets were taken after than before the lesion, inducing an eventual bias, such as described in the chapter treating of the evolution of the performance along the task. However, when looking at the raw data, it appeared that this explanation is to reject, as the monkeys did not retrieve fewer pellets in the recovery plateau than before the lesion. Nevertheless, in order to avoid such an eventual effect, it would be judicious in the future to consider the same number of pellets and to perform the comparisons by using the Wilcoxon non-parametrical statistical test. Concerning the better post-lesion performance for the horizontal wells in these two monkeys, this remains a perplexing phenomenon. Further detailed analyses of the way the monkeys grasped the pellets could bring a light; indeed, the explanation could be found in an eventual compensatory prehension strategy.

Mk-AV, a control monkey, showed a complete recovery for all the tasks, with however remaining deficits for the horizontal wells in the modified Brinkman board task on the contact time parameter, and on the score parameter, as well as in the hidden Brinkman board task without visual feedback, where he had already more difficulties before the lesion.

Thus, the horizontal wells remained a difficulty for this monkey, although he recovered globally well from his mainly in PM lesion.

For his part, the control monkey Mk-WI, for which only the modified Brinkman board task was analyzed, showed a complete recovery, both for the score and the contact time, for the vertical, the horizontal and the total wells.

It seems thus that the diverse tasks did not represent the same degree of difficulty for the different monkeys. Indeed, although there are common points, such as the greater difficulty to perform the hidden Brinkman board task without vision and the lower contact times before the lesion in the hidden Brinkman board task with vision and the modified Brinkman board task, the differences between the tasks, such as the larger space to perform the modified Brinkman board task compared to the hidden Brinkman board task for which the hand is more confined and which contains fewer pellets, or the moving aspect of the rotating Brinkman board task, did not affect the monkeys in a systematic way. For example, in the rotating Brinkman board task, the monkey can either feel in a greater difficulty due to the rotation, such as Mk-JA after the lesion compared to before or to the modified Brinkman board task, or feel in a facilitated situation to grasp the pellets in an optimal well orientation, such as Mk-JO after the lesion compared to the other tasks having a visual feedback but remaining static.

6 ANNEXES

6.1 TIME COURSE OF EXPERIMENTAL PROTOCOL, ICMS, LESION AND CELL REIMPLANTATION

All monkeys were first trained to sit into a primate chair and then to learn various manual dexterity motor tasks (see "General Material and Methods" part).

6.1.1 MONKEY MK-JO

	T	B+C	L	I	cl	S
	Beginning of training	Biopsy Implantation of Chamber	Lesion left hemisphere	Cells Reimplantation	Chamber loss	Sacrifice
MK-JO	11.10.2004	25.01.2005	08.03.2005	23.03.2005	22.06.2005	22.08.2005

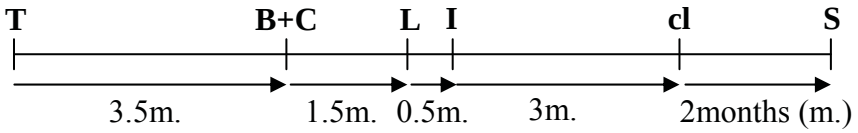
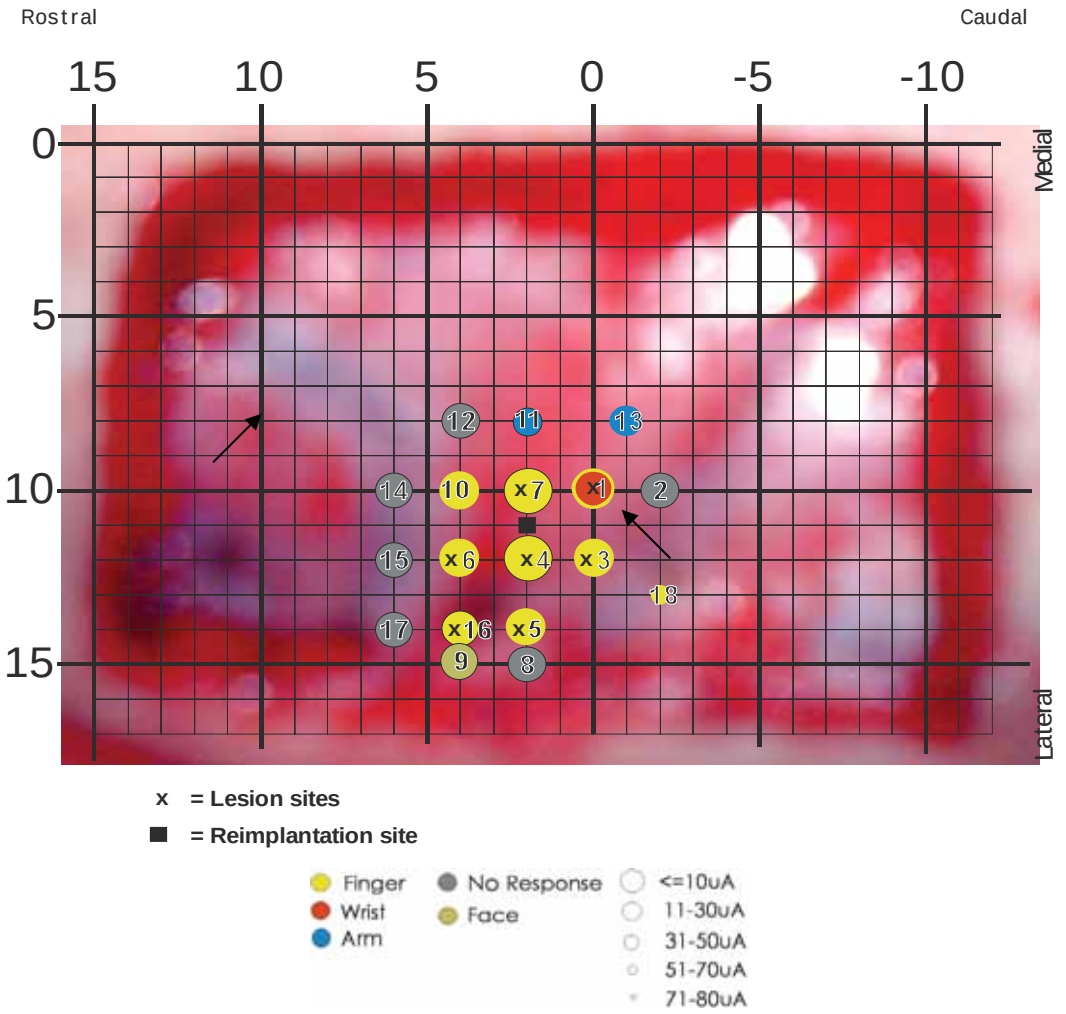


Figure 6.1.1 : Time course of the main events in the protocol for monkey MK-JO.

As shown in figure 6.1.1, three months and a half after the beginning of training sessions, as Mk-JO reached a behavioural plateau, a right dlPFC biopsy was performed surgically to collect adult progenitor cells for subsequent autotransplantation; at the same time, an electrophysiological chronic recording chamber was implanted above the sensorimotor area on the left hemisphere. One month and a half after the biopsy and the implantation of the chronic chamber, a lesion aimed at the hand representation's area in M1 was performed in the left hemisphere (fig. 6.1.2 A, B and C). Fifteen days later, reimplantation of cells was performed into the lesioned site[3] (fig. 6.1.2 D). Three months after the cells reimplantation, Mk-JO lost his chronic chamber. Five months after reimplantation, Mk-JO was sacrificed. Thus, in total, the experimental protocol on Mk-JO lasted about ten months and a half.

[3] At the time of the experiments conducted on MK-JO and MK-AV, the samples containing cells or just culture medium were coded by another member of the laboratory in order to keep the experimenter blind to what was injected.

A



B

depth	Tract 1	tract 3	tract 4	tract 5	tract6	tract 7	tract 10	tract 16	tract 18
			12345						
2	0 (70)	0 (70)	(17)	0 (70)	a (40)	0 (70)	0 (70)	0 (70)	0 (70)
	345								
3	(59)	w (47)	345 (9)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)
		2345							
4	w (35)	(18)	2345 (3)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)
		2345	2345			345			
5	w (31)	(22)	(10)	1 (30)	0 (70)	(28)	0 (70)	1 (15)	0 (70)
6	w (15)	0 (70)	2 (2)	1 (20)	1 (66)	3 (30)	0 (70)	2 (12)	0 (70)
7	w (29)	0 (70)	2 (6)	1 (36)	1 (32)	1 (20)	1 (30)	1 (40)	0 (70)
	2345		12345						
8	(27)	1 (20)	(15)	0 (70)	1 (32)	23 (20)	1 (58)	1 (40)	1 (65)
9	w (11)	0 (70)	1 (32)	w (70)	12 (20)	2 (8)	1 (50)	f (40)	1 (56)
10			w (57)		f (25)	1 (8)	f (45)	0 (70)	0 (70)
11						2 (36)			
12						5 (45)			
13						15 (60)			

C

Tract 1	8mm : 1.5µl
Tract 3	4mm : 1.5µl
Tract 4	8mm : 1.5µl 6mm : 1.5µl 4mm : 1.5µl
Tract 5	6mm : 1.5µl
Tract 6	9mm : 1.5µl
Tract 7	10mm : 1.5µl 6mm : 1.5µl
Tract 16	6mm : 1.5µl

D

Surface	Profondeur	Quantité
18	11 (7mm)	5µl
18	12 (6mm)	5µl
18	13 (5mm)	5µl
18	14 (4mm)	5µl

(α = 30°) ; 20µl (1µl/min.). Référence: ML 21.4 ; RC 65.1. Tract: ML 32.4 ; RC 67.1.

