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# Multisensory integration in non-human primates and humans

## Thesis

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By

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Fribourg,

| Thesis supervisor : | Dean : |
|---|---|
| Prof. Eric Rouiller | Prof. Fritz Müller |

**“Qu’importe la surdité de l’oreille, quand l’esprit entend? La seule surdité, la vraie surdité, la surdité incurable, c’est celle de l’intelligence”**

**Victor Hugo à Ferdinand Berthier**

**“Ce qui est essentiel à la perception sensorielle n’est pas ce qui sépare les sens les uns des autres, mais ce qui les unit entre eux; à chacune de nos expériences internes (même non sensorielles) et à notre environnement.”**

**Erich von Hornbostel,**
***The Unity of the Senses*, 1927.**

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# Contents

# ***Foreword***

The multisensory integration concept does not date from the 80s or the 90s of the past century, but it takes its origin in much older times. Indeed, this concept was developed by Aristote (384-322 BC), Greek philosopher. His question was: "How the human acquires the events perceptual experience of the outside world?" In modern terms, we could ask how we can perceive our outside environment by integrating various stimuli resulting from different modalities.

It seems that this perception cannot occur without our 5 senses and without a coordinator, the brain, able to integrate this information and process it in order to generate an adequate action (behavioural response). To achieve such a complex integration task, various regions of the brain are devoted to each of our sensory modalities. Generally, every modality is optimized for what it is defined. Nevertheless, and according to Aristotle's rules, the brain would it not gain to create a synergy between these modalities, with the aim of improving our perception? This question turns into an affirmation, because when an event, an action implies several modalities at the same time, and in the corresponding space, the brain is able to integrate this various information in a unified perception. This phenomenon, commonly named "***multisensory integration***", underlies certain interactions during the integrative processes. These effects can be expressed as profits (e.g. decrease of the reaction time for the task execution, better performance) or as deficits (illusions: ventriloquism, McGurk effect).

To understand these multisensory integration phenomena, it is necessary, from a neurological point of view, to determine where and how these various attributes are processed. Since around thirty years, numerous studies conducted on animals allowed to better address this issue and to establish general principles of multi-modal integration. These studies also allowed to demonstrate that the multisensory integration does not occur only in associative areas, but earlier already in unisensory areas (e.g. auditory areas, visual area, etc). Several studies showed the existence of intra-cortical connectivity between the auditory and visual area, but few investigations so far were focused on a possible role played by the premotor cortex, a sensorimotor area of convergence (auditory, visual information and somatosensory), leading to the preparation and the organization of a motor (behavioural) response. Indeed, the premotor area is very important in the initiation of voluntary movements, especially when activated by external stimuli. To assure a fast behavioural response, particularly a reaction time decreased when triggered by a multimodal stimulation, the presence of connections between the premotor cortex and subcortical areas, such as the superior colliculus and the thalamus seems plausible. The goal of the present study was first to validate a bimodal behavioral detection task in primates (both monkeys and human subjects) and collect behavioral and electrophysiological data in non-human primates (n=2) and in humans (n=14). We evaluated various multisensory criteria bound to the multisensory behavior (reaction time, performance, errors) and, in a second step, derived electrophysiological data from the premotor cortex in the non-human primates and from the whole brain in human subjects, all when performing the same behavioral detection task.

The behavioural results obtained in this study demonstrated a reaction time decrease in multimodal condition (visuo-acoustic), as compared with a unisensory condition (visual or acoustic alone). In animals (monkeys), this bimodal gain, or facilitatory effect, followed the principle of inverse effectiveness: the higher the intensity of the stimuli, the smaller is the facilitatory effect. This principle of inverse effectiveness was not verified in the human subjects in the present experimental conditions. Concerning the electrophysiological results, the single unit recordings conducted on monkeys in the premotor cortex showed this presence of neurons responding to acoustic or to visual stimuli, however with a change of response when these two stimuli were presented simultaneously. This observation suggests the existence of multisensory integration process in the premotor cortex at neuronal level. The neuronal activities observed in relation to the detection behavioral paradigm in the premotor cortex allowed us to define two classes of neurons, namely "Response pattern” and “Modulation pattern”. In humans, the results derived from EEGs revealed the presence of early neural activities possibly linked to multisensory integration and taking place from 60 msec post-stimulation.

This work has allowed us to better understand some of the neuronal processes involved in multi-modal integration, in particular at the level of the premotor cortex in the non-human primates, as a contributor to a larger network possibly involving also subcortical areas like the thalamus. Furthermore, the studies conducted in parallel in human subjects, using the same protocol as in the non-human primates, will allow future transfer of knowledge towards clinical questions.

# ***Avant-Propos et résumé***

Le concept d'intégration multisensorielle ne date pas des années 80 ou 90 mais remonte à des temps bien plus anciens. En effet, ce concept a été développé par le philosophe grec Aristote (384-322 av. J.-C.) qui se demandait «Comment l'homme acquiert l'expérience perceptuelle des évènements du monde extérieur?». En des termes plus contemporains on pourrait se demander comment on peut percevoir notre environnement extérieur en intégrant différents stimuli provenant de modalités différentes.

Il apparaît que cette perception extérieure ne peut pas avoir lieu sans nos sens et sans un chef d'orchestre, le cerveau, capable d'intégrer ces informations et de les traiter pour générer une action adéquate (réponse comportementale). Pour ce faire, différentes régions du cerveau sont dévolues à chacune de nos modalités sensorielles. D'une manière générale, chaque modalité est optimisée pour ce pour quoi elle est définie. Néanmoins, et selon les préceptes d'Aristote, le cerveau ne gagnerait-il pas à créer une synergie entre ces modalités dans le but d'améliorer notre perception? Cette question est une affirmation, car lorsqu'un événement, une action implique plusieurs modalités en même temps, et dans un même espace, le cerveau est capable d'intégrer ces différentes informations de manière unifiée. Ce phénomène, communément nommé «***intégration multisensorielle***», induit certaines interactions lors des processus intégratifs. Ces effets peuvent se traduire en tant que bénéfices (par exemple, diminution du temps de réaction pour l'exécution d'une tâche, augmentation de la performance) ou en tant que déficits (illusions: ventriloquisme, effet McGurk).

Pour comprendre ces phénomènes d'intégration multisensorielle, il s'avère nécessaire, d'un point de vue neurologique, de déterminer où et comment ces différentes informations sont traitées. Depuis une trentaine d'années, de nombreuses études menées chez les animaux ont permis de mieux appréhender ce point et ainsi d'établir les principes fondamentaux de l'intégration multi-modale. Ces études ont également permis de démontrer que l'intégration multisensorielle n'a pas lieu que dans des aires associatives mais également plus en amont au niveau d'aires unisensorielles (e.g. aire auditive, aire visuelle,….). Plusieurs études ont mis en avant cette intra-connectivité entre l'aire auditive et l'aire visuelle, mais peu se sont intéressées au cortex prémoteur, lieu de convergence sensoriel (informations auditives, visuelles et somatosensorielles) en vue de la préparation et de l'organisation d'une réponse motrice. Cette aire motrice est en effet très importante dans l'initiation du mouvement, déclenché à partir de stimuli externes. Pour assurer une réponse comportementale rapide, plus particulièrement un temps de réaction raccourci suite à la présentation d'une stimulation multimodale, l'existence de connections entre le cortex prémoteur et des régions sous corticales telles que le colliculus supérieur et le thalamus paraît vraisemblable. C'est cette analyse qui a été entreprise dans le présent travail en effectuant différents tests, dont des enregistrements comportementaux et électrophysiologiques chez le primate non-humain (n=2) et des enregistrements comportementaux et de potentiels évoqués chez l'humain (n=14). Nous avons étudié différents critères multisensoriels liés au comportement (temps de réaction, performance, erreurs) et aux données électrophysiologiques collectées lors de l'intégration audio-visuelle au niveau du cortex prémoteur chez le primate non-humain et de l'ensemble du cerveau chez l'humain.

Les résultats comportementaux obtenus dans ce travail ont démontré une diminution du temps de réaction en stimulation multimodale en comparaison aux conditions unisensorielles. Chez les animaux ce gain bimodal, ou effet facilitateur communément appelé principe d'efficacité inverse, établit que plus l'intensité de stimulation augmente moins l'effet facilitateur est présent. Ce principe n'a pas été retrouvé chez l'être humain dans les conditions expérimentales testées dans le présent protocole. En ce qui concerne les résultats électrophysiologiques, les enregistrements effectués chez les animaux ont démontré que le cortex prémoteur comprend bien des neurones qui sont influencés par des stimuli aussi bien auditifs que visuels et que, surtout, ces réponses sont différentes lorsque que ces mêmes stimuli sont présentés de manière simultanée. Cette observation suggère la présence d'une intéraction multisensorielle au niveau neuronal dans le cortex prémoteur. Les enregistrements neuronaux dans le cortex prémoteur ont montré la présence de deux grandes classes de neurones influencés par la tâche de détection comportementale, que l'on a désignées par patrons de «réponses» et patrons de «modulations». Chez l'humain, pour la même tâche comportementale, les résultats obtenus par EEG révèlent des activités neuronales précoces à partir de 60 msec post-stimulation, en relation avec une possible interaction bimodale.

Ce travail nous a permis de mieux comprendre les processus neuronaux impliqués dans l'intégration multi-modale au niveau du cortex prémoteur et s'intègre dans une étude globale visant à mettre en relation ces processus propres à cette région cortical et ceux régissant l'intégration multisensorielle dans d'autres régions du cerveau faisant partie d'un même réseau, par exemple au niveau du thalamus. Ceci dans un but d'approfondir d'un point de vue électrophysiologique les découvertes faites préalablement en anatomie. De plus, les études menées en parallèle chez l'homme avec le même protocole que celui utilisé chez les primates non-humains permettront dans le futur d'effectuer un transfert direct de la connaissance acquise vers d'éventuelles applications cliniques.

## *Glossary*

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|---|---|
| **A** | **Auditory stimulus** |
| **AD** | **Alhzeimer’s disease** |
| **AP** | **Action Potential** |
| **AES** | **Anterior Ectosylvian Sulcus** |
| **AEV** | **Anterior Ectosylvian Visual area** |
| **ASD** | **Autism spectrum disorders** |
| **FAES** | **Auditory field of the Anterior Ectosylvian Sulcus** |
| **BM** | **Basilar Membrane** |
| **CN** | **Caudal Nucleus** |
| **CNS** | **Central Nervous System** |
| **CIC** | **Central nucleus (Inferior Colliculus)** |
| **DSC** | **Deep layer of Superior Colliculus** |
| **DCIC** | **Dorsal Cortex (Inferior Colliculus)** |
| **EAM** | **External Auditory Meatus** |
| **ECX** | **External Cortex (Inferior Colliculus)** |
| **EEG** | **Electroencephalogram** |
| **EP** | **Evoked potential** |
| **ERP** | **Event-related Potential** |
| **FG** | **Fusiform Gyrus** |

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| **fMRI** | **Functional magnetic resonance imaging** |
| **FST** | **Fundus of the Superior Temporal visual area** |
| **GFP** | **Global Field Power** |
| **IC** | **Inferior Colliculus** |
| **ICC** | **Inferior Colliculus central nucleus** |
| **ICP** | **Pericentral nucleus of the Inferior Colliculus** |
| **ICX** | **External nucleus of the Inferior Colliculus** |
| **IHCs** | **Inner Hair Cells** |
| **IT** | **Inferior Temporal cortex** |
| **ITG** | **Inferior Temporal Gyrus** |
| **LGN** | **Lateral Geniculate Nucleus** |
| **LIP** | **Lateral Intraparietal area** |
| **LSO** | **Lateral Superior Olive** |
| **MGN** | **Medial Geniculate Nucleus** |
| **MIP** | **Medial Intraparietal area** |
| **MNTB** | **Medial Nucleus of the Trapezoid Body** |
| **MSO** | **Medial Superior Olive** |
| **MST** | **Medial Superior Temporal visual area** |
| **OHCs** | **Outer Hair Cells** |
| **PhR** | **Photoreceptors** |

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|---|---|
| **PM** | **Premotor Cortex** |
| **PMd** | **Dorsal Premotor Cortex** |
| **PMv** | **Ventral Premotor Cortex** |
| **POI** | **Period of Interest** |
| **PPC** | **Posterior Parietal Cortex** |
| **ROI** | **Region of Interest** |
| **RT** | **Reaction Time** |
| **SEM** | **Scanning Electron Microscope** |
| **SIV** | **Somatosensory area of AES** |
| **SAI** | **Stratum Album Intermedium** |
| **SAP** | **Stratum Album Profundum** |
| **SC** | **Superior Colliculus** |
| **SGI** | **Stratum Griseum Intermedium** |
| **SGP** | **Stratum Griseum Profundum** |
| **SGS** | **Stratum Griseum Superficiale** |
| **SN** | **Substantia Nigra** |
| **SO** | **Stratum Opticum** |
| **SO** | **Superior Olive** |
| **SOAs** | **Stimulus Onset Asynchronies** |
| **SOC** | **Superior Olivary Complex** |

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|---|---|
| **STP** | **Superior Temporal Polysensory area** |
| **STS** | **Superior Temporal Sulcus** |
| **SZ** | **Stratum Zonale** |
| **TBW** | **Temporal Binding Window** |
| **V** | **Visual stimulus** |
| **VA** | **Visuo-acoustic stimulus** |
| **VIP** | **Ventral Intraparietal area** |

**Cross modal stimuli:** Stimuli from two or more different sensory modalities. This must not be mixed up with the word “multisensory”.