Figure 6.1.2: (A) After trepanation, view of the dura surface on the left hemisphere in MK-JO before implantation of the chronic chamber. Blue structures (arrows) correspond to the approximate location of the arcuate (rostrally) and central (caudally) sulci (scale is in mm). Coloured circles represent the sites at which movements were elicited at lower intensity threshold, which is represented by circles' size. Crosses represent sites of ibotenic acid injections (see C). Square represents site of cells reimplantation (see D). Table in panel B represents the pre-lesional ICMS responses elicited at each depth; the first letter indicates the activated territory, except numbers indicating the activated fingers; the numbers in parentheses give the ICMS threshold. Based on the pre-lesional ICMS map and on the Table in panel B, sites of ibotenic acid injections (C) were chosen. Later, cells reimplantation was performed into the lesioned site (D). In total, 250'000 cells were transplanted, at a concentration of 12'500 cells/µl.

6.1.2 MONKEY MK-AV

	T	B+C	L	I	cl	S
	Beginning of training	Biopsy Implantation of Chamber	Lesion left hemisphere	Culture medium Reimplantation	Chamber loss	Sacrifice
MK-AV	20.09.2004	09.02.2005	10.03.2005	23.03.2005	29.03.2005	29.08.2005

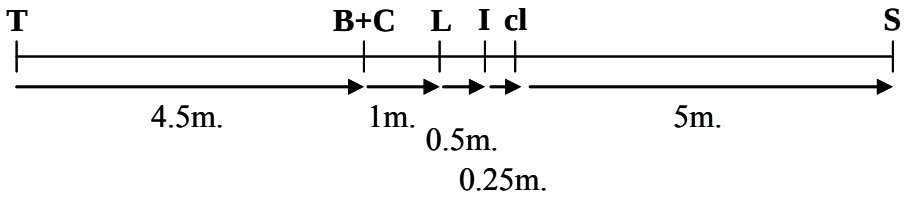
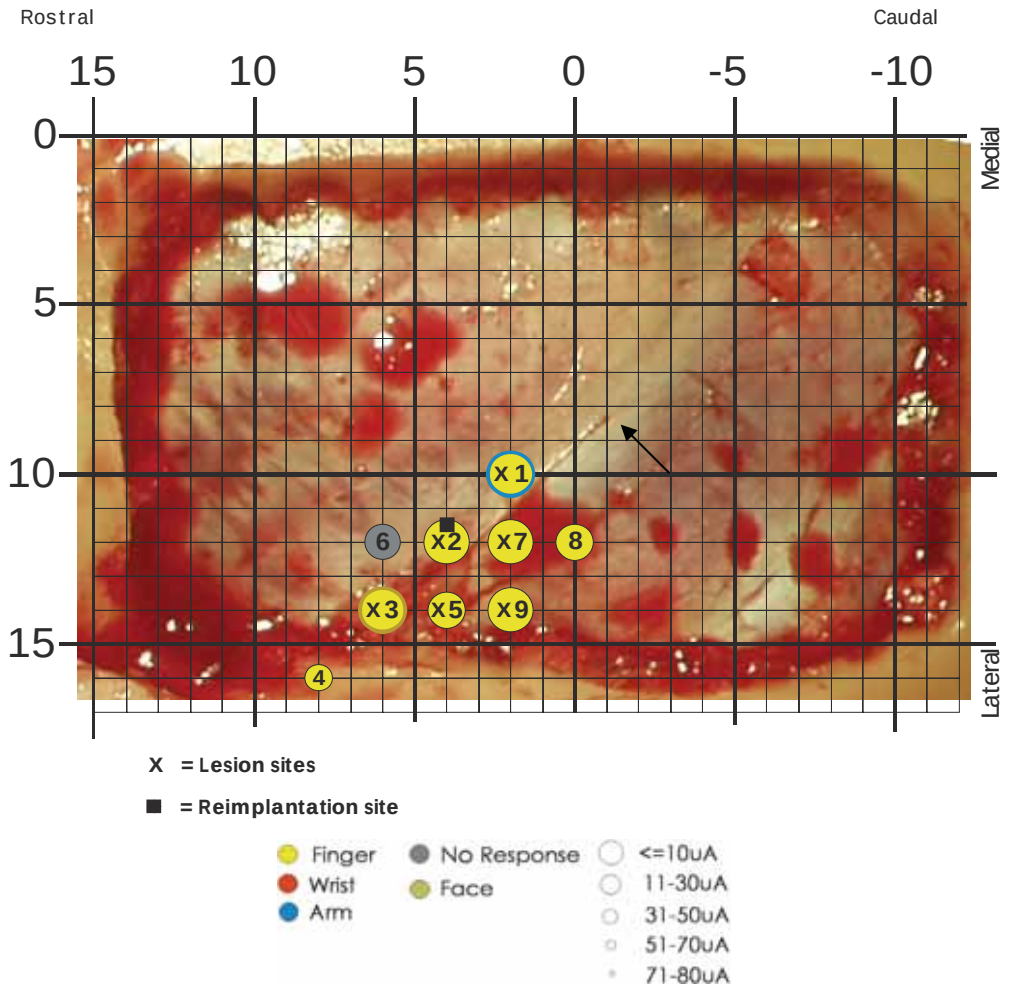


Figure 6.1.3 : Time course of the main events in the protocol for monkey MK-AV.

Mk-AV reached a behavioural plateau four months and a half after the beginning of training sessions (fig. 6.1.3). Then, the right dlPFC biopsy was performed, at the same time as the chronic chamber implantation was performed on the left hemisphere. One month later, a lesion of the hand representation's area was performed in the left hemisphere, unfortunately mainly in PM (fig. 6.1.4 A, B andC). Thirteen days later, implantation of culture medium was performed into the lesioned site (fig. 6.1.4 D). Six days after the implantation, Mk-AV lost his chamber. About five months after implantation, Mk-AV was sacrificed. Overall, the experimental protocol on MK-AV lasted about eleven months and a half.

A



B

Depth	tract 1	tract 2	tract 3	Tract 4	Tract 5	tract 7	tract 8	tract 9
2	a (60)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)
3	a (23)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)	0 (70)
4	a (40)	0 (70)	0 (70)	0 (70)	0 (70)	345 (55)	0 (70)	0 (70)
5	a (20)	0 (70)	0 (70)	0 (70)	23 (48)	2345 (13)	0 (70)	0 (70)
6	a (12)	w (36)	a (60)	0 (70)	2345 (26)	45 (1)	0 (70)	0 (70)
7	w (12)	1 (9)	1 (28)	1 (42)	w (18)	45 (6)	0 (70)	0 (70)
8	45 (9)	12 (10)	2 (31)	1 (40)	1 (17)	345 (9)	0 (70)	1 (38)
9	w (9)	1 (13)	f (24)	0 (70)	1 (17)	4 (15)	0 (70)	5 (5)
10	w (18)	1 (13)	f (48)	0 (70)	0 (70)	34 (9)	0 (70)	1 (14)
11	w (20)	1 (28)			45 (31)	1 (20)	345 (30)	1 (43)
12	w (26)	34 (19)			45 (26)	a (40)		
13	0 (70)	345 (36)			45 (15)			

C

Tract 1	8mm : 1.5µl
Tract 2	8mm : 1.5µl 10mm : 1.5µl
Tract 3	7mm : 1.5µl
Tract 5	6mm : 1.5µl 8mm : 1.5µl
Tract 7	5mm : 1.5µl 7mm : 1.5µl 9mm : 1.5µl
Tract 9	9mm : 1.5µl

D

Surface	Profondeur	Quantité
12	4 (8mm)	5µl
12	5 (7mm)	5µl
12	6 (6mm)	5µl
12	7 (5mm)	5µl

(α = 30°) ; 20µl (1µl/min.). Reference: ML 17.8 ; RC 69.8. Tract: ML 29.3 ; RC 73.3

Figure 6.1.4: (A) After trepanation, view of the dura surface on the left hemisphere in MK-AV before implantation of the chronic chamber. Beige structure (arrow) corresponds to the approximate location of the central (caudally) sulcus (scale is in mm). Coloured circles represent the sites at which movements were elicited at lower intensity threshold, which is represented by circles' size. Crosses represent sites of ibotenic acid injections (see C). Square represents site of culture medium implantation (see D). Table in panel B represents the pre-lesional ICMS responses elicited at each depth; the first letter indicates the activated territory, except numbers indicating the activated fingers; the numbers in parentheses give the ICMS threshold. Based on the pre-lesional ICMS map and on the Table in panel B, sites of ibotenic acid injections (C) were chosen. Later, culture medium implantation was performed into the lesioned site (D).

6.1.3 MONKEY MK-JA

	T	C1	L1	B	cl	C2	L2	I1	I2	S
	Beginning of training	Implantation of chamber	1st lesion left hemisphere	Biopsy	Chamber loss	Reimplantation of chamber	2nd lesion left hemisphere	Cells reimplantation Chamber put away	2nd cells reimplantation without chamber	Sacrifice
MK-JA	19.12.2005	29.06.2006	11.08.2006	18.08.2006	14.09.2006	20.09.2006	06.03.2007	10.07.2007	28.08.2007	13.03.2008

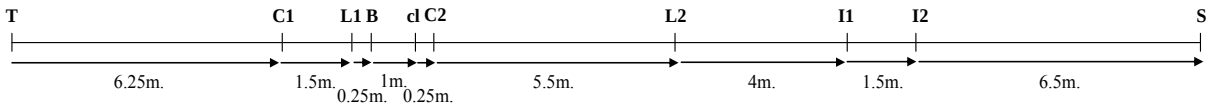
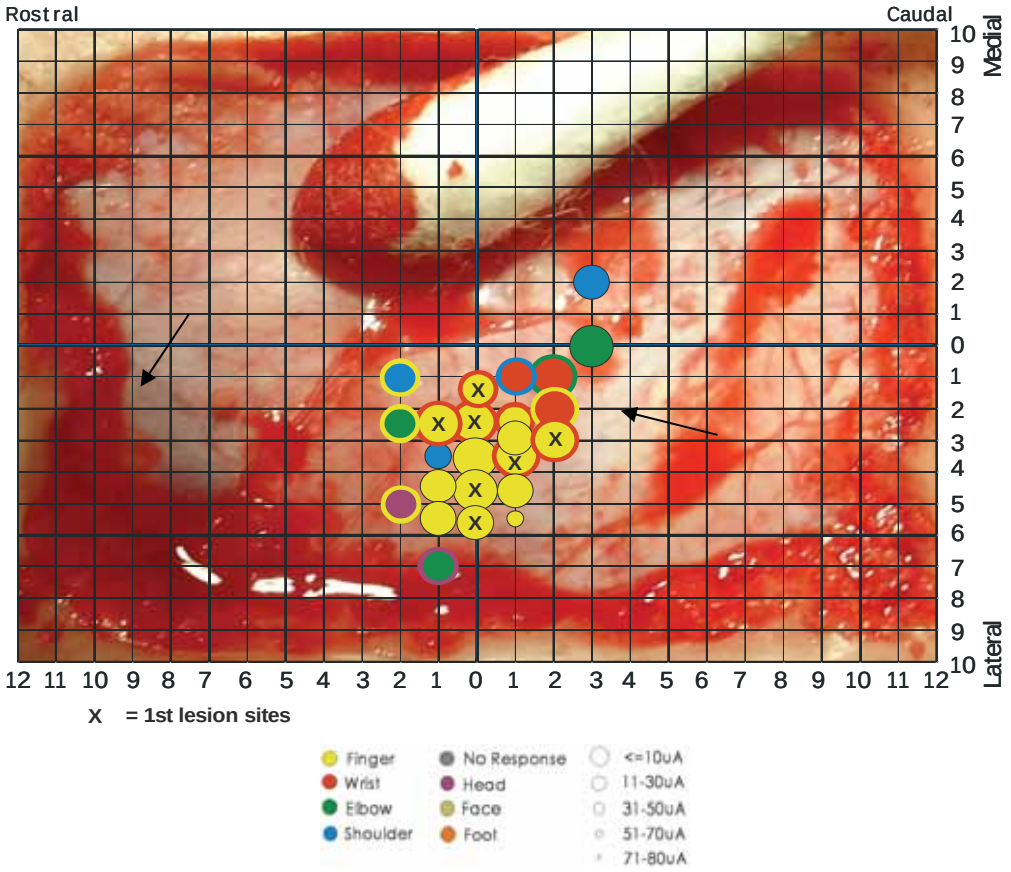


Figure 6.1.5 : Time course of the main events in the protocol for monkey MK-JA.

Figure 6.1.5 illustrates the time course of the experiments in Mk-JA. This monkey reached a behavioural plateau after about six months after the beginning of training sessions. Then, the chronic recording chamber was implanted above the sensorimotor area of the left hemisphere. One month and a half later, a lesion of the hand representation's area was performed in the left hemisphere (fig. 6.1.6). One week later, the right dlPFC biopsy was performed. One month after the biopsy, Mk-JA lost his chamber, which was reimplanted one week after the loss. As Mk-JA suffered epileptic seizures following the first lesion he was subjected to, he received an antiepileptic drug (0.025mg Luminal, GABA agonist), which we suspected later to have a counteracting effect to the ibotenic acid excitotoxic effect. Thus, after the first motor cortex lesion he was subjected to, Mk-JA recovered unusually quick and good.

Five months and a half later, a second lesion was performed, in the same left hemisphere (fig. 6.1.7 A, B and C). Four months later, cells were reimplanted into and near the lesioned site (fig. 6.1.7 D), and the chronic chamber was removed. One month and a half later, a second reimplantation of cells was performed near the lesioned site (fig. 6.1.7 E). Six months and a half after the cells reimplantation, Mk-JA was sacrified. Overall, the experimental protocol on Mk-JA lasted about twenty-seven months.

A

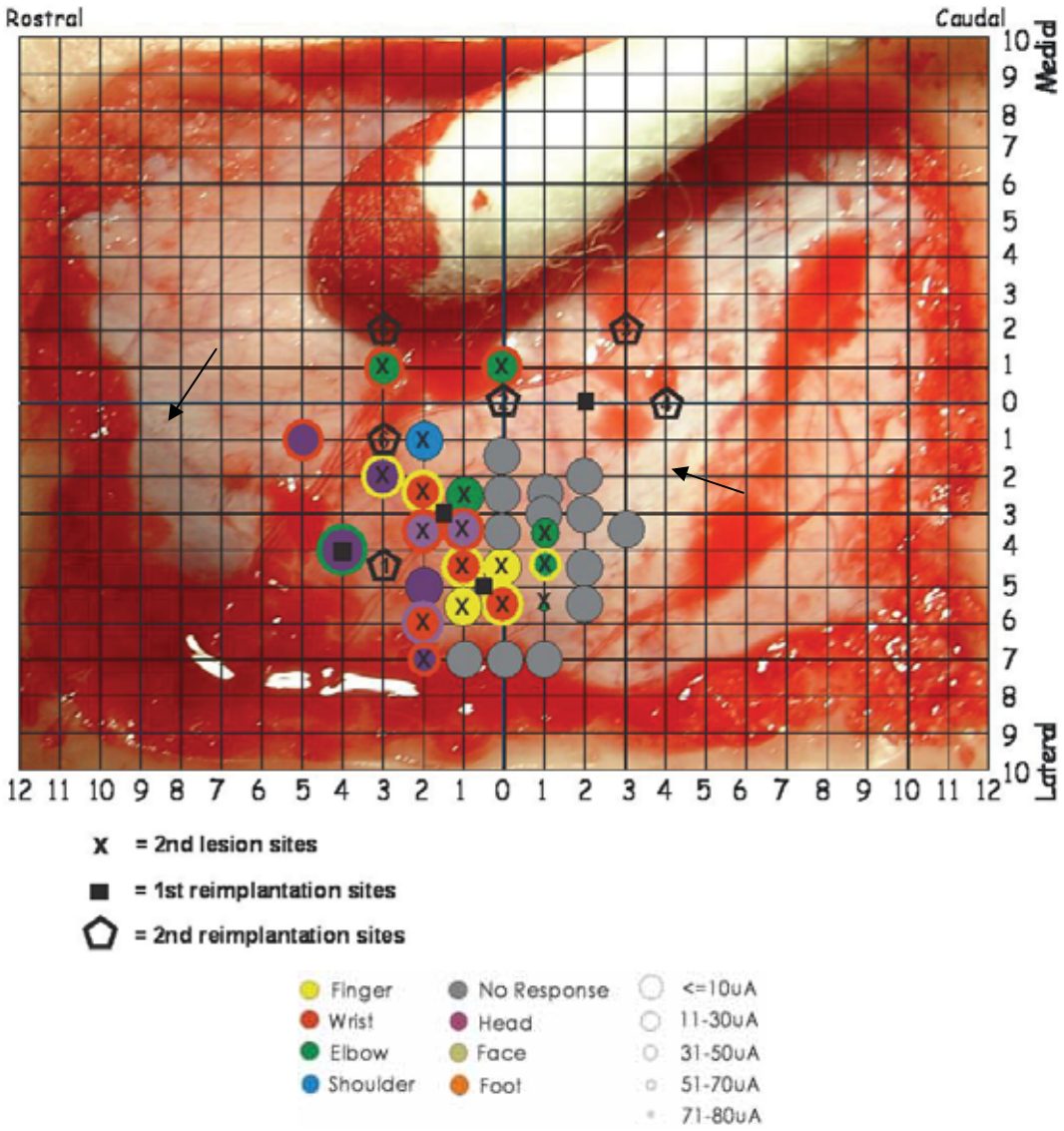


B

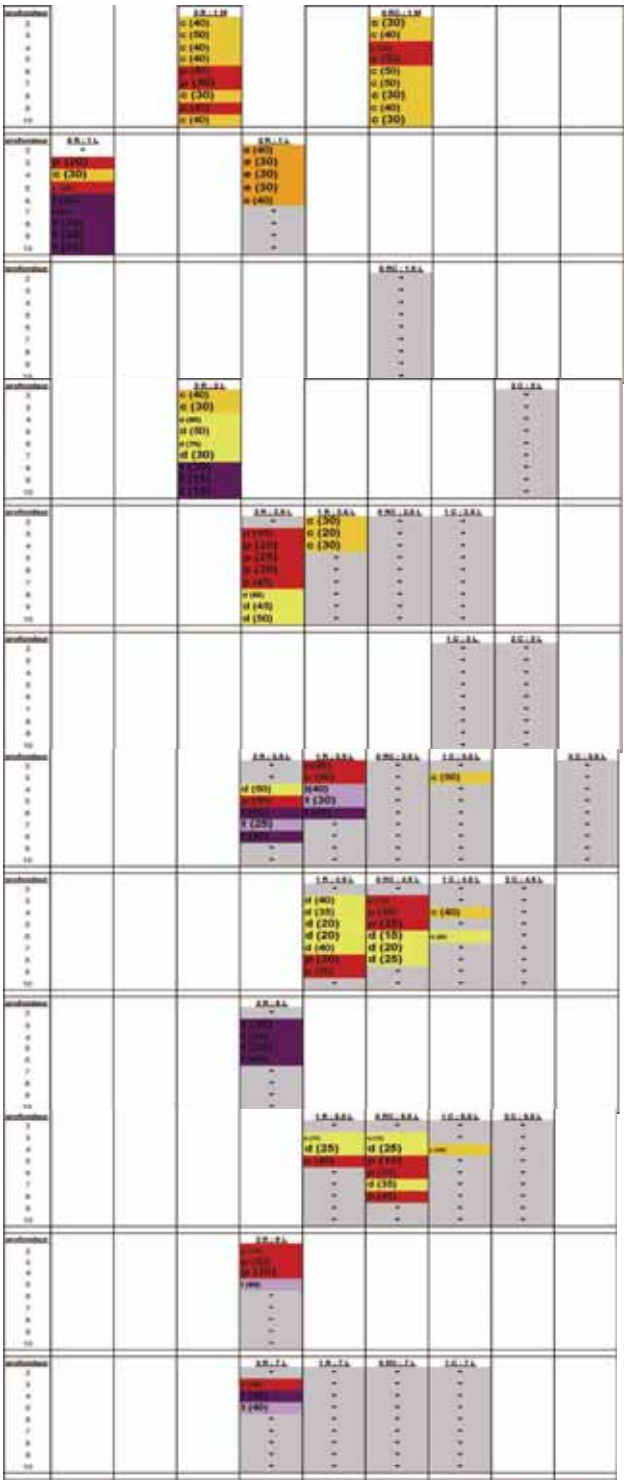
Tract	Coordonnée	Profondeur	Qté µl
Tract 9	**1 R**ostral / **2.5 L**atéral	10 mm	1.5
Tract 6	**0** RC / **1.5 L**atéral	10 mm	1.5
Tract 10	**0** RC / **2.5 L**atéral	3 mm 9 mm	1.5 1.5
Tract 18	**0** RC / **4.5 L**atéral	6 mm 9 mm	1.5 1.5
Tract 22	**0** RC / **5.5 L**atéral	8 mm	1.5
Tract 16	**1 C**audal / **3.5 L**atéral	7 mm 10 mm	1.5 1.5
Tract 13	**2 C**audal / **3 L**atéral	9 mm 11 mm	1.5 1.5
Total		11 sites	16.5