**Multisensory integration:** Neuronal process by which information is integrated and processed from cross modal stimuli.

**Multisensory neuron:** Neuron responding to stimuli originating from two or more sensory modalities.

**Additivity:** Response which is the exact arithmetic sum of the responses to the separate unisensory stimuli.

**Subadditivity:** Response which is below the arithmetic sum of the responses to the separate unisensory stimuli.

**Superadditivity:** Response which exceeds the arithmetic sum of the responses to the separate unisensory stimuli.

**Multisensory enhancement:** When the response from the cross-modal stimulus is greater than the most effective of its unisensory stimuli.

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| **Multisensory depression:** | When the response from the cross-modal stimulus is less than the most effective of its unisensory stimuli. |
| **Inverse effectiveness:** | The degree of multisensory integration increases as the responsiveness to unisensory stimuli decreases. |
| **Receptive field:** | Represents a region of space in which a stimulus can alter the neuronal spiking. This concept can be extended to the neural system. |

# I. Introduction

Before going into the depth of various concepts regarding multisensory integration, we shall briefly introduce the three main sensory modalities (auditory, visual and somatosensory), which have been considered in the multisensory literature, and briefly present here each of them from an individual point of view. These few introductory paragraphs aim at grasping the fundamental principles rather than describing each sensory modality into great details.

The first part of this basic introduction will be dedicated to the auditory system and has been extracted from my Master's degree thesis conducted in the same laboratory.

## I.1 Auditory System

### I.1.1 Theoretical considerations

The auditory system consists of several structures arranged in series and in parallel controlling different functions, all aiming towards a common point which is the acoustic signal perception. The auditory system includes a peripheral part, the ear, and a central part, the central auditory system. The ear can be further split into three different structures (external, middle and inner ear), both from anatomical and functional point of views (Bear et al., 2002; Faller et al., 1999; Haines, 2002; Monney-Jacomme, A.-V., 2004). The part of the inner ear devoted to the control of balance (vestibular system) is not considered here.

#### I.1.1.1 Anatomy

**External ear**

The external ear (Figure 1), the visible part of the auditory system, is composed of the pinna (or auricle) and the external auditory meatus (EAM). The border between the external and the middle ear is a thin membrane, the tympanic membrane (or the eardrum) able to vibrate in response to a variation of air pressure in the environment.

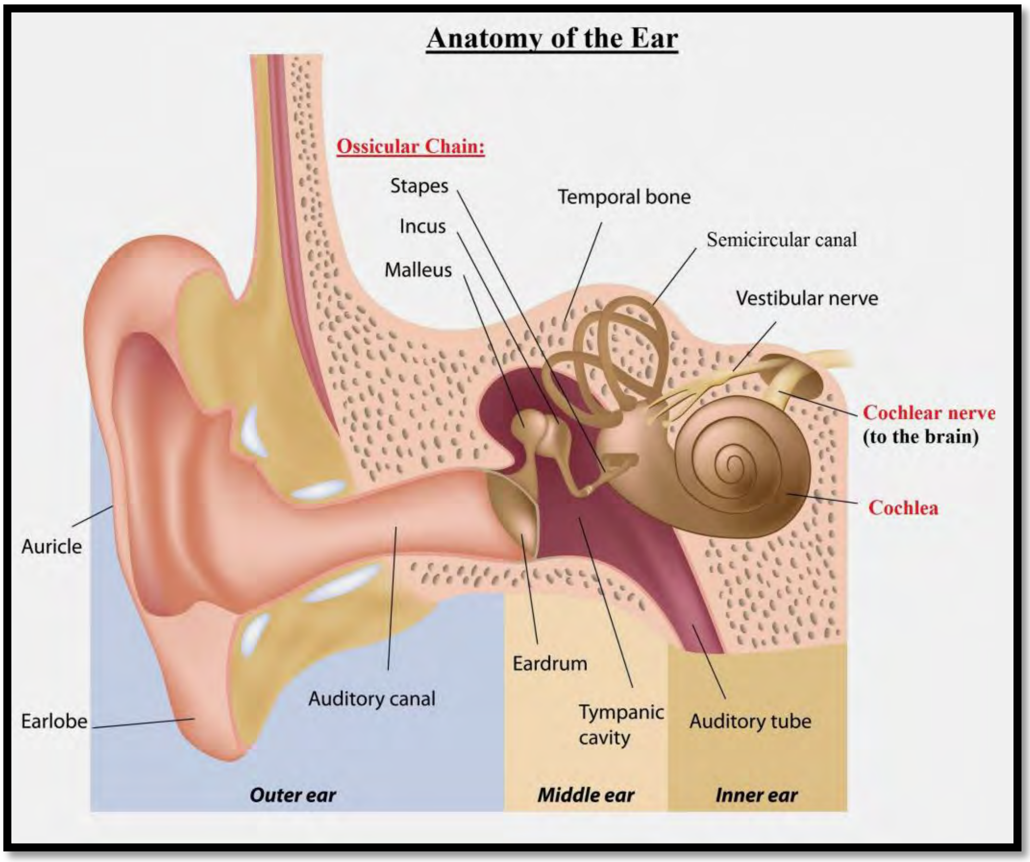


**Figure 1: Anatomy of the peripheral auditory system. Modified from http://www.yournursingtutor.com/wp-content/uploads/2012/06/9727799_l.jpg.**

### Middle ear

The middle ear (Figure 1) is formed by four structures: the tympanic membrane, the tympanic cavity, the ossicular chain and the Eustachian tube.

The tympanic membrane forms a hermetic separation between the EAM and the tympanic cavity which is an air-filled space in the temporal bone. The first bone of the ossicular chain, the malleus, is in contact with the tympanic membrane. The two other ossicles, the incus and the stapes, associated with the malleus close the connection with the oval window. This chain of ossicles, maintained in place with two small muscles (tensor tympani and stapedius) forms the arms of a control lever, contributing to the amplification of the acoustic signal (1.4 times). However, the main amplification of the acoustic signal in the middle ear is performed by the principle of surface ratio “tympanic membrane / oval window membrane”, with a gain of about 17 times. The equal air pressure distribution between the external and the middle ear is ensured by the Eustachian tube which communicates with the nasopharynx.

**Inner ear**

The middle ear is separated from the inner ear by the membrane of the oval window. The inner ear (Figure 1) is located in the petrous part of the temporal bone, and can be divided into an acoustic and a vestibular (not treated here) part.

The acoustic part is located in the spiral osseous labyrinth: the cochlea. Within the cochlea two membranes separate three scala: the scala vestibuli, the scala media and the scala tympani (Figure 2). The scala vestibuli is separated from the scala media by the Reissner's membrane and the scala media from the scala tympani by the basilar membrane (BM). The scala tympani and the scala vestibuli contain an extracellular fluid, the perilymph, for which the ionic composition is comparable to the one observed in extracellular fluid, such as plasma or cerebrospinal fluid (in other words rich in sodium ($Na^+$). The scala media contains endolymph, an extracellular fluid paradoxically rich in potassium ($K^+$).

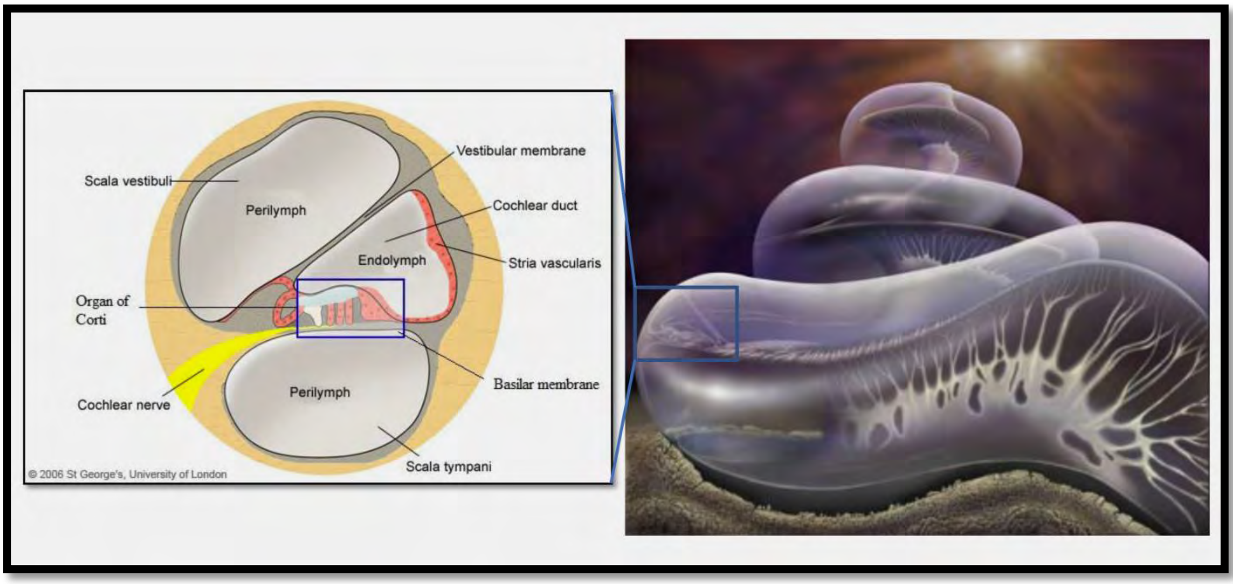


**Figure 2: Artistic view of a human cochlea. Modified from http://www.elu.sgul.ac.uk/rehash/guest/scorm/183/package/content/cochlea_cross_section.html and http://lyricsdog.eu/s/biomedical%20illustration.**

The BM supports many sensory and accessory cells, which all form the organ of Corti (Figure 2). The sensory cells are hair cells, supporting on their surface bundles of approximately 100 stereocils interconnected by tip links which control the opening of cations channels (mainly potassium). The hair cells can be distinguished according to their localization and their arrangement on the BM (Figure 3). The inner hair cells (IHCs) form a simple row from base to apex, whereas the outer hair cells (OHCs) form three parallel rows which follow the same course. The organ of Corti is covered by fine colourless gelatinous fibers, the tectorial membrane, which is attached to the limbus of the osseous spiral lamina (Figure 3), close to the vestibular canal. This tectorial membrane is in contact with the stereocils of the OHCs but not with the stereocils of the IHCs.

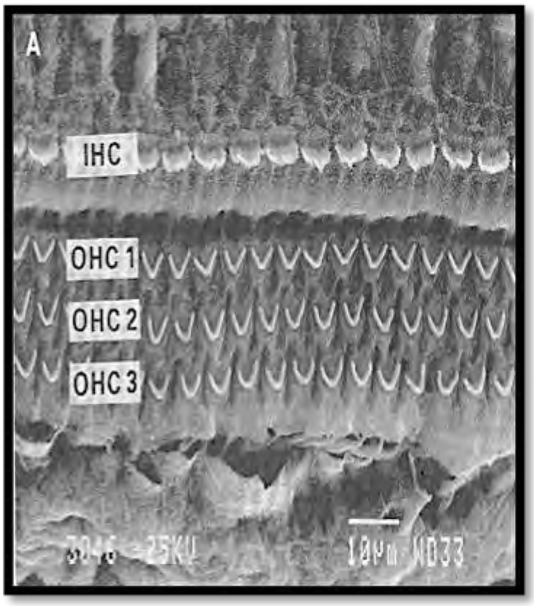


**Figure 3: Scanning electron microscope (SEM) microphotography (X1300) of the OHC and IHC from Loquet et al., 1999 (reproduced with permission).**

### I.1.1.2 Physiology of hearing

The pinna contributes to the localization of the sound source. Sound is then transmitted to the eardrum by the external auditory meatus (EAM), which selectively amplifies specific frequencies (from 0.5 to about 5 kHz in human). Under the influence of the sound, the eardrum vibrates and transmits its oscillatory movements via the ossicular chain (middle ear) to the membrane of the oval window. This chain plays a role in the amplification and attenuation (role of ear protection in response to high intensity) of the oscillatory movement. The ossicular chain allows the passage of sound wave from aerial to liquid environment (internal ear), with a theoretical loss of energy however compensated by the two mechanisms of amplification taking place in the middle ear (see above).