Figure 6.1.6: (A) After trepanation, view of the dura surface on the left hemisphere in MK-JA before implantation of the chronic chamber. Arrows indicate the appropriate location of the arcuate (rostrally) and central (caudally) sulci (scale is in mm). Coloured circles represent the sites at which movements were elicited at lower intensity threshold, which is represented by circles' size. Crosses represent sites of ibotenic acid injections (B).

A


Rostral
Caudal
Medial
Lateral
12 11 10 9 8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10 11 12
10 9 8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10
X = 2nd lesion sites
■ = 1st reimplantation sites
⬠ = 2nd reimplantation sites
Finger
Wrist
Elbow
Shoulder
No Response
Head
Face
Foot
<=10uA
11-30uA
31-50uA
51-70uA
71-80uA

B



C

1) 1 médial ; 3 rostral :
a) 3mm
b) 5mm
c) 7mm
d) 9mm

2) 1 médial ; 0 rostro-caudal :
a) 3mm
b) 5mm
c) 7mm
d) 9mm

3) 1 latéral ; 5 rostral :
a) 2mm

4) 1 latéral ; 2 rostral :
a) 3mm
b) 5mm

5) 2 latéral ; 3 rostral :
a) 3mm
b) 5mm
c) 7mm

6) 2.5 latéral ; 2 rostral :
a) 4mm
b) 6mm
c) 8mm
d) 10mm

7) 2.5 latéral ; 1 rostral :
a) 3mm

8) 3.5 latéral ; 2 rostral :
a) 4mm

9) 3.5 latéral ; 1 rostral :
a) 2mm

10) 3.5 latéral ; 1 caudal :
a) 3mm

11) 4.5 latéral ; 1 rostral :
a) 4mm
b) 6mm
c) 8mm

12) 4.5 latéral ; 0 rostro-caudal :
a) 4mm
b) 6mm
c) 8mm

13) 4.5 latéral ; 1 caudal :
a) 4mm
b) 6mm

14) 5.5 latéral ; 1 rostral :
a) 4mm

15) 5.5 latéral ; 0 rostro-caudal :
a) 4mm
b) 6mm
c) 8mm

16) 5.5 latéral ; 1 caudal :
a) 4mm

17) 6 latéral ; 2 rostral :
a) 2mm
b) 4mm

18) 7 latéral ; 2 rostral :
a) 3mm

D

Into the lesioned site (PKH-26)	
1. 1.5 rostral; 3 lateral:	a. 6mm : 25µl
	b. 8mm: 25µl
2. 0.5 rostral; 5 lateral:	a. 6mm : 25µl
	b. 8mm: 25µl
Near the lesioned area (PKH-67)	
1. 2 caudal; 0 medio-lateral:	e. 6mm : 25µl
	f. 8mm: 25µl
2. 4 rostral; 4 lateral:	g. 6mm : 25µl
	h. 8mm: 25µl

E

Near the lesioned site (PKH-67)	
Tract 1	a. 3mm : 5µl
Tract 2	b. 3mm : 5µl c. 5mm : 5µl
Tract 3	d. 5mm : 5µl e. 3mm : 5µl
Tract 4	f. 5mm : 5µl g. 3mm : 5µl
Tract 5	h. 3mm : 7µl
Tract 6	i. 3mm : 7µl

Figure 6.1.7: (A) Post-first lesion and pre-second lesion ICMS map of MK-JA (scale is in mm). Coloured circles represent the sites at which movements were elicited at lower intensity threshold, which is represented by circles' size. Crosses represent sites of ibotenic acid injections (see C). Squares represent sites of first cells reimplantation (see D). Polygons represent sites of second cells reimplantation (see E). Table in panel B represents the ICMS responses elicited at each depth; the first letter indicates the activated territory, except numbers indicating the activated fingers; the numbers in parentheses give the ICMS threshold. Based on this second ICMS map and on the Table in panel B, sites of ibotenic acid injections (C) were chosen. Later, cells reimplantation was performed twice. First, two populations of cells were reimplanted into and near the lesioned site (D). Second, a population of cells was reimplanted near the lesioned site (E). In total, 600'000 cells and 150'000 cells were transplanted at the first and the second reimplantation time points, respectively, at a concentration of 3'000 cells/µl.

6.1.4 MONKEY MK-WI

	T	B+L1(r)	L2(r)	L3(r)	L4	C	L5	S
	Beginning of training	Biopsy 1st Lesion right hemisphere	2nd lesion right hemisphere	3rd lesion right hemisphere	4th lesion left hemisphere	Implantation of chamber	5th lesion left hemisphere	Sacrifice
MK-WI	13.06.2005	24.02.2006	09.03.2006	06.04.2006	26.04.2006	02.10.2006	23.03.2007	04.04.2008

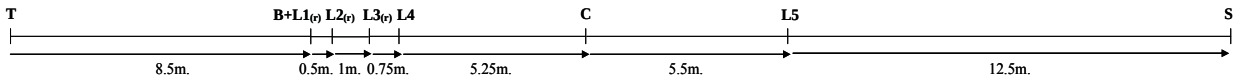
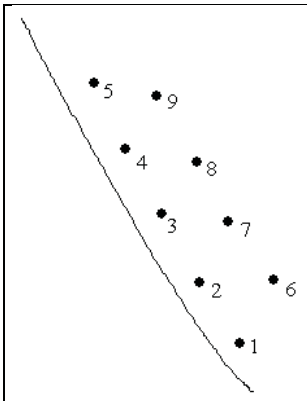


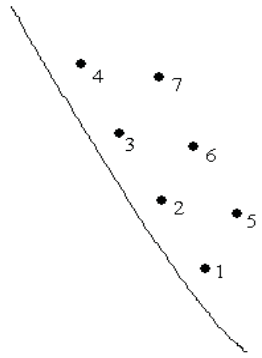
Figure 6.1.8 : Time course of the main events in the protocol for monkey MK-WI.

Figure 6.1.8 illustrates the time course of the main events concerning Mk-WI. This monkey reached a behavioural plateau six months and a half after the beginning of training sessions. Then the right dlPFC biopsy was performed; at the same time, a lesion of the hand representation's area in M1 was performed in the right hemisphere (fig. 6.1.9A). This lesion was performed stereotaxically, that is based on the knowledge of the macaque's M1 somatotopy (see "General Material and Methods" part); to note that after the opening of the skull and then of the dura on both sides, no sulcus centralis was clearly apparent. Two weeks later, as Mk-WI was treated with an antiepileptic drug (0.025mg Luminal; GABA agonist), he recovered unusually good and quickly. Several lesions (two in the right hemisphere, one in the left hemisphere; fig. 6.1.9B, C and D) were thus performed in order to try to obtain the expected effects following an M1 lesion. The interval between these lesions varied between a half and one month. After each lesion, Mk-WI showed symptoms, very short-lasting, and he recovered faster after each lesion. About five months after the fourth lesion, an electrophysiological chronic recording chamber was implanted above the sensorimotor area of the left hemisphere. Five months and a half later, a fifth lesion was performed in the left hemisphere (fig. 6.1.10). Inbetween, we suspected the Luminal to have a counteracting effect to the ibotenic acid excitotoxic effect; indeed, an anti-epileptic drug was reported to reduce the lesion size after stroke (Farber et al., 2002; Rogawski et al., 2004). Thus, Mk-WI did not receive any antiepileptic treatment around this last (fifth) M1 lesion. As he recovered again fast 100% spontaneously (perhaps due to a pre-conditioning effect induced by the previous lesions), no cell transplantation was performed. Twelve months and a half after this fifth lesion, Mk-WI was sacrificed. Overall, the experimental protocol on Mk-WI lasted about thirty-two months.

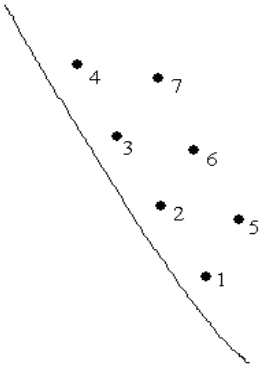
A

 Tract	Coordonnée	Profondeur	Qté µl
Tract 1		7.5mm 4.5mm 2mm	1 1 1
Tract 2	plus médial que tract 1	7.5mm 4.5mm 2mm	1 1 1
Tract 3	plus médial que tract 2	7.5mm 4.5mm 2mm	1 1 1
Tract 4	plus médial que tract 3	7.5mm 4.5mm 2mm	1 1 1
Tract 5	plus médial que tract 4	7.5mm 4.5mm 2mm	1 1 1
Tract 6	plus rostral; ML entre tracts 1 et 2	2mm	1
Tract 7	plus médial que tract 6	2mm	1
Tract 8	plus médial que tract 7	2mm	1
Tract 9	plus médial que tract 8	2mm	1
Total		19 sites	19

B

 Tract	Profondeur	Qté µl
Tract 1	8mm 3mm	2 2
Tract 2	8mm 3mm	2 3
Tract 3	8mm 3mm	2 2
Tract 4	8mm 3mm	2 2
Tract 5	3mm	2
Tract 6	3mm	2
Tract 7	3mm	3
Total	11 sites	24

C

 Tract	Profondeur	Qté µl
Tract 1	8mm 3mm	2 2
Tract 2	8mm 3mm	2 2
Tract 3	8mm 3mm	2 2
Tract 4	8mm 3mm	2 2
Tract 5	3mm	2
Tract 6	3mm	2
Tract 7	3mm	2
Total	11 sites	22

D

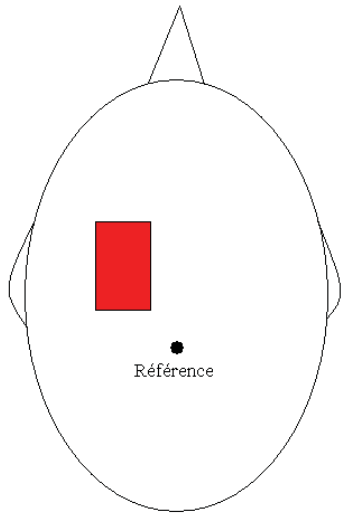
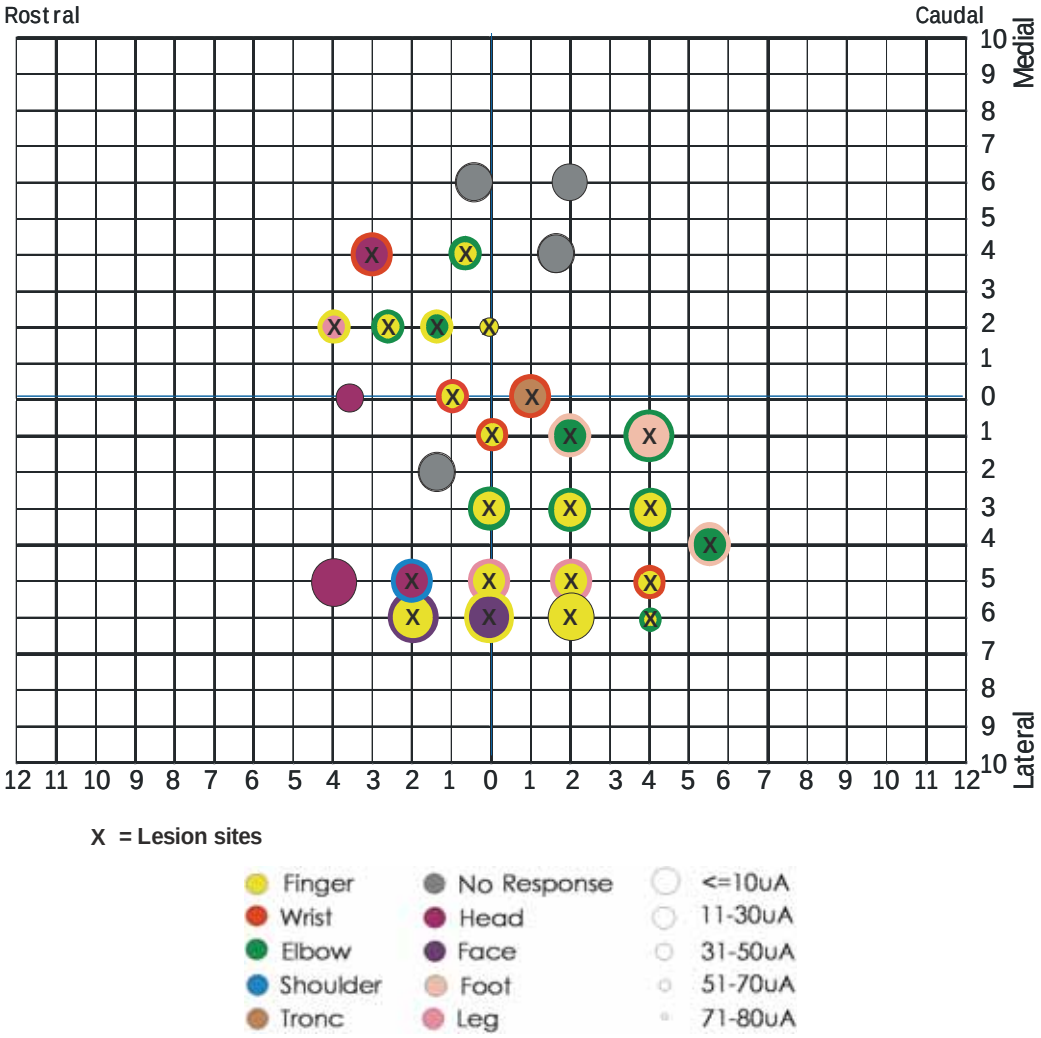
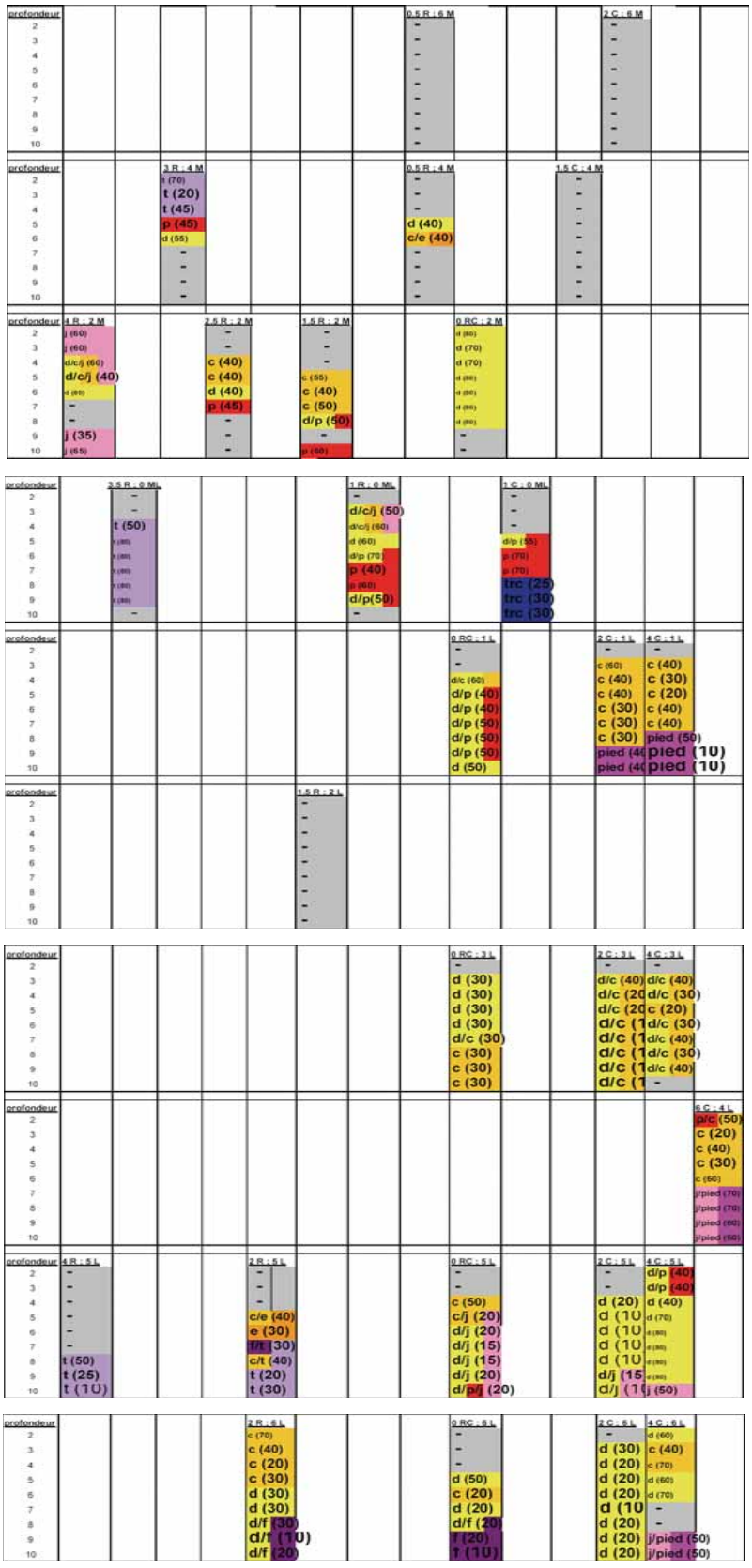
 Tract	Coordonnée	Profondeur	Qté µl
Référence	**ML 39.5 ; RC 29**		
Tract 1	ML 48.7 ; RC 37.7	2.4mm 4.4mm 6.4mm	1.5 1.5 1.5
Tract 2	ML 48.7 ; RC 39.7	2.5mm 4.5mm	1.5 1.5
Tract 3	ML 48.7 ; RC 41.7	2.5mm 4.5mm	1.5 1.5
Référence	**ML 39.5 ; RC 29.7**		
Tract 4	ML 46.5 ; RC 40.4	2mm 4mm 6mm	1.5 1.5 1.5
Tract 5	ML 46.5 ; RC 42.4	2mm 4mm 6mm	1.5 1.5 1.5
Total		13 sites	19.5

Figure 6.1.9: (A) First lesion in MK-WI, performed in the right hemisphere. Ibotenic acid injection: 1µl/tract; 10mg/ml in PBS, 0.1M, 7.2pH. (B) Second lesion in MK-WI, performed in the right hemisphere. Ibotenic acid injection: 2-3µl/tract; 10mg/ml in PBS, 0.1M, 7.2pH. (C) Third lesion in MK-WI, performed in the right hemisphere. Ibotenic acid injection: 2µl/tract; 10mg/ml in PBS, 0.1M, 7.2pH. (D) Fourth lesion in MK-WI, performed in the left hemisphere. Ibotenic acid injection: 1.5µl/tract; 10mg/ml in PBS, 0.1M, 7.2pH.