When the membrane of the oval window begins to vibrate during the sound emission, the engendered movements of the endolymph creates waves of propagation through the BM up to the apex. The amplitude and the distribution of the wave depend on the intensity and the frequency of the sound. For the amplitude; higher the intensity of a stimulus is, higher the deformed part of the BM is. The distribution distance of the wave depends on BM's rigidity gradient which decreases from base to apex (Figure 4). For high frequencies, the maximum of BM oscillations takes place at the base. Although the distribution of the wave is going to continue up to the apex, its amplitude is decreasing in an exponential manner along the membrane. In contrast, low frequencies will engender a distribution up to the apex but with a maximum of BM oscillations located in the apical region.

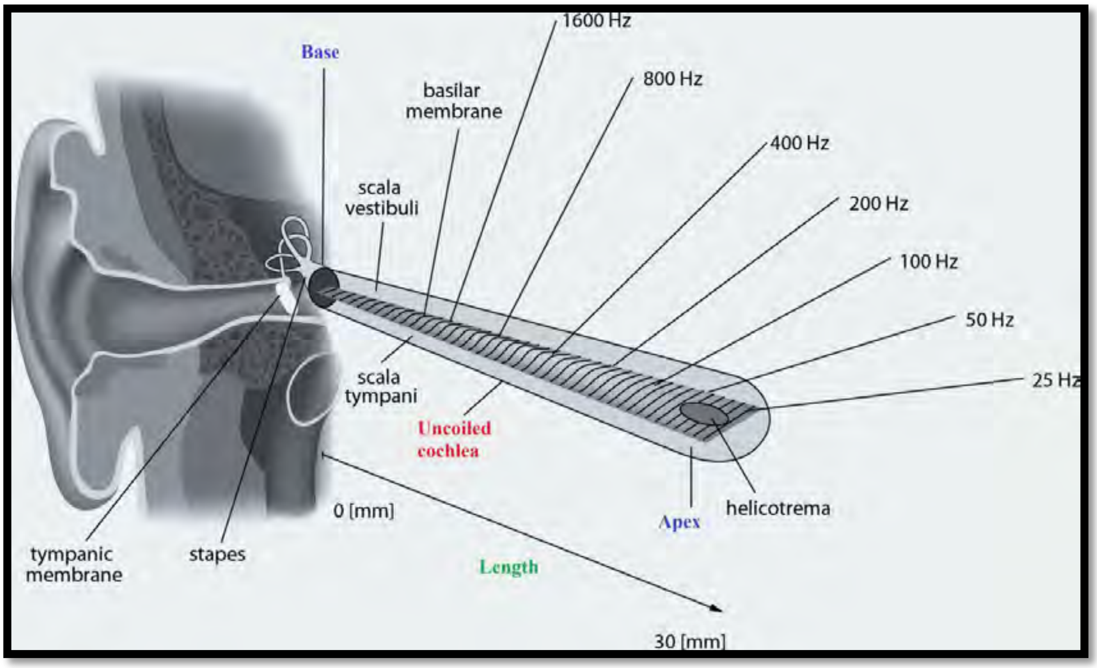


**Figure 4: Localization of BM deformation during an acoustic stimulation (place coding mechanism). Modified from Kern et al., 2008.**

As a consequence of the intrinsic rigidity property of the BM (decreasing gradient from base to apex), specific frequencies cause a maximum deformation of the BM at a specific place. This well described mechanism has been named the ***place coding mechanism*** (Nicholls et al., 2001). This creates a local deformation which causes a deflection of the stereocils of both OHCs and IHCs, the former before the latter however. It induces a stretch of the tip links which opens the transduction channels and allows a $K^+$ influx from the endolymph into the hair cells (Figure 5). This engenders a depolarization of the hair cells (receptor potential), opening voltage-gated calcium channels located in the cell membrane and an increase of intracellular calcium concentration, which facilitates the release of neurotransmitters (glutamate). This conversion of a mechanical energy applied to hair cells into an electrical energy (receptor potential) is known as the ***transduction phenomenon***. In addition, the hair cells are tuned mechanically because the fibers along the BM are specifically activated when a frequency is received. This selectivity principle is named ***cochleotopic*** or ***tonotopic map*** for frequency. These mechanical properties should be understood with the electric characteristics of the cells' membrane. Indeed IHCs depolarizations play a role in sound detection and inform the brain about vibration occurrences within the organ of Corti. OHCs have a role essentially restricted locally in the cochlea, in the coding of signal amplitude and frequency selectivity through the well-known ***cochlear amplifier*** mechanism. This role of the OHCs relies on the electromotility properties of the OHC bodies thanks to a transmembrane protein, the prestine (Médecine/science, 2001; Tang et al., 2005), identified by Zheng et al. in 2000.

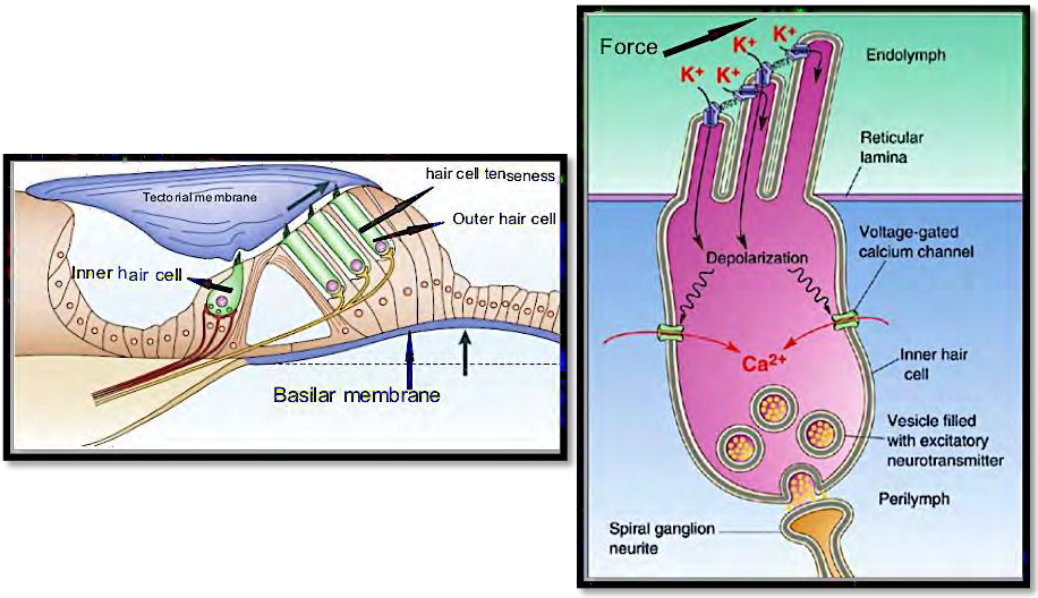


**Figure 5: Principle of electromotility. a) When the basilar membrane undergoes a deformation the tectorial membrane slides over the hair cells and deforms the stereocils. b) The stereocils bending opens the k+ channels and depolarizes the hair cells. Modified from *From Neuron to brain* (Nicholls et al., 2001).**

It is important to note that the site of the BM deformation is the same for all stimuli in the range of 70 Hz to 200 Hz where no neurons were found. This shows that frequency selectivity cannot only be explained with mechanical and electrical mechanism (Bear and al., 2002). The complementary system to the cochleotopic map that has been assumed is the phase-locking mechanism (Köppl C., 1997), which is a general mechanism in sensory physiology. In the auditory system, this mechanism shows, in response to tones of low frequencies, a close correlation (synchronization) between neuronal discharges and each period of a sinusoidal sound wave (Figure 6). Phase-locking (temporal coding) is believed to contribute frequency analysis for frequencies going from 20 Hz to about 4 kHz. For intermediate frequencies, stimulus frequency coding is performed by a tonotopic mechanism plus a phase-locking one. For high frequencies, the phase-locking synchronisation disappears and only tonotopic mechanisms become effective. This complementary system has been confirmed by Moore (1973) who has demonstrated that place and temporal mechanisms play together a role in pitch discrimination. According to Moore (1973), the temporal coding mechanism would be more efficient for frequencies below 5 kHz, whereas the place mechanism is in charge of frequencies above 5 kHz.

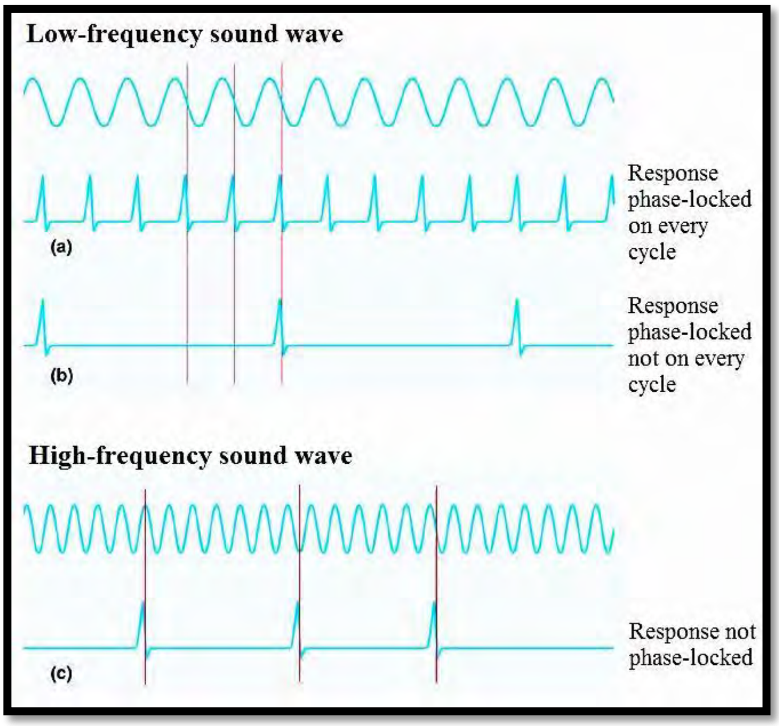


**Figure 6: Phase-locking mechanism as a function of sound wave's -frequency. Modified from Bear et al., 2002.**

### I.1.1.3 Ascending auditory pathway

Neural inputs, which originate from the ganglion of Corti, travel through the tract of axons which form the cochlear root of the auditory nerve (VIII$^{th}$ cranial nerve, Figure 7). This nerve contains around thirty thousand of axons (originating from the thirty thousands of primary auditory neurons in the spiral ganglion, distributed into type I (dominant, about 90%) fibres and type II nerve fibres (about 10%). These axons follow a pathway punctuated with groups of neurons, the cochlear nuclei, which aim at refining the processing of the auditory information. The first encountered nucleus is the cochlear nucleus (obligatory synapse on the secondary auditory neurons) and the entry point is the ventral root (giving access to the more voluminous ventral cochlear nucleus), followed by the dorsal cochlear nucleus. These nuclei considered as the first site of neuronal processing are situated, in the auditory region of the fossa rhomboidea. The following nucleus along the auditory pathways, located in the pons, is the superior olivary complex (SOC), which represents an important component in both ascending and descending auditory pathways. In addition, the SOC is the main first station at which the information from each ear converges. The SOC further splits between the lateral superior olives (LSO), the medial superior olive (MSO) and the medial nucleus of the trapezoid body (MNTB). In the SOC, the interaural time difference is discriminated mainly by the MSO and the interaural intensity difference mainly by the LSO. A further (third) essential nucleus along the auditory pathways is the inferior colliculus (IC), representing an obligatory relay for most of the afferent information that will be transferred later to the auditory thalamus. Some neurons of the cochlear nuclei and SOC may project indirectly to the IC, via the nucleus of the lateral lemniscus (Figure 7). From an anatomical point of view, the IC is subdivided into three parts (Rockel et al., 1973 a and b): the central nucleus (ICC), the pericentral nucleus (ICP) and the external nucleus (ICX). From a physiological point of view, the IC allows the detection and the discrimination of a sound wave amplitude change, named pitch detection. Interestingly with regard to the theme of this thesis, it was demonstrated that the ICX responds to sensory stimuli other than acoustics. In particular, Aitkin et al. (1978) demonstrated that some bimodal neurons respond to auditory and tactile stimuli. The following relay is the medial geniculate bodies, component of the thalamus (thalamic relay system). These relays are reached thanks to the lateral lemniscus: tract of axons having the form of a ribbon. The lemniscus is a beam of ascending and crossed fibers for the greater part. The lemniscus, as mentioned previously of ends in the medial geniculate body, which turns out to be the seat of the central neurons. From there, axons pass by the acoustic radiation to terminate in the primary auditory cortex, being in the superior temporal convolution. The primary auditory cortex is the first region to receive auditory input at cortical level.