A


Rostral
Caudal
Medial
Lateral
12 11 10 9 8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10 11 12
10 9 8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10
X = Lesion sites
Finger
Wrist
Elbow
Shoulder
Tronc
No Response
Head
Face
Foot
Leg
<=10uA
11-30uA
31-50uA
51-70uA
71-80uA

B


profondeur
0.5 R : 6 M
2 C : 6 M
3 R : 4 M
0.5 R : 4 M
1.5 C : 4 M
d (40)
c/e (40)
4 R : 2 M
2.5 R : 2 M
1.5 R : 2 M
0 RC : 2 M
c (40)
c (40)
d (40)
p (45)
c (40)
c (50)
d/p (50)
j (35)
3.5 R : 0 ML
1 R : 0 ML
1 C : 0 ML
t (50)
d/c/j (50)
p (40)
d/p(50)
trc (25)
trc (30)
trc (30)
0 RC : 1 L
2 C : 1 L
4 C : 1 L
d/c (60)
d/p (40)
d/p (40)
d/p (50)
d/p (50)
d/p (50)
d (50)
c (40)
c (30)
c (20)
c (40)
c (30)
c (30)
pied (50)
pied (10)
pied (10)
1.5 R : 2 L
0 RC : 3 L
2 C : 3 L
4 C : 3 L
d (30)
d (30)
d (30)
d (30)
d/c (30)
c (30)
c (30)
c (30)
d/c (40)
d/c (40)
d/c (30)
d/c (40)
d/c (30)
d/c (40)
6 C : 4 L
p/c (50)
c (20)
c (40)
c (30)
4 R : 5 L
2 R : 5 L
0 RC : 5 L
2 C : 5 L
4 C : 5 L
c/e (40)
e (30)
f/t (30)
c/t (40)
t (20)
t (30)
c (50)
c/j (20)
d/j (20)
d/j (15)
d/j (15)
d/j (20)
d/p/j (20)
d/p (40)
d/p (40)
d (40)
d (20)
d (10
d/j (15
d/j (50)
t (50)
t (25)
t (10)
2 R : 6 L
0 RC : 6 L
2 C : 6 L
4 C : 6 L
c (40)
c (20)
c (30)
d (30)
d (30)
d/f (30)
d/f (10)
d/f (20)
d (50)
c (20)
d (20)
d/f (20)
f (20)
t (10)
d (30)
d (20)
d (20)
d (20)
d (10
d (20)
d (20)
c (40)
d (20)
j/pied (50)
j/pied (50)

C

1) 4 médial ; 3 rostral :
a) 6mm

2) 4 médial ; 0.5 rostral :
a) 5mm

3) 2 médial ; 4 rostral :
a) 5mm

4) 2 médial ; 2.5 rostral :
a) 5mm
b) 7mm

5) 2 médial ; 1.5 rostral :
a) 5mm
b) 7mm
c) 9mm

6) 2 médial ; 0 rostro-caudal :
a) 2mm
b) 4mm
c) 6mm

7) 0 médio-latéral ; 1 rostral :
a) 3mm
b) 5mm
c) 7mm

8) 0 médio-latéral ; 1 caudal :
a) 5mm

9) 1 latéral ; 0 rostro-caudal :
a) 5mm
b) 7mm
c) 9mm

10) 1 latéral ; 2 caudal :
a) 3mm
b) 5mm
c) 7mm

11) 1 latéral ; 4 caudal :
a) 3mm
b) 5mm
c) 7mm

12) 3 latéral ; 0 rostro-caudal :
a) 4mm
b) 6mm
c) 8mm
d) 10mm

13) 3 latéral ; 2 caudal :
a) 4mm
b) 6mm
c) 8mm
d) 10mm

14) 3 latéral ; 4 caudal :
a) 4mm
b) 6mm
c) 8mm

15) 4 latéral ; 6 caudal :
a) 3mm
b) 5mm

16) 5 latéral ; 2 rostral :
a) 6mm

17) 5 latéral ; 0 rostro-caudal :
a) 5mm
b) 7mm
c) 9mm

18) 5 latéral ; 2 caudal :
a) 5mm
b) 7mm
c) 9mm

19) 5 latéral ; 4 caudal :
a) 3mm
b) 5mm
c) 7mm
d) 9mm

20) 6 latéral ; 2 rostral :
a) 3mm
b) 5mm
c) 7mm
d) 9mm

21) 6 latéral ; 0 rostro-caudal :
a) 6mm
b) 8mm

22) 6 latéral ; 2 caudal :
a) 4mm
b) 6mm
c) 8mm
d) 10mm

23) 6 latéral ; 4 caudal :
a) 3mm
b) 5mm

Figure 6.1.10: (A) Pre-fifth lesion ICMS map of MK-WI's left hemisphere (scale is in mm). Coloured circles represent the sites at which movements were elicited at lower intensity threshold, which is represented by circles' size. Crosses represent sites of ibotenic acid injections (see C). Table in panel B represents the pre-lesional ICMS responses elicited at each depth; the first letter indicates activated territory, except numbers indicating the activated fingers; the numbers in parentheses give the ICMS threshold. Based on this ICMS map and on the Table in panel B, sites of ibotenic acid injections (C) were chosen.

6.1.5 REFERENCES

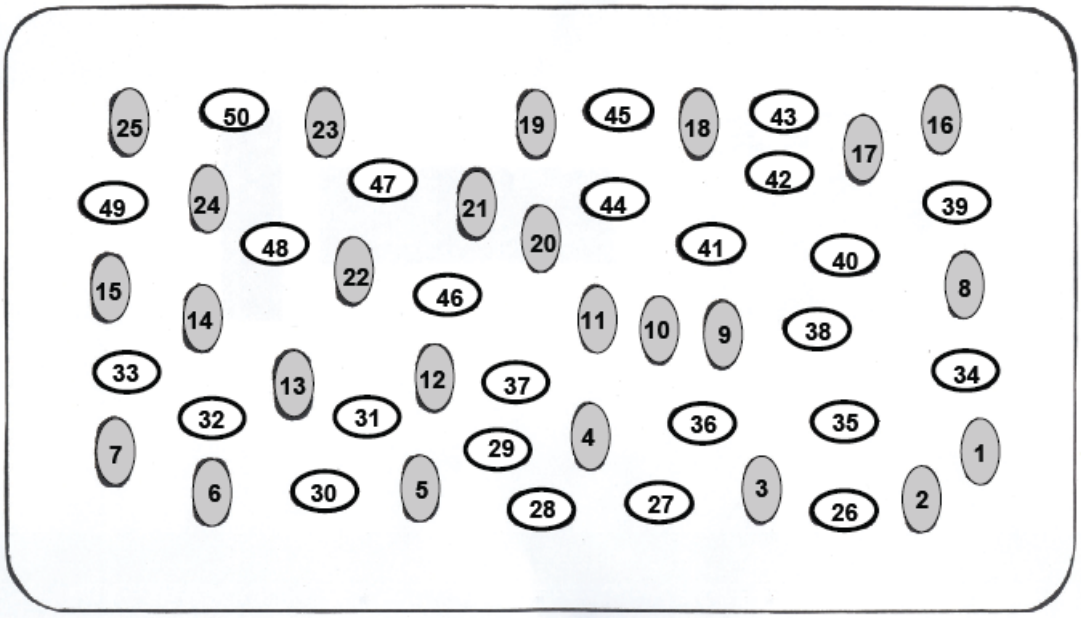
Farber, N. B., Jiang, X. P., Heinkel, C., and Nemmers, B. (2002). Antiepileptic drugs and agents that inhibit voltage-gated sodium channels prevent NMDA antagonist neurotoxicity. *Mol.Psychiatry* 7, 726-733.

Rogawski, M. A. and Loscher, W. (2004). The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. *Nat.Med.* 10, 685-692.