Based on this simplified introduction, we can notice that the auditory system is complex, and that it is totally adapted to the perception of complex sounds, in particular it can analyse efficiently and discriminate the frequency (Hz), the intensity (dB), and the localization of sounds.

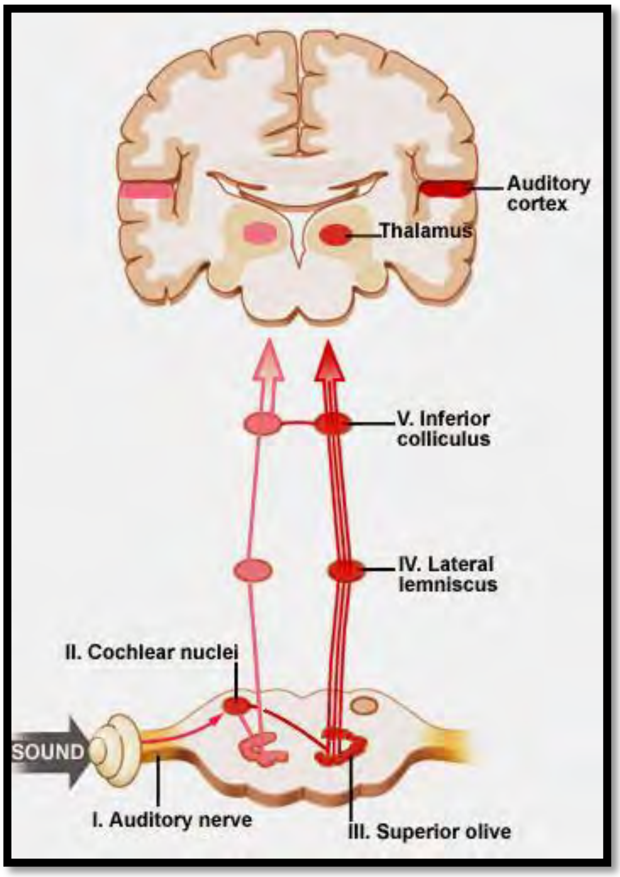


**Figure 7: Representation of the auditory pathways. From the website: http://www.neuroreille.com/promenade/english/audiometry/ex_ptw/explo_ptw.htm. (All rights reserved © 1999 – 2007).**

## I.2 Visual system

This second introductory chapter is dedicated to the visual system. As mentioned earlier the aim is not to get into details but to make a brief overview of the visual system.

### I.2.1 Theoretical considerations

Light is a set of electromagnetic waves visible for the human eyes. We are speaking here of wavelengths ranging from 380 to 780 nm, where every wavelength corresponds to a colour (Figure 8). For example, blue is located around 400 nm, green around 500 nm and red around 700 nm. It is Isaac Newton who demonstrated during the XVIIIth century that the mix of the wavelengths emitted by the sun is perceived as a white colour, whereas a light source of a single wavelength produces a single colour belonging to the rainbow's palette.

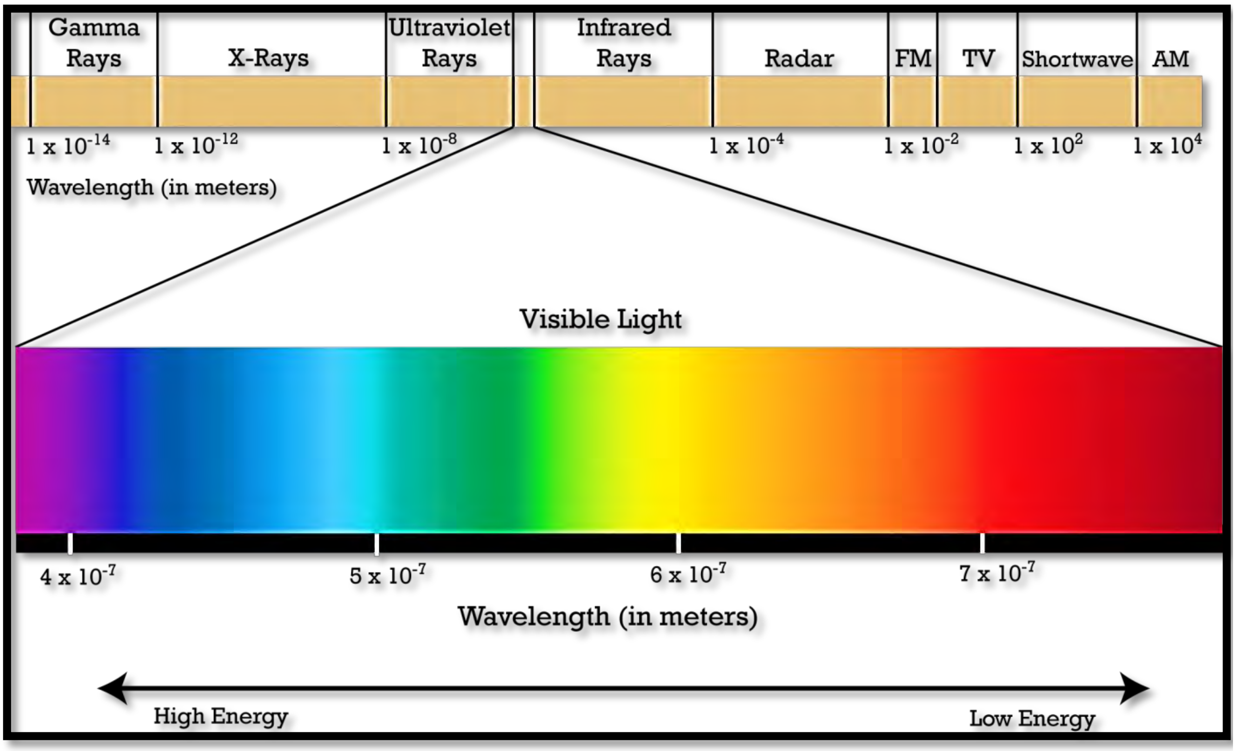


**Figure 8: Wave spectrum. From the site: http://www.pion.cz/en/article/electromagnetic-spectrum.**

In space, light moves straight till it encounters obstacles like objects and molecules. There it interacts through either:

- **A reflection** by changing its movement direction (as a function of the angle between the incident beam of light and the object surface),
- **An absorption** by transferring its energy to a particle or a surface,
- **A refraction** when the beam of light is deviated when passing from a more or less transparent environment to another.

This latter type of interaction takes place in the eye where differentiation of darkness and colours happens.

As introduced in the auditory system and in analogy to this paragraph (in the § I.1.1), the visual system is composed of structures arranged in series and/or in parallel controlling different functions and processing the visual information to obtain a complex and unified visual cue perception.

The visual system includes a peripheral part, the eye, and a central part, the central visual system (Bear et al., 2002; Faller et al., 1999). Contrary to the auditory system it is not possible to divide the eye according to its anatomy or its function. Indeed, as the ear, the eye receives the physical stimulus (light in that case) but, in addition and in contrast to the ear, already performs in the eye a highly sophisticated processing of the incoming information at the level of the retina (in the § I.2.1.2).

### I.2.1.1 Anatomy

The peripheral part of the visual system, the eye, consists in different parts (Figure 9). On the exposed side of the eyeball, the pupil is an opening which allows the entry of the light inside the eyeball up to the retina. The pupil looks black due to the presence of retinal pigments which absorb the light. The pupil acts as an optical system where the diaphragm controls the quantity of light which is entering by modifying the diameter of its opening. Such opening of the pupil is controlled by a circular muscle: the iris. The colour of the iris is determined by the concentration of melanin which is also an essential pigment of the skin and the hair. The iris and pupil are covered by the cornea which receives its blood supply from a point situated below the aqueous humor. The cornea is prolonged by the white of the eye or the sclera which forms the stiff wall of the eyeball. The eyeball is mobile and its movements are guaranteed by three pairs of muscles inserted into the sclera. However these muscles are not visible because they are behind a transparent mucous membrane which hides the inside of the eyelids and unites them with the eyeball. This membrane produces the mucus which greases the surface of the eye. In the back of the eye is a surface of about 0.5 mm in thickness and covering 75 % of the eyeball: it is the retina. It forms the sensory part of the visual receptor. Indeed, this layer allows, via specialized photoreceptors, the transformation of light (electromagnetic energy) into bio-electric energy (receptor potentials first and then action potentials). This transduction phenomenon happens within the retina which is considered to be an integral part of the brain. From a functional point of view, the eye is very similar to a camera by catching a sharp image of our world onto the retina. The optical nerve, formed by the axons originating from the retina (the so-called ganglion cells), leaves the eye from the back, goes out of the orbits, and travels at the base of the brain near the pituitary gland. It joins the central visual pathway which will be more detailed in paragraph I.2.1.3.

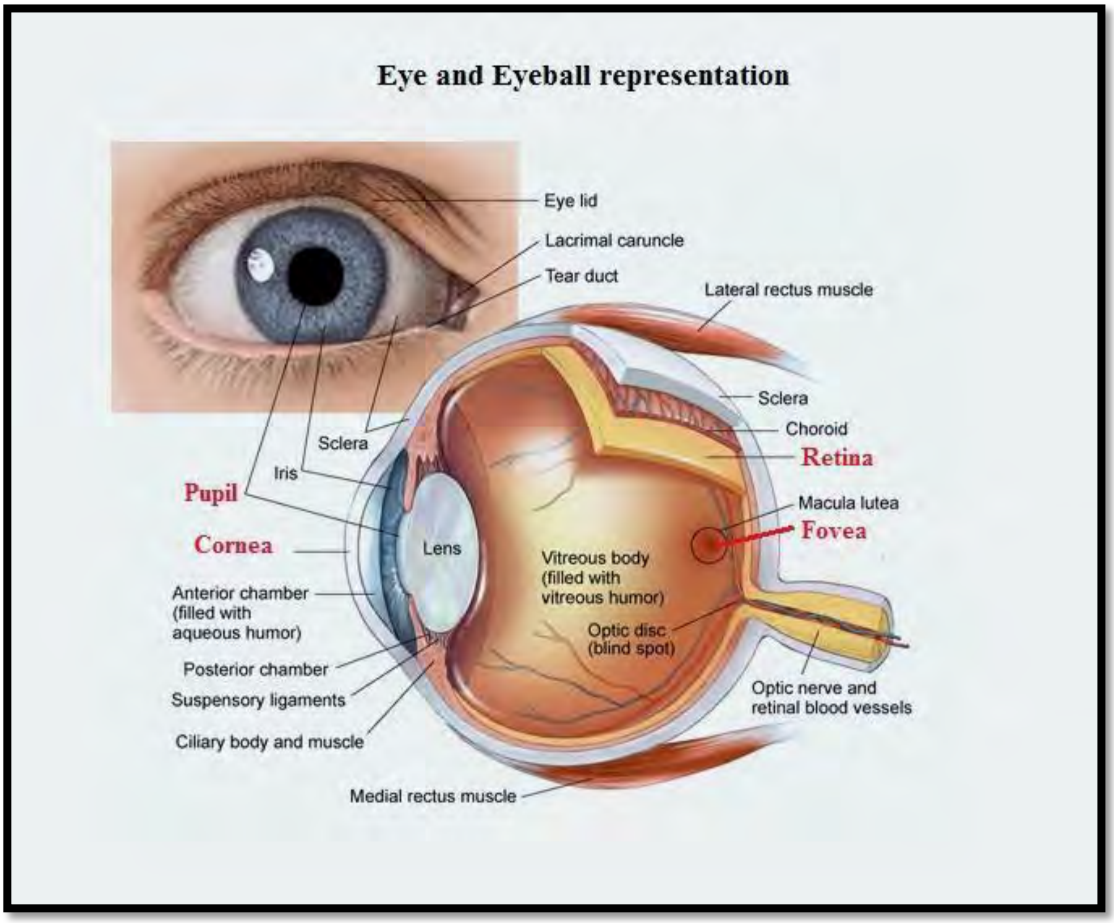


**Figure 9. Representation of the eyeball structure. Modified from the site: http://www.eyezonemedical.com/?page_id=386.**

### I.2.1.2 Physiology of the vision

Visual perception requires the existence of photoreceptors (PhR) which are photosensitive cells. These receptors can be divided into two classes: cones and rods. These two photoreceptors present similar structure and function (transduction of the light in the external segment), but are different in their response delays and their sensitivity.

1 ° **Cones**: Cones contain one specific pigment which turns out to be sensitive to a certain range of wavelengths. Generally, across cones, there are three different pigment types which absorb green light (531 nm), blue light (419 nm), or red light (559 nm). This sensitivity to three colours allows humans and non-human primates to see in colour during daylight and that is called photopic vision (or macular). The absence of one or several cone types makes the retina insensitive to the corresponding wavelengths. It is the case for patients suffering from color-blindness, dischromatopsies or achromatopsies. From a quantitative point of view,

the number of cones decreases as we go away from the centre of the fovea. As a consequence, colour vision corresponds to the central vision (fovea).

2° **Rods**: In contrast to the cones, the rods have slow responses and express a sensitivity in night-vision (known as scotopic vision or peripheral vision). By definition, this vision is only in black, white and nuances of grey, because rods do not absorb colours. However rods play a role in movements' detection in association with the visual cortex. Rods' density is maximal in periphery of the retina and decreases when we approach the fovea.

The visual information resulting from the phototransduction made by the photoreceptors is transmitted to other cells that can be considered as kind of interneurons (although they do not all generate action potentials, but rather slow potentials, such as the bipolar cells). These cells correspond to the different layers of bipolar cells, amacrines cells and horizontal cells and are connected to the ganglion cells (true neurons), which axons will form the optic nerve. The ganglion cells play an important role in the temporal and spatial coding of the light admitted onto the retina. It is important to note that the light must cross all the retinal (transparent) layers first before reaching the photoreceptors.

Retinal ganglion cells: These neurons have a receptive field of circular shape and formed by photoreceptors, horizontal cells and bipolar cells. This receptive field is usually divided into two parts: the center and the surround with an antagonism center-surround. More precisely, there are two types of ganglion neurons: center-ON and center-OFF. Cells center-ON present an excitation when the center is stimulated by a light whereas an inhibition is produced when the surround area is reached by light. On the contrary, center-OFF are excited when the light reaches the surround, and inhibited when it reaches the center. Consequently, the ganglion cells are responsible for contrasts' detection. It is important to note that ganglion neurons are of two main types: type P cells (parvus) and type M cells (magnus). These cells have well defined roles and are at the origin of different visual pathways, which are described in the next paragraph (in the § I.2.1.3). The M cells represent approximately 5 % (or 10 % according to the literature (e.g. Baseler and Sutter, 1997)) of the ganglion neurons and emit AP in the case of stimuli of low contrasts. These cells are adapted to movement discrimination. On the contrary, type P cells (around 80 and 90 % of the ganglion cell population) have receptive fields smaller and more sustained AP. These cells are sensitive to the shape and to various wavelengths. The remaining percentages of cells are not-P and not-M cells which play a role in colors detection. P cells give rise to the ventral neuronal paths of visual system, whereas M cells give rise to the dorsal neuronal paths (in the § I.2.1.3 for details).

### I.2.1.3 Visual Ascending Pathways

Neuronal inputs originating from cones and rods are transmitted to the visual cortex through the ascending visual pathways (Figure 10). These pathways consist first in the left and right optical nerves formed by the temporal and nasal bundle of axons. These fibers merge at the level of the optic chiasma situated in front of the infundibulum. The nasal fibres cross at the level of the chiasma but not the temporal fibres. At the chiasma level takes place a decussation of the optical ways, which allows a crossed processing of the information. It means that the right tract conveys the information concerning the visual left hemifield and the left tract conveys the information concerning the visual right hemifield. Both optical tracts end in the lateral geniculate nucleus (LGN) in the thalamus. From a cytoarchitectonic point of view, the type P cells are connected with layer P of the LGN, whereas the type M cells are connected with layer M of the LGN. This nucleus is considered as the first sensory relay of the visual information. One can note that the LGN receives feedback connections from the primary visual cortex, but also from the optic tectum, also named superior colliculus (SC). In addition to what has already been said in the section "Auditory system", some other information concerning the SC (developed in details in § I.4.5.3.1) and the visual system will be introduced here. One of the main functions of the SC, situated in the midbrain, is the coordination of the eye movements relative to the head. Consequently one of SC’s role is to direct gaze (Sparks, 1986; Meredith and Stein, 1983) via saccadic eye movements, and the head towards the source of stimulation. These studies demonstrate the spatial aspects controlled by the SC and especially in terms of orientation. Furthermore the sensory inputs are not limited to the visual system, but are also coming from both the auditory and the somatic systems (Gordon, 1973; Middlebrooks and Knudsen, 1984). These are the three sensory systems which were investigated in the present work.

Following the transfer of information to the SC and to the LGN, through the optic radiation, the visual inputs will reach the visual cortex. From an anatomical point of view, the visual cortex is located in the occipital lobe of the brain and is the largest cerebral cortex devoted to a specific sensory modality; in the non-human primates, the visual cortical areas cover until 50 % of the neocortex (Sereno et al., 1994). Usually the visual cortex refers to both the primary visual cortex, also known as the striate cortex (Baseler and Sutter, 1997) or V1 (area 17 according to Brodmann’s classification (Brodmann, 1909; Falchier et al., 2002)) and the secondary visual cortex, known as the extrastriate visual cortex (V2, V3, V4 and V5). The visual cortex then transmits information to the inferior temporal and to the posterior parietal cortex, via two separate pathways, named the dorsal and the ventral streams. These pathways are engendered by both types of ganglionar cells (M and P cells) (in the § I.2.1.2). Generally, the dorsal stream reaching the posterior parietal cortex, is called the «where » pathway and plays a role in the spatial visual information processing, whereas the ventral stream reaching the inferior temporal cortex is called the «what» pathway and plays a role in the recognition and identification of visual stimuli (objects; including color).

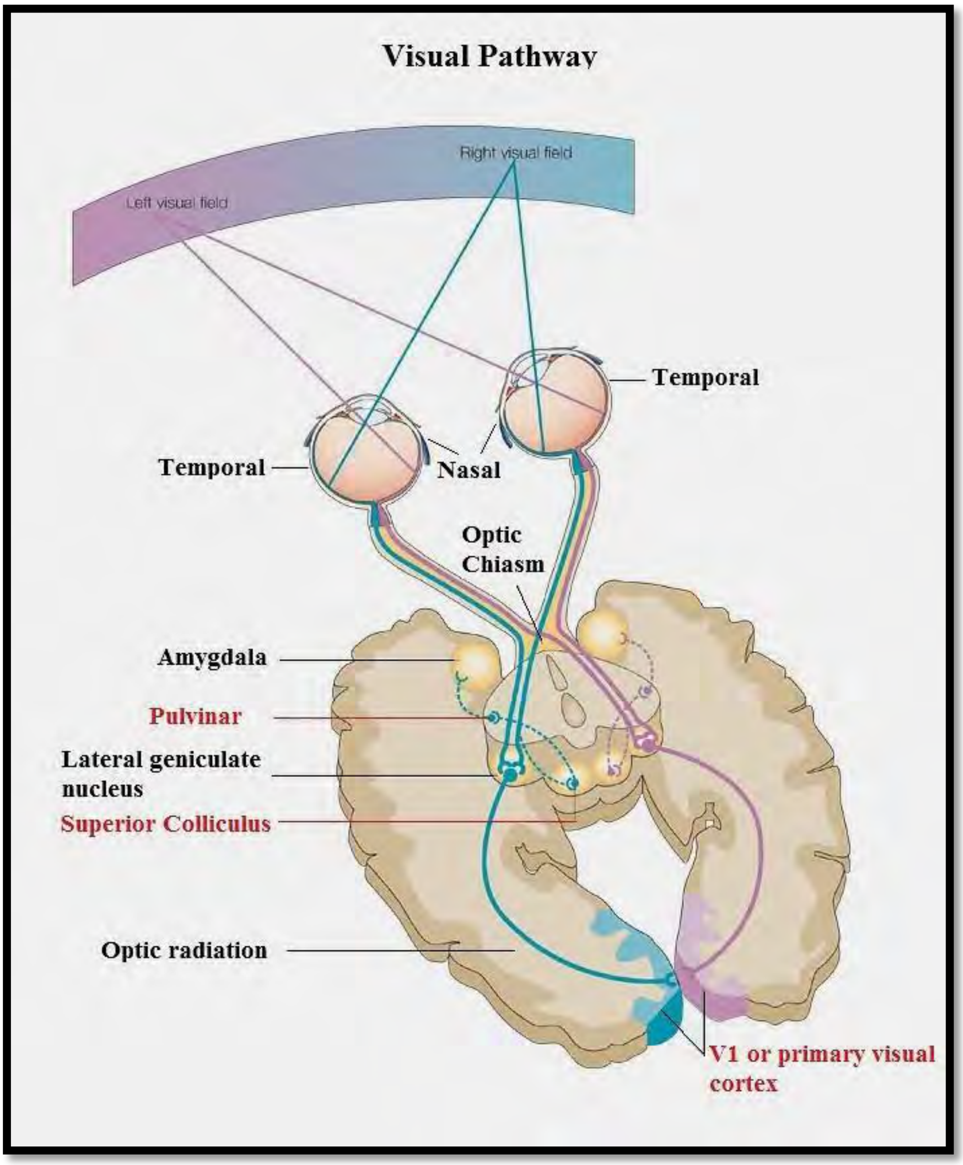


**Figure 10: Representation of the visual pathways. Modified from the article Deborah et al., 2005.**

Through this chapter we showed that the visual system is well adapted to its function and allows the perception of shapes, colours, movements, and intensities differences.

## I.3 Somatosensory system

The somatosensory system, also called sensitivity of the body, is the main sensory system in humans, considering the surface of its receptor organ (the skin). However, compared to the visual and the auditory systems which correspond to individual modalities, the somatosensory system is multimodal because it integrates mechanical, thermal and chemical inputs. Here are the different modalities which are part of the somatosensory system:

1° The proprioception: this function provides indications to the central system about the body position in space. One particularity of this modality is that it can be conscious or unconscious. The conscious perception implicates different submodalities, such as the sensation of vibrations and tactile information. The unconscious perception depends on receptors responding to contractions or tensions of respectively muscles and tendons.

2° The kinesthesis or kinesthesia: This function concerns the conscious perception of movements by giving information concerning the position and the movements of the body. This modality is independent from tactile and visual senses. The information originates from receptors placed in joints and muscles and is transmitted to the central nervous system in a continuous manner through sensory afferents. This function allows subjects for example to reach an object, to grasp it precisely, or to walk. All this process happens without conscious actions. Indeed, we do not have to think about the various movements to be performed when we walk.

3° The cutaneous senses or commonly named sense of touch: this function represents the ability to perceive through body contacts objects from the environment and forces.

4° The nociception: this function allows informing the CNS about pain thanks to pain receptors, collectively called nociceptors. These receptors are located at various spots over the body and can be divided into cutaneous, muscular and articular receptors. Nociceptors can detect the thermal, mechanical, and chemical changes, and then transport this information to the brain via afferent nerves. As cross-modal neurons, nociceptors can be polymodal. Indeed, these receptors respond to mechanical, chemical and thermal stimuli.

From an anatomical point of view, the somatosensory system is the only system which is linked by efferents with the brain and the spinal cord (Willis et al., 1991). At the cortex level, the somatosensory area is located in the postcentral gyrus of the parietal lobe. As for all other sensory areas, there is a spatial map (somatotopic), called in this case the homunculus (Figure 11) where tactile information is preferentially treated. It is important to note that the processing of somatosensory information is spread out all over the CNS (Table 1). For example the spinal cord combines motor and sensory nerves and elaborates reflexes on the basis of somatosensory inputs. At the level of the cerebellum somatosensory information play a role in motricity and balance.