6.2 ANALYSES PROTOCOLS

6.2.1 MODIFIED BRINKMAN BOARD TASK

Nom: Main: Date:

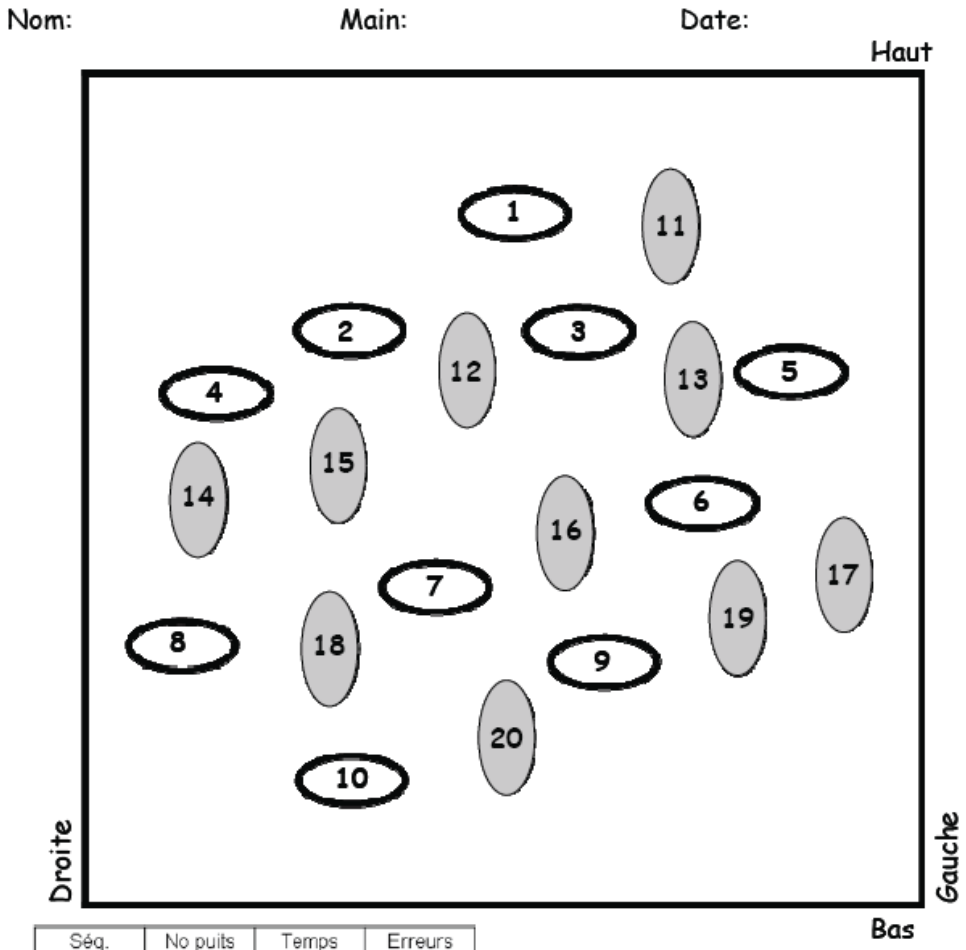


No	Dr.	Séq.	Entrée	Sortie	I/E	P/I	2	Err.	No	Dr.	Séq.	Entrée	Sortie	I/E	P/I	2	Err.
1	V				-	-			26	H							
2	V				-	-			27	H							
3	V				-	-			28	H							
4	V				-	-			29	H							
5	V				-	-			30	H							
6	V				-	-			31	H							
7	V				-	-			32	H							
8	V				-	-			33	H							
9	V				-	-			34	H							
10	V				-	-			35	H							
11	V				-	-			36	H							
12	V				-	-			37	H							
13	V				-	-			38	H							
14	V				-	-			39	H							
15	V				-	-			40	H							
16	V				-	-			41	H							
17	V				-	-			42	H							
18	V				-	-			43	H							
19	V				-	-			44	H							
20	V				-	-			45	H							
21	V				-	-			46	H							
22	V				-	-			47	H							
23	V				-	-			48	H							
24	V				-	-			49	H							
25	V				-	-			50	H							

Remarques:

Figure 6.2.1 : Protocol sheet for the analysis of the modified Brinkman board task.

6.2.2 HIDDEN BRINKMAN BOARD TASK



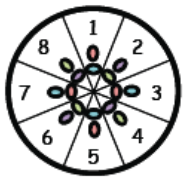
Séq.	No puits	Temps	Erreurs
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Remarques:

Figure 6.2.2 : Protocol sheet for the analysis of the hidden Brinkman board task.

6.2.3 ROTATING BRINKMAN BOARD TASK

Nom: Date: Main: Sens:



Orientations:

H1
H5
V3
V7

H3
H7
V1
V5

H2
H6
V4
V8

H4
H8
V2
V6

Séq.	V/H	No cercle	No entrée	Or. entrée	Tps entrée	No sortie	Or. sortie	Tps sortie	E/I	P/I	2 à la fois	Erreur	Remarques
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
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Figure 6.2.3 : Protocol sheet for the analysis of the rotating Brinkman board task.

CURRICULUM VITAE

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Prénom	**Mélanie**
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Date de naissance	04 décembre 1979
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ETUDES

Faculté des Sciences, Fribourg :	Doctorat en neurosciences	2004-2010
Faculté de Psychologie, Genève :	Certificat complémentaire plurifacultaire en neurosciences cognitives	2003-2004
Faculté des Lettres, Fribourg :	Licence en philosophie branche secondaire	2003-2004
Faculté de Psychologie, Genève :	Licence en psychologie	2001-2003
Faculté des Lettres, Fribourg :	Demi-licence en psychologie Demi-licence en philosophie branche secondaire	1999-2001
Collège Ste-Croix, Fribourg :	Maturité fédérale et Baccalauréat (section langues modernes)	1995-1999
Ecole primaire et Cycle d'orientation, Marly :	Certificat d'études secondaires	1985-1995

DOMAINES D'EXPÉRIENCES PROFESSIONNELLES

Enseignement universitaire : Charge de cours (étudiants de psychologie : Neuropsychologie cognitive et clinique)
Travaux pratiques (étudiants de médecine et de sciences biomédicales)
Supervision de travaux de bachelor (étudiants de biologie)

Enseignement privé : (cours d'appui)	Français	niveaux secondaire et maturité fédérale
	Allemand	niveau secondaire
	Anglais	niveaux secondaire et maturité fédérale
	Mathématiques	niveau secondaire
	Espagnol	niveau maturité fédérale
	Piano	

Edition, Animation musicale, Vente, Restauration, Sécurité

LANGUES

Français : langue maternelle
Allemand : niveau maturité fédérale
Anglais : niveau maturité fédérale
Espagnol : niveau maturité fédérale

INFORMATIQUE

Word, Excel, Powerpoint, GraphPad, SigmaPlot/Sigma Stat, SPSS, Corel Draw, Adobe Photoshop, Microsoft Photoshop.

CENTRES D'INTERET

Piano : conservatoire de Fribourg	1988-1998
Chant : conservatoire de Fribourg	2000-2004

Cuisine, musique, théâtre, voyages

FORMATION CONTINUE

2003-2010

Formations :

FARP (Formation des Associations Romandes et tessinoise des Psychologues) :
Neuropsychologie clinique, Auto-hypnose, La fratrie, Formation à la thérapie familiale et à l'intervention systémique, Développement des contenants de pensée (DDCP) de B.Douet.

IECF (Institut d'Etudes du Couple et de la Famille, GE) :
La loyauté et la réécriture des scénarios dans les familles, dans les institutions et dans les relations entre les familles et les intervenants, Transformer les liens du couple et de la famille, Ethique du changement, Approche systémique et troubles psychosomatiques.

Institut de la famille (GE) :
Du corps à la parole, Le jeu de l'Oie (Loi) systémique, Violences sur soi : suicides, auto-mutilations et autres atteintes à son propre corps.

CERFASY (Centre de Recherches Familiales et Systémiques, NE):
Les thérapeutes et l'opéra.

SPsyAJ (Séminaire Psychanalytique de l'Arc Jurassien):
Concepts fondamentaux de la psychanalyse.

Cours, Workshops :

Université de Fribourg :
Psychopharmacothérapie, Toxicodépendances, Prévention des troubles psychiques, Neurobiology Seminars.

Université de Genève :
Analyse multivariée, Techniques projectives.

Université de Lausanne :
Entretien systémique, Intervention systémique.

CERFASY (Centre de Recherches Familiales et Systémiques, NE):
Le Corps en Jeu.

Cours Romand de Formation à l'Expérimentation Animale, Module I, CHUV.

CHUV Research Days :
« Immunity, Inflammation and Infection », janvier 2005 ; « Neurosciences et Psyché », février 2006 ; « Biomedical Imaging », février 2007; « Regenerative Medicine », janvier 2008; « Genes and Disease », janvier 2009.

SSN (Swiss Society of Neuroscience) :
Annual meeting: Lausanne, septembre 2004; Zürich, septembre 2005; Bâle, janvier 2006; Berne, mars 2007; Fribourg, mars 2009; Lausanne, mars 2010.

BENEFRI Neurosciences (Universités de Berne, Neuchâtel et Fribourg) :
« Brain Mapping : From in vitro multisite recording to functional imaging », Berne, mars 2005.
« Brain, Behavior and Plasticity », Fribourg, février 2006.
« Neurotransmitters and Emotion », Berne, mars 2007.
« Animal Models in Brain Research », Fribourg, janvier 2008.
« Functions of Cerebral Cortex », Berne, février 2009.
« Animal Models for Human Nervous Diseases », Fribourg, janvier 2010.

SSCN (Swiss Stem Cells Network):
Annual Meeting : Zürich, décembre 2007; Genève, février 2009; Bâle, février 2010.

NCCR (National Center of Competence in Research) Neural Plasticity and Repair Symposium:
Ittingen, mars 2005; Ittingen, mars 2006; Berlingen, janvier 2009; Berlingen, janvier 2010.

Congrès :

REDIF, IFF et ASTHEFIS (Réseau Européen des Instituts de la Famille, Institute for family research and counseling, Association Suisse des Thérapies de Familles et Interventions Systémiques):
« Divorce : médiation, audition, parentalité », Fribourg, septembre 2003.

SGHR/SSRM, DAHTH, GEMMSOR (Schweizerische Gesellschaft für Handrehabilitation/Société Suisse de Rééducation de la Main, Deutsche Arbeitsgemeindschaft für Handtherapie, Groupe d'Etude de la Main et du Membre Supérieur en Orthèses et en Rééducation):
« 2ème Congrès de Rééducation de la Main des trois pays Suisse, Allemagne, France », Bâle, novembre 2004.

FENS (Federation of European Neurosciences):
« 5th Forum of European Neuroscience », Vienne, juillet 2006.
« 6th Forum of European Neuroscience », Genève, juillet 2008.
« 7th Forum of European Neuroscience », Amsterdam, juillet 2010.

IFCN (International Federation of Clinical Neurophysiology):
« 28th International Congress of Clinical Neurophysiology », Edinburgh, septembre 2006.

ESSFN (European Society for Stereotactic and Functional Neurosurgery) :
« 17th Congress of the European Society for Stereotactic and Functional Neurosurgery », Montreux, octobre 2006.