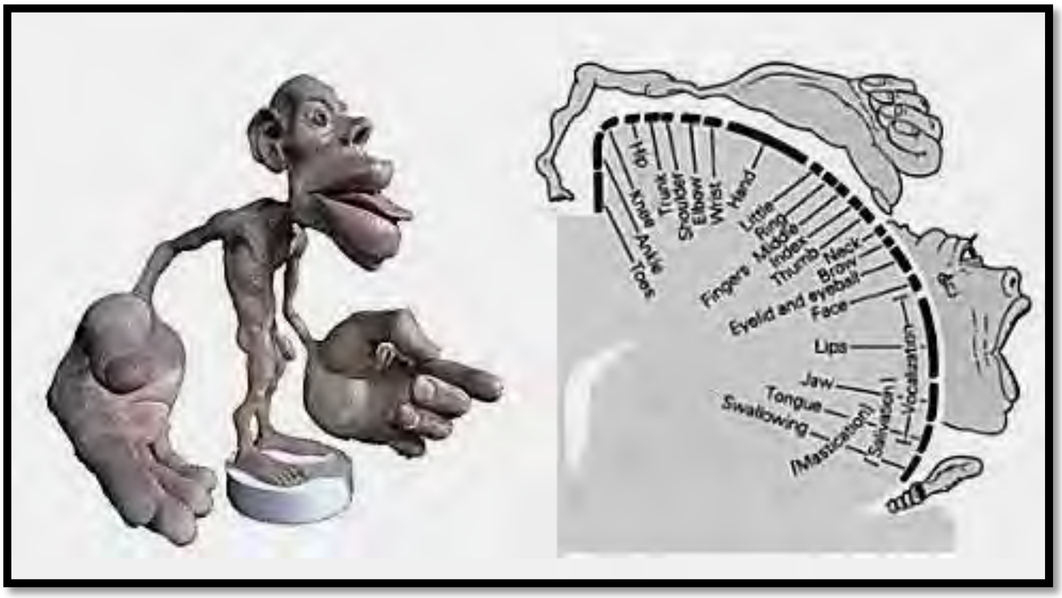


**Figure 11: Representation of the Penfield Homunculus. From http://sciblogs.co.nz/misc-ience/files/2010/06/penfield-homunculus.jpg.**

**Table 1: Distribution of the somatosensory information processing in the CNS.**

| | |
|---|---|
| **Cerebral cortex** | Tactile information (→ homonculus) |
| **Cerebellum** | Control of balance and motricity |
| **Spinal cord** | Motor Reflex |
| **Thalamus** | Cognitive treatement and forwarding information to the cortex |
| **Hypothalamus** | Hormonal and neuroendrinal control |
| **Reticular system** | Attention control |
| **Limbic system** | Emotion |

The ascending somatosensory pathways form a neuronal chain going from the receptors to the cerebral cortex. This path is formed by three neurons:

- The primary neuron: with its cell body located in the peripheral ganglion (spinal or cranial),

- The secondary neuron: which makes a synapse with a third neuron in the spinal cord or in the thalamus,
- The tertiary neuron: which ends at the level of the cerebral cortex.

Through this chapter, we can say like Sherrington in 1924 that the somatosensory "*receptors are points of the organism specialized for the surrounding world to act upon*" and that this system is highly contributing to complex responses including motor movements, behaviors and social interactions. Like visual and auditory systems, the somatosensory system interacts with other modalities to improve the environmental perception.

## I.4 Multisensory integration

### I.4.1 Generalities

Since the mid of 19th century till 1950s scientists were dividing the neocortex, into three functional subdivisions : the motor, the sensory and the association areas (see review in Masterton and Berkley, 1974). This view is illustrated in Figure 12 where cortical areas have been investigated on the basis of the dual principle of perception (by the senses) and reaction. Such neocortical divisions were contradicted by the review of Masterton and Berkley (1974) which reports a collection of evidences during 20 years for demonstrating that cortical areas are more heterogeneous across auditory, visual, motor, somatosensory, frontal and temporal cortex. During the last half of the 20th century, technological developments have considerably helped neurosciences' research. During this period the neuroscientific approach was focussed on the 5 senses individually: hearing, vision, touch, taste and smell (in line with Aristotle's view). Through this process physiologists have even identified other sensory modalities connected to new receptors, such as the vestibular system, the proprioceptors, and the thermo receptors. Thereby the perception has been considered to be "modular". For example, the hearing system consists of various modules sensitive to various characteristics like the frequency, the intensity and some more. This manner of studying the various sensory systems went on during the 80s and 90s. Recently, such an approach has been contested because it could not bring any satisfying answer to the fundamental questions about mechanisms underlying the ability to globally perceive the environment. This questioning brought together for the first time neuroscientists and philosophers of science in order to start thinking about what is called now “multisensory processes”. The pioneer work of Meredith and Stein (Meredith and Stein, 1983; 1986) on multisensory processes can be briefly summarized here. In particular, their observations that neurones in the superior colliculus (SC) were able to respond to both visual and auditory stimuli. This cross-modal response resulted from a modification of the neuronal activities. This modification can be expressed by an enhancement or an inhibition of the neuronal spiking, as compared with the basic activity.

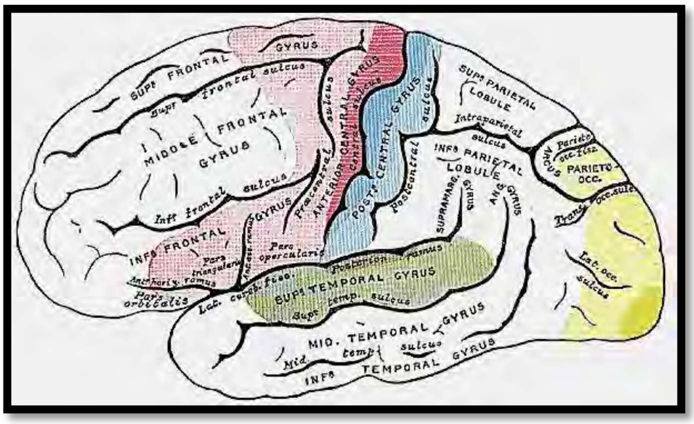


**Figure 12: Functional organization of the neocortex according to Gray's Anatomy book (1858).**

Following these advances, evidences accumulate for demonstrating that our senses are functioning together and sensory information coming from different systems is integrated. In an attempt to define what multisensory integration is, Reynaud Emmanuelle (2013) suggests, on her webpage, that it is a question of: "the capacity which has the cognitive system to integrate information coming from various sensory receivers in a unified representation". According to Stein and Stanford (Stein and Stanford, 2008) the multisensory integration is: "the neural process which participates in the information synthesis of cross-modal stimuli". At this level, a clarification concerning the use of “cross-modal” must be made too. According to previous reports (e.g. Meredith and Stein, 1986 and 1987), cross-modal integration or cross-modal convergence occurs on a given neuron if a response can be induced by stimuli from different modalities (e.g. visual and auditory) presented in a way isolated, or if the neuronal activity obtained by a stimulus can be modulated (enhanced or decreased) by another modality stimulus. Many questions are still open in the multisensory research field and researches conducted today try to fill those gaps. This is the case for recent studies which describe distinct factors per modality: an individual and a collective factor. To illustrate this, we will choose two well-known modalities like vision and audition. Taken individually, each modality is efficient when stimulated with the appropriate stimulus. For example with the visual system humans are able to detect with precision a shift of a visual stimulus. Moreover, a little change in the direction of a stimulus which is represented by two levels, the azimuth (horizontal level) and the elevation (vertical level), is easily identified. On the other side, the auditory system allows humans to perceive sounds coming from 360° (individual modality). However, it has been demonstrated that the resolution of the auditory system is lower than the one of the visual system. Put into a "collective" perspective, the senses of vision and hearing are together going to interact to increase the precision of localization of the direction change. We can also mention the increase of the probability of detection of this change when both stimuli are perceived. To add another relevant example we can discuss about speech. Spontaneously, we could think that speech implicates only an auditory process (McGurk and McDonald, 1976), but it includes visual lip movements too (McGurk and McDonald, 1976; Driver, 1996). Furthermore, in a congruent situation (i.e. if the lip-movements and the sounds from the speaker reflect the same event) the lip-reading contributes to better speech perception (Sumby and Pollack, 1954; Saito et al., 2005; Rouger et al., 2007 ; Ross et al., 2007). The estimated gains thanks to this multimodal combination raise the ratio acoustic signal over noise from 11 to 20 dB, which results in better understanding speech, especially in presence of background noise.

We know that human and non-human primates have evolved in a complex environment generating multiple sensory inputs. Vertebrate nervous system has evolved to process information from different modalities. One important role of the brain is to decode multiple information from the environment caught by peripheral sensory organs, then integrate and finally encode them with the aim to create a coherent perception. To do that, the brain dedicated regions able to integrate converging information from several modalities (Stein and Meredith, 1993).

The effects of multisensory integration are multiple. Indeed, according to Driver and Noesselt (2008), the stimulation of a modality can affect the appreciation of a property normally applied to another modality. That is what we will introduce in the following paragraphs multisensory effects when environmental parameters are well controlled and give rise to illusions or when it brings clear benefits, namely facilitatory effects, to the subject.

### I.4.1.1 Illusions

Concerning the "multisensory" illusions specifically during a visuo-acoustic task like the one investigated in the present thesis, we have found two categories:

1) when visual processing can influence the auditory experience,
2) when auditory processing can alter the visual experience.

In the first category, the point is about visual illusions, which happen when there is a dissociation between the perception of an object or an event and the physical reality. In other words we can speak about a perception of something that does not exist like in the following examples.

- **The ventriloquism**: This word originates from a Latin phrase “venter” (belly) and “loqui” (speak) which means “speaking from the stomach”. Historically the ventriloquism was a religious practice (Schmidt, 1998). Indeed the ventriloquist was able to interpret sounds resulting from stomach and was able to predict future. From a more rational point of view, ventriloquism is an act in which a person changes his voice so that it gives the illusion that the voice is coming from elsewhere. So we can state that the direct vision of some articulatory lips movements influence the spatial discrimination of a sound source (Driver, 1996). Moreover the ventriloquist’s art consists usually in minimizing the lips movement (the compellingness) in order that the audience associates the word (resulting from the ventriloquist) with the lips movements of the dolly; although keeping a consistence between the dolly lips movement and what is heard. So we can add another statement which is that the percept of an auditory stimulus is captured by the spatial location of a visual stimulus. Therefore we can conclude like Recanzone (2009) that the efficiency of an illusion depends on three factors: the timing of the two stimuli, the spatial disparity and the compellingness.

- **The McGurk effect**. This phenomenon was found accidently and was described for the first time in 1976 by Harry McGurk and John MacDonald (McGurk and McDonald, 1976). This illusion occurred when mismatching auditory (e.g., /apa/) and visual (e.g., /aka/) syllables were presented (http://www.youtube.com/watch?v=G-lN8vWm3m0). In this example subjects perceived a different syllable (/ata/) which is a syllable that is neither seen, nor heard. A recent study (Szycik et al., 2012) using functional MRI tried to define the brain areas involved in the McGurk effect. The active brain areas were found in the posterior part of STS, bilaterally. According to some studies, certain neurological disorders produce a significantly reduced McGurk effect. This has been observed in young people affected by dyslexia (Bastien-Toniazzo et al., 2010), in patients affected by Alhzeimer’s disease (AD) (Delbeuck et al., 2007) and in children with specific spectrum disorders (ASD) (Mongillo et al., 2008). Concerning the mechanisms involved, the report of Delbeuck et al. (2007) suggests that AD people have a dysfunction in the interactions between brain areas which could be due to a reduction of corpus callosum’s size (dysfunction between hemispheres communication). ASD patients, especially children, show difficulties in using visual information from the face in particular in speech perception conditions. Along the same line, other authors (Schultz et al., 2000; Kikuchi et al., 2009) underline that ASD children face emotional problems linked to information processing from faces as described in autism. In such patients fMRI analysis done by Schultz et al. (2000) showed a reduction of the activity in the fusiform gyrus (FG) area and an increase of the activity in the inferior temporal gyrus (ITG) (Figure 13). In children affected by a specific language impairment (SLI) the McGurk effect is significantly reduced compared to control children (Norrix et al., 2007).

Using the McGurk effect as an investigation tools in such patients allows the scientists to understand better the various steps of signal processing in the context of visual perception. Indeed, this type of illusion represents an incapacity to recreate the physical reality and therefore open-up our understanding of the visual information steps.

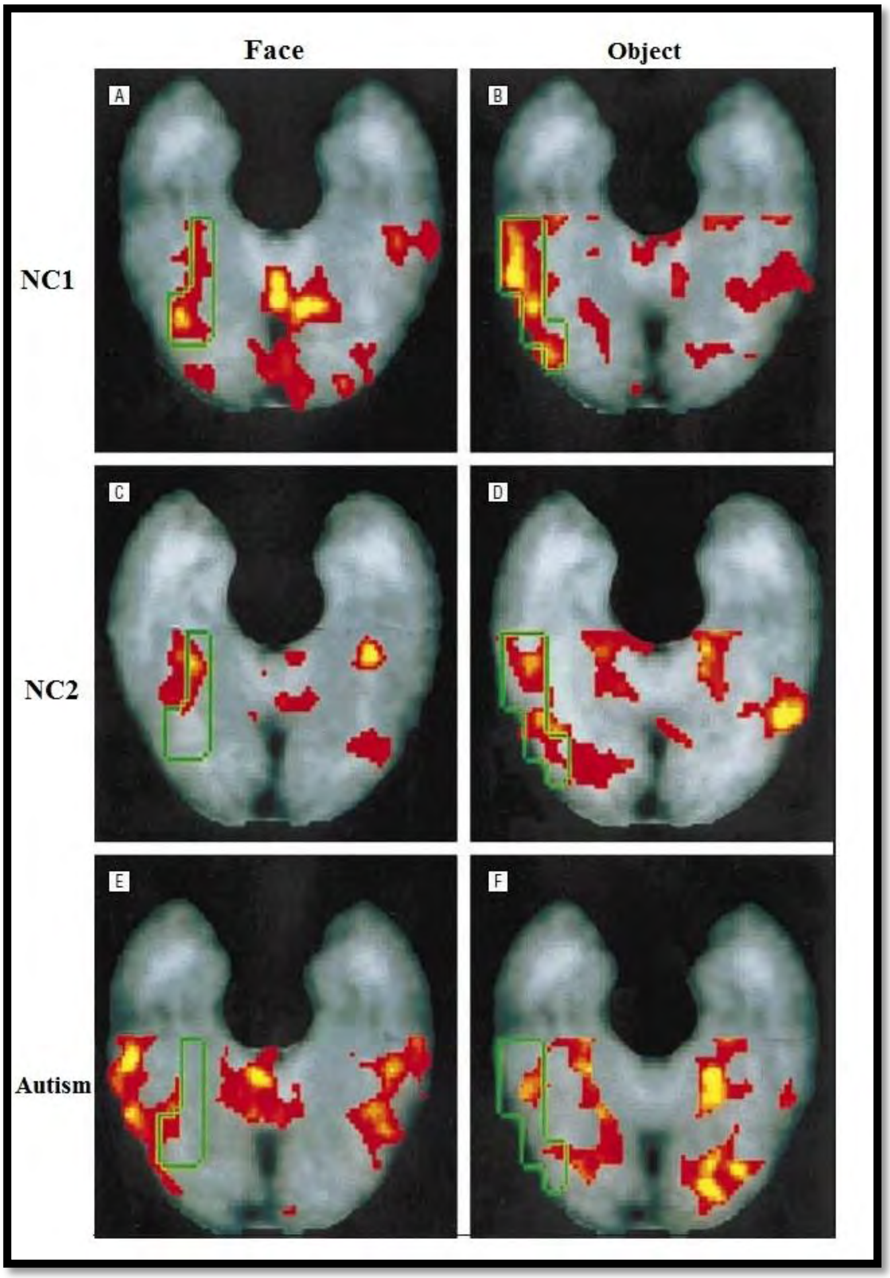


**Figure 13: Composite activation maps superimposed on averaged anatomical images by group (NC1, NC2, autism) and task (Face, Object) using regions of interest outlined in green. Activations in normal control group NC1 and NC2 (A, B, C, D) are not significantly different. Right and left are reversed by radiologic convention. Note the inferior temporal gyrus (ITG) activity during face processing in the autism group (E, F). Modified from Schultz et al., 2000.**

The illusions mentioned above (Ventriloquism and McGurk effect) show that the vision can alter the hearing perception. Reciprocally, certain illusions show us that hearing can alter the visual perception.

- **Illusory flashing:** This illusions demonstrated in 2002 by Shams et al., (2002) (Figure 14) happens when a flash is accompanied by several beeps. The flash is surprisingly not perceived as being unique but multiple as it is in reality for the beeps.

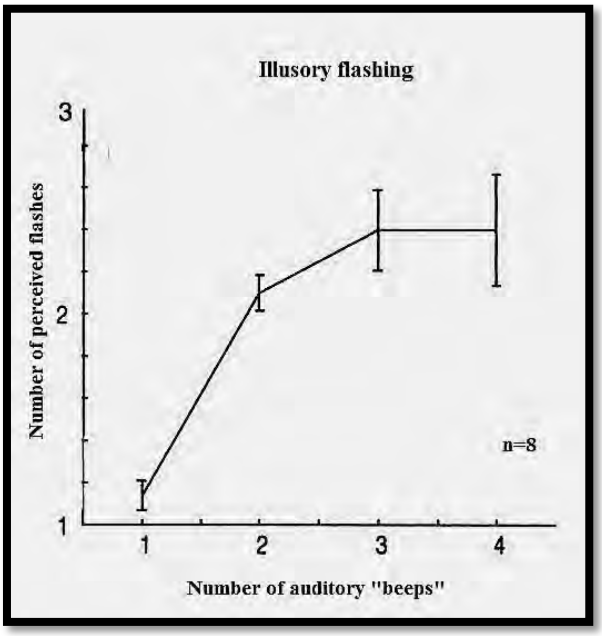


**Figure 14: Illusory flashing. Modified from Shams et al., 2002**

Until now we discussed alteration between the vision and the hearing perception, however other modalities, such as the balance or the touch, can be altered. We shall illustrate this by the next two examples.

- **Oculogravic illusion**: This illusion is due to a combined effect of gravity and when the body of a subject is pushed by an acceleration This occurs in fighter pilots for example who get a wrong information from the otolith organs when the aircraft accelerates (or decelerates) giving them a feeling of a false climb/descent.
- **Parchment skin illusion:** This illusion is due to an audiotactile interaction and happens when the sound is synchronized with hand-rubbing. This modifies the tactile sensations (Jousmäki and Hari, 1998).

In conclusion, these few examples and descriptions of illusion phenomena demonstrate that they are obtained under specific conditions (e.g. spatio-temporal conditions, which be treated later), and they are part of the phenomena of cross modal integration.

### I.4.1.2 Benefits

In contrast with the previous approach where environmental parameters are changed in order to mislead the integrative centres (illusions), it was observed that cross-modal integration brings significant benefits (Stein and Meredith, 1993; Kayser et al., 2007a). Indeed, from a behavioural point of view, studies showed that cross-modal integration allows an improvement of RTs (Giard and Peronnet, 1999; Reynolds and Day, 2007; Rowland et al., 2007; Brandwein et al., 2011), an improvement of performances and an increase of detection probabilities of an event (Meredith and Stein, 1986; Giard and Peronnet, 1999; Calvert and Thesen, 2004; Wallace and Stein, 2007; Stein and Stanford, 2008). In everyday life such integrative function happens for example during driving which requires inputs from several modalities among which we find the visual, the auditory, the somatosensory, the vestibular and the motor systems. In particular, the visual system allows us to get a dynamic image of the road; the auditory system helps us to remain alert to the engine and the environment; the somatosensory system sends messages to the SNC about the car's movements, the vestibular system about acceleration, and all that results in activation of the motor system to move and adjust the gears. Interestingly, one can note that such an ability to integrate cross-modal stimuli is not innate but appears through a learning process (McIntosh et al., 1998; Stein and Stanford, 2008). To support this, the study of Wallace and Stein in 1997 demonstrated in the cat that during the first postnatal days (n=10) neurons in the superior colliculus (SC) are unable to process cross-modal stimuli, when animals are raised in darkness (Wallace et al., 2004). Therefore they concluded that early sensory experiences are crucial for developing such ability by the SNC. The acquisition of a senses' synergy through experience have been claimed by Wallace and Stein in 2007 and confirmed by the study of Yu et al. in 2010. In this latter study it was demonstrated that the neuronal responses from the SC deep layers show gradually ability in cross-modal integration as a function of postnatal cross-modal experiences. Without these experiences multisensory neurons have the ability to respond to different modalities but no inputs are integrated. This ability appears during a postnatal period when rapid and numerous changes take place in the neuronal architecture. This period is known as a "sensory" period. In addition it has been noticed that certain multisensory tasks are delayed until the superior colliculus neurons have the capacity for cross-modal integration (Yu et al., 2010). This would suggest learning by stages, which means that the strength of the integrated responses increases gradually as a function of cross-modal experience. This phenomenon may be active as well in reaction times following cross-modal integrations.

### I.4.1.3 Reaction time

The first studies of RTs during psychological experiments appeared at the beginning of the 19th century (Bessel, 1823). Then a new interest for these studies was observed in the 1950s in particular with Dubois-Reymond (Figure 15) who tried to measure the speed of the neuronal transmission. Indeed, RTs' studies are important to investigate the processes between stimuli and their responses. However RTs are subject to certain variability on the basis of different factors:

**Figure 15: Emile Dubois-Reymond**

A) **Cue intensity:** several studies demonstrated that the more the intensity of the stimulus increased the shorter the response time became. This relation was established by Piéron in the 20s (Piéron, 1920) and was formulated as follow: $RT = (a/i^n) + k$ where *i* corresponded to the intensity, *a* to the time, *k* to a constant and *n* to a variable which differed according to the stimulation type and the different conditions. The reaction is influenced by the duration of the signal and by the area that is the spatial field. For example, an increase of the spatial area (=special summation) or/and of time (=temporal summation) improves reaction times.

B) **Sensory modality:** this factor is essentially linked to peripheral mechanisms rather than central processes. It is about:

1) Differences in time relatives to the afferent conduction. For example if we compare the hearing and visual systems, it will take 8 to 10 msec for an auditory

cue to reach the SNC, whereas it will take between 20 and 40 msec for a visual cue to reach the SNC,

2) The change of state which is longer in certain sensory systems. For example, the changes in the visual system are slower compared to those in the hearing system.
3) There is also a significant variability among sensory systems (see Table 2).

**Table 2: Reaction times for different sensory modalities. Table 1.2. modified from Welford (1980)**

| Study | Stimulus | Reaction Time (msec) |
|---|---|---|
| *Woodworth and Schlosberg* (1954) | Light | 180 |
| | Sound | 140 |
| *Robinson* (1934) | Touch | 155 |
| *Kiesow* (1903) | Salt on tip of tongue | 308 |
| | Sugar on tip of tongue | 446 |
| *Baxter and Travis* (1938) | Rotation of the person | 520 |
| | Change in the direction of rotation of the person | 720 |
| *Wright* (1951) | Intense radiant heat | 330 |

C) **Stimulus complexity:** This last point refers to the stimulation information process. The less we have to integrate separate stimulus attributes (e.g. shape, angles, colour), the faster the central organization of the sensory activity.

To complete the picture, here is a brief summary about RT and its measurement:
- First the signal is received by the adequate sense organ then conveyed to the brain via the afferent channel. This stage is relatively short and takes about few milliseconds.
- Second the cue is identified by the brain,
- Third the cue is compared with prior sensory representations and a choice of response is made,
- Fourth: the response is initiated through an action.

So the RT is the time difference between the perception of the signal (step 1 above) and the motor response (step 4 above). One can note that during RT measurement the longest stages are steps 2 and 3. To shorten RT, Hershenson demonstrated in 1962 that using a bimodal stimulation in place of an unimodal stimulation facilitates reactions. This is what we will describe in the following paragraph from a statistical perspective.

### I.4.2 Probabilistic models

Historically the decrease of RT observed in multisensory contexts has been linked to a facilitation induced by two or more different stimuli and described as the redundant signal effect (RSE). This effect has been studied through three different classes of models:

1) The ***race model*** (Raab, 1962), or the separate activation model, in which it is assumed that shorter RTs are linked to the modality which first detects the signal. In this model, the facilitation in response to two competing stimuli is predicted according to a statistical phenomenon, described as a probability summation. Indeed, and as written in Cappe et al., 2010:"the likelihood of either of the two stimuli yielding a fast RT is higher than that from either stimulus alone". Consequently it is the reaction times of the fastest modality that is considered. The weakness of this model is that it does not consider neural convergence and interactions.

2) The ***coactivation model*** (Miller, 1982) predicts that the interaction between unimodal channels happens somewhere in the processing system before the initiation of the behavioural response. A possible integrative phenomenon is tested with Miller's inequality which predicts that the cumulative response time distribution for the combined stimuli never exceeds the summed distribution of the single stimuli. The equation is expressed as following : $P(\mathrm{RT(VA)}) < (P(\mathrm{RT(A)}) + P(\mathrm{RT(V)})) - (P(\mathrm{RT(A)}) \times P(\mathrm{RT(V)}))$ (Corballis, 1998; Cappe et al., 2010 a). Violation of this inequality demonstrates the presence of integrative processes.

3) The ***time-window-of-integration (TWIN) model*** (Colonius et al., 2004, 2009). This model is a combination of the two previous ones and is based on three stages' assumptions:

A° *First Stage Assumption*: It corresponds to the early sensory processing, where there is a "race" among the peripheral neural excitations in the different sensory pathways generated by the cross-modal stimulus.

B° *Second Stage Assumption*: It corresponds to the neuronal integration of the different inputs and the preparation of the adequate response. For example, in the case of saccadic eye movements, this stage comprises the preparation of an ocular motor response.

The multisensory effect acts at the processing time level, either by a decrease or by an increase.

C° *Time-Window-of-Integration Assumption*: The “window of integration” corresponds to a given period in which the first stage’s process must be finished. In this case a multisensory integration occurs. This “window of integration” can be considered as a temporal filter between the various sensory inputs which determines the multisensory integration. As a consequence, this model allows fixing a temporal rule during multisensory integration which was not the case in the first two models.

### I.4.3 Spatiotemporal perspective

As shown in the previous paragraphs, the behavioural effects of cross-modal integration have been well demonstrated by different authors. In the following section, we would like to recall about the multisensory effects which are dependent on spatiotemporal relations between the different sensory inputs (Wallace et al., 2004).

1° **Temporal influence**. With regard to the present work, we will focus on auditory and visual stimuli. In this context, one can note that a facilitation (=latency reduction) happens regarding the timing of the visual versus the auditory stimulus. Most of the studies using an audiovisual combination reported a facilitation when the auditory stimulus was presented after the visual stimulus (Miller, 1986; Slutsky and Recanzone, 2001). In contrast, Frens and al. (1995) found that the optimal or maximal facilitation was obtained when the auditory stimulus was presented slightly before or synchronously with the visual stimulus. The range of the various temporal intervals which can engender a beneficial effect, that is facilitation, is known in terms of “temporal binding window” (TBW) (Stevenson and Wallace, 2013). The TBW could be defined as follow: this is the “range of stimulus onset asynchronies [SOAs] in which multiple sensory inputs have a high probability of altering responses” (Stevenson and Wallace, 2013). The TBW can be modulated according to two characteristics: the type of stimulus and the task chosen. In the case of asynchrony discrimination between the presentation of an auditory and a visual stimulus, the subjects can present a certain tolerance. In other words, the subjects cannot perceive the asynchrony as it is. This tolerance applies when the visual stimulus precedes the auditory stimulus. It happens when the auditory stimulus is speech (Dixon and Spitz, 1980) since there is a difference of speed propagation between the vision and sound waves. Indeed the brain learns how to manage this discrepancy and take into account the lower speed propagation of the sound wave. In case of an auditory stimulus precedes a visual stimulus, such effect of tolerance has no impact. Consequently the asynchrony is better detected.

According to several authors (Stevenson et al., 2010; Vroomen and Keetels, 2010), the temporal relationship between stimuli is a very important criterion in the way inputs are integrated and what strength is resulting from this integration. This importance can be clearly seen when the temporal processing between modalities is impaired as it is the case in patients affected by autism or dyslexia.

2° **Spatial influence**. In parallel to these temporal aspects, the facilitation is also dependent on the spatial origin of the various stimuli. According to Stein et al. (1988) (study conducted in cats with an orientation/reaction task) the best performance is obtained when the cues of various modalities are presented in a spatial alignment. In other words spatial scattering tends to diminish performances when some multisensory integration is happening. This is supported by some studies like the one of Lee et al. (1991) which demonstrates that saccadic RTs decrease when a tone (auditory cue) was spatially aligned with the visual targets.

This section confirms that more detailed studies concerning the role of spatiotemporal conditions in multisensory integration are necessary. Besides, some knowledge keeps growing on the different types of crossmodal integration and this will be the topic of the next part.

## 1.4.4 Types of crossmodal integration

### 1.4.4.1 Stochastic resonance

The discovery of stochastic resonance is quite recent (beginning of 80s) and is a phenomenon whereby a signal becomes better identifiable (enhanced) if noise is added to it (Harper, 1979). In the context of multisensory integration Harper demonstrated in 1979 that the use of a white noise could facilitate the sensibility to visual flicker in human subjects. More recently Manjarrez et al. (2007) and Sasaki et al. (2008) reached the same conclusions. According to Manjarrez et al. (2007) a sub-threshold visual stimulus (flashes) is enhanced when a certain amount of white noise is added. In contrast, the same visual stimulus delivered in supra-threshold conditions does not improve the perception. To explain this phenomenon three neural hypotheses have been suggested:

1° Synaptic convergence: This facilitation can be described as the synaptic convergence of inputs from different modalities on multisensory neurons, such as the auditory and visual afferent inputs. This is in line with the evidence formulated for the SC (in the § I.4.5.3.1) in which multisensory neurons exhibit an overlap of different receptive fields; in this case auditory and visual (e.g. Wallace et al., 1993).

2° Impact on the "dynamic range" of SC neurons: We can recall that the dynamic range is the stimulus intensity range within which neurons are discharging and which goes from threshold up to saturation (May and Sachs, 1992; Manjarrez et al., 2007). Therefore according to the latter authors the addition of white noise could spread the dynamic range of multisensory neurons. This view is perfectly in line with studies from Perrault et al. (2003; 2005) who reported that the integrative function of a neuron is correlated to its dynamic range As a consequence, every unisensory dynamic range is smaller than multisensory dynamic ranges.

3° Coherence Resonance or internal stochastic resonance (Manjarrez et al., 2002): To show a stochastic resonance the brain needs a certain amount of external noise plus a stimulus. However non-linear systems can, in case of no external stimuli, still engender a stochastic resonance which results for example in a behavior. This concept refers to the notion of internal stochastic resonance.

#### 1.4.4.2 Phase coherence

Neural oscillations at the level of large groups of neurons can be observed in electroencephalograms (EEG technique). Such activities generally arise from neurons which synchronize their firing patterns as in sensori-motor and cognitive functions (Sendowski et al., 2008). These oscillations in neuronal ensembles happen at different frequencies than the firing frequency of individual neurons and have been divided in 5 frequency bands:

- the delta activity from 0.5 to 3.5 Hz,
- the theta activity from 4 to 7 Hz,
- the alpha activity from 8 to 12 Hz,
- the beta activity from 13 to 30 Hz,
- the gamma activity which is superior to 30 Hz

It has been demonstrated that gamma activities were involved in sensory modalities like vision, hearing, somatosensory system and olfaction. Therefore the analysis of neuronal signals coherence should allow us to establish the existing relations between sensory modalities. Studies performed with a protocol using audiovisual stimuli (e.g. Stein et al., 1999; Senkowski, 2005) showed an enhancement of the evoked oscillations, in particular the beta waves, during crossmodal stimulations. This enhancement is superior to the sum of unisensory evoked responses in certain brain regions that were called Regions Of Interest (ROI). During the referring to an object with words that has been presented orally or visually, von Stein et al. (1999) demonstrated that during the crossmodal integration there was an enhanced coherence of 13-18 Hz activity (beta waves) between electrodes located on the temporal and parietal cortex. This study does not show an increase in signal amplitude but rather an overlapping of activities originating from different sites. In other words we can speak about an enhancement of the phase-locking between neuronal activities from different cortical areas. In contrast the work of Posner et al. (1988) with a similar task but with PET scan acquisition did not show any overlapping activities between brain areas.

## I.4.5 Neuronal perspective

### I.4.5.1 Bayes integration concept

Before getting into multisensory integration at a neuronal level, I would like first to introduce an approach used since Helmholtz (1911) to describe the processes of integration. That is the Bayesian inference which is a statistical method for describing the systems of perception and cognition in a mathematical way. This approach establishes the probability of an event from the probability of other events which were beforehand estimated. In other words, this theory takes into account the multiple inputs the brain has to manage and which keep varying. While integrating such information the brain creates a coherent perception of our environment. According to the concept of "Bayes optimal integration" (Klemen and Chambers, 2011) certain levels in the brain hold internal probabilistic models of events which are updated by inputs from lower hierarchical level (forward connections). According to these authors “higher order areas, in turn, supply lower level areas with predictions, or in Bayesian terms, “priors” (backward connections). On this basis authors have built the model of cue combination which assumes that the sensory cues originate from the same object (Murray et al., 2012). Integrating information in such a context is beneficial but probably less when the cues originate from two different sources. For example while driving it would be beneficial to perceive a horn noise and to see the car in front of us: we would increase our alertness. However if the horn was originating from a car behind us and we would still see the car in front of us, it would make no sense if our brain was rather integrating these two signals originating from the same object. In this case several modalities are involved and constantly "forward" cues to the brain that must be processed regardless if they result from the same object or from several objects. Sometimes the brain is facing a problem and must decide if it is necessary to combine or not the various signals. For the brain the answer lies in a causal inference to determine if these cues result from one source, an event or from several sources. For that the brain uses several criteria bound to these cues such as the temporal and spatial influence (in the § I.4.3), but also the interactions between these cues. Concerning the temporal and spatial influences, we saw (in the § I.4.3) that the larger the discrepancy between sources is, the less the brain is able to process a unified perception. According to Murray et al. (2012) these discrepancies can be classified according to three classes: 1 ° the fusion when there is no difference (two signals resulting from the same source), 2 ° the partial integration when the difference is moderated and, 3 ° the segregation, when there is a lack of interaction due to a too large discrepancy. When the discrepancy is very low or there is none (fusion in this case) the integration of the cues is from a mathematical point of view linear. When the discrepancy is moderate or large, the integration is not any more linear and a probabilistic model shall be used (Shams et al., 2005; Angelaki et al., 2009; Murray et al., 2012). Such a model allows determining the probability that two modalities interact and the formula is as follow (modified from Shams et al., 2005):

$$P(S1, S2/x1, x2) = \frac{P\,(x1/S1)\;P\,(x2/S2)\;P\,(S1/S2)}{P\,(x1)\;P(x2)}$$

where S1 and S2 corresponds respectively to two sources for the sensory cues x1 and x2; P (x1/S1) and P (x2/S2) represent respectively the likelihood that the cue x results from the source S. P (S1/S2) is a prior probability often called the prior and, representing the events x1 and x2 in the environment. To summarize this model we can write that it allows an estimation of the environment compared with the received cues.

### I.4.5.2 Multisensory integration concept

Anatomically the neocortex is the outside layer of both hemispheres and can be subdivided into three functional types (Masterton and Berkley, 1974): the sensory, the motor and the associative cortex. The neocortex is also organized in 6 layers numbered from I to VI and involved in functions such as the sensory perception, the generation of motor commands, spatial reasoning, thoughts as well as the language. Until the end of the 20th century one thought that the neurons from the sensory areas were strictly unimodal whereas the neurons of the associative superior areas were specific responding to combined stimulations. Following the pioneer work of Stein and Meredith in 1993 in the SC the scientific community accumulated evidences that during cross-modal integration the neurons of the associative areas send signals back to the lower areas (backward connections), to the unisensory areas, as well as in regions involved in the perception and the execution of actions. Different methods have been used to characterize multisensory areas:

A° ***Anatomical***: neuronal tracing studies allowed to establish the various connections between the specific sensory areas (Rouiller et al., 1998; Rouiller and Durif, 2004; Cappe and Barone, 