PUBLICATIONS ET PRESENTATIONS

Publications :

Kaeser M., Brunet J.F., Wyss A.F., Belhaj-Saif A., Liu Y., Hamadjida A., Rouiller E.M., Bloch J. Autologous adult cortical cell transplantation enhances functional recovery following unilateral lesion of motor cortex in primates: a pilot study. *Neurosurgery* (under minor revision).

Bloch J., **Kaeser M.**, Sadeghi Y., Rouiller E.M., Redmond D.E., Brunet J.F. Doublecortin-positive cells in the adult primate cerebral cortex and possible role in brain plasticity and development. *J Comparative Neurology* (accepted for publication).

Kaeser M., Wyss A.F., Bashir S., Hamadjida A., Liu Y., Bloch J., Brunet J.F., Belhaj-Saif A., Rouiller E.M. 2010. Effects of unilateral motor cortex lesion on ipsilesional hand's reach and grasp performances in monkeys: relationship with recovery in the contralesional hand. *J. Neurophysiology* 103: 1630-1645.

Présentations de poster :

Kaeser M., Brunet J. F., Wyss A. F., Hamadjida A., Bashir S., Belhaj-Saif A., Liu Y., Bloch J., Mir A., Schwab M. E. and Rouiller E. M. 2010. Strategies to enhance hand's functional recovery after unilateral lesion of motor cortex in non-human primates. Forum of European Neuroscience (FENS) Meeting, Abstract book, Amsterdam 2010.

Badoud S., Chatagny P., Wannier T., Schmidlin E., Belhaj-Saif A., Hamadjida A., Gindrat A. D., **Kaeser M.** and Rouiller E. M. 2010. Comparison of hand dominance between non-human primates (macaque monkeys) and human subjects in the context of manual dexterity. Forum of European Neuroscience (FENS) Meeting, Abstract book, Amsterdam 2010.

Kaeser M., Brunet J.F., Wyss A.F., Hamadjida A., Bashir S., Belhaj-Saif A., Bloch J., Mir A., Schwab M.E. and Rouiller E.M. 2010. Effect of autologous adult brain progenitor cell transplantation and of anti-Nogo-A antibody treatment on functional recovery of hand dexterity after unilateral lesion of motor cortex in macaque monkeys. Swiss Society of Neuroscience (SSN) Meeting, Abstract book, Lausanne 2010.

Kaeser M., Brunet J.F., Liu, Y., Bloch J. and Rouiller E.M. Effect of autologous adult neural progenitor cells transplantation on functional recovery of manual dexterity after lesion of motor cortex in macaque monkeys. Swiss Society of Neuroscience (SSN) Meeting, Abstract book, Fribourg 2009.

Rouiller E.M., **Kaeser M.**, Wyss A.F., Hamadjida A., Bashir S., Liu Y., Bloch J., Brunet J.F. and Belhaj-Saïf A. 2009. Role of the primary motor cortex (M1) in the control of fine manual dexterity of the homolateral hand as assessed in a model of unilateral lesion of M1 in macaque monkeys. Swiss Society of Neuroscience (SSN) Meeting, Abstract book, Fribourg 2009.

Kaeser M., Brunet J.F., Liu Y., Bloch J. and Rouiller E.M. 2009. Effect of autologous adult neural progenitor cells transplantation on functional recovery of manual dexterity after lesion of motor cortex in macaque monkeys. Swiss Stem Cells Network (SSCN), Abstract book, Geneva 2009.

Kaeser M., Brunet J.F., Wyss A.F., Hamadjida A., Bashir S., Belhaj-Saif A., Bloch J., Mir A., Schwab M.E. and Rouiller E.M. 2010. Effects of anti-Nogo-A antibody treatment and of autologous adult brain progenitor cell transplantation on manual dexterity recovery after unilateral lesion of motor cortex in macaque monkeys. International Neurorehabilitation Symposium, Zurich 2009.

Brunet J.-F., **Kaeser M.**, Rouiller E.M., Redmond D., Levivier M. and Bloch J. The primate adult brain cells in vitro originate from doublecortin positive cells in adult primate cortex. Journée de recherche du CHUV, Abstract book, Lausanne, 2009.

Brunet J.F., **Kaeser M.**, Rouiller E.M., Redmond D.E.Jr., Levivier M. and Bloch J. 2008. The primate adult brain cells in vitro originate from doublecortin positive cells in adult primate cortex. Society for Neuroscience, Abstract book, Washington (USA) 2008.

Kaeser M., Wannier T., Bloch J., Brunet J.F., Wyss A.F. and Rouiller E.M. 2008. Changes of motor strategies following a right dorsolateral prefrontal cortical biopsy in macaque monkeys. Forum of European Neuroscience (FENS) Meeting, Abstract book, Geneva 2008.

Bashir S., **Kaeser M.**, Wyss A.F., Hamadjida A., Liu Y., Belhaj-Saif A. and Rouiller E.M. 2008. Effects of Unilateral Lesion of the Primary Motor Cortex on Ipsilesional Hand Dexterity in Adult Monkeys. Forum of European Neuroscience (FENS) Meeting, Abstract book, Geneva 2008.

Bashir S., Beaud M.L., Freund P., **Kaeser M.**, Wyss A., Belhaj-Saif A., Bloch J., Debatisse D., Mir A., Pralong E., Rouiller E.M., Schwab M.E. and Wannier T. 2007. Characterization of early somatosensory evoked potentials (SEPs) to electrical stimulation of the median nerve in adult macaque monkeys: intact and spinal lesioned animals. Joint Meeting Swiss Society for Neuroscience (SSN), NCCR Neuro and Swiss Society of Multiple Sclerosis, Abstract book, Bern, 2007.

Kaeser M., Wannier T., Wyss A.F., Belhaj-Saif A., Bloch J., Brunet J.-F., Debatisse D., Pralong E. and Rouiller E.M. 2007. Cortical plasticity assessed by somatosensory evoked potentials (SEPs) parallels functional recovery of manual dexterity after lesion of primary motor cortex (M1) in monkeys. Joint Meeting Swiss Society for Neuroscience (SSN), NCCR Neuro and Swiss Society of Multiple Sclerosis, Abstract book, Bern, 2007.

Bashir S., **Kaeser M.**, Beaud M.-L., Freund P., Wyss A., Belhaj-Saif A., Bloch J., Debatisse D., Pralong E., Lavenex P., Rouiller E.M. and Wannier T. 2007. Effects of a combination of ketamine and medetomidine hydrochloride (KM) anaesthesia on somatosensory evoked potentials (SEPs) obtained by electrical stimulation of the median nerve in macaque monkeys. Society for Neuroscience Meeting (SfN), Abstract book, San Diego, USA, 2007.

Kaeser M., Brunet J.F., Rouiller E., Wannier T. and Bloch J. Primate adult brain cell autotransplantation for brain repair. Journée de recherche du CHUV, Abstract book, Lausanne, 2007.

Bloch J., **Kaeser M.**, Rouiller E., Wannier T. and Brunet J. Primate adult cortical cell autotransplantation, where do these cells come from? Journée de recherche du CHUV, Abstract book, Lausanne, 2007.

Kaeser M., Wannier T., Bloch J., Debatisse D., Pralong E., Brunet J.F. and Rouiller E.M. 2006. Cortical plasticity parallels functional recovery of manual dexterity after lesion of motor cortex in monkeys: long-term neuro-monitoring by using ECoG. International Congress of Clinical Neurophysiology (ICCN) meeting, Abstract book, Edinburgh, 2006.

Brunet J.F., **Kaeser M.**, Rouiller E.M., Wannier T., Villemure J.G. and Bloch J. 2006. Primate adult brain cell autotransplantation for brain repair. Joint Meeting Swiss Society for Neuroscience (SSN) and Swiss Society for NeuroRadiology, Abstract book, Basel, 2006.

Kaeser M., Brunet J.F., Wannier T., Villemure J.G., Bloch J and Rouiller E.M. 2006. Primate adult brain cell autotransplantation : a new tool for brain repair ? BENEFRI workshop, Fribourg, 2006.

Kaeser M., Brunet J.F., Rouiller E.M., Wannier T., Villemure J.G. and Bloch J. 2006. Primate adult brain cell autotranspantation for brain repair. Forum of European Neuroscience (FENS) Meeting, Abstract book, Vienna 2006.

Brunet J.F., **Kaeser M.**, Rouiller E.M., Wannier T., Villemure J.G. and Bloch J. 2005. Primate adult brain cell autotransplantation : a new tool for brain repair ? Journée de recherche du CHUV, Abstract book, Lausanne, 2006.

Kaeser M., Brunet J.F., Wannier T., Villemure J.G., Bloch J and Rouiller E.M. 2005. Primate adult brain cell autotransplantation : a new tool for brain repair ? Swiss Stem Cells Network (SSCN), Geneva, 2005.

Brunet J.F., **Kaeser M.**, Rouiller E.M., Wannier T., Villemure J.G. and Bloch J. 2005. Primate adult brain cell autotransplantation for brain repair, Society for Neuroscience Meeting (SfN), Abstract book, Washington DC, USA, 2005.

Présentations orales :

Kaeser M., Brunet J.F., Wyss A.F., Belhaj-Saif A., Liu Y., Hamadjida A., Rouiller E.M. and Bloch J. Autologous adult cortical cell transplantation enhances functional recovery following unilateral lesion of motor cortex in primates. CHUV, Lausanne 2009.

Kaeser M., Brunet J. F., Wannier T., Bloch J. and Rouiller E.M. Primate adult brain cell autotransplantation for brain repair. BENEFRI workshop, Bern, 2007.

Kaeser M., Brunet J.F., Rouiller E.M., Wannier T., Villemure J.G. and Bloch J. 2006. Autotransplantation de cellules cérébrales adultes chez le macaque. CHUV, Lausanne 2004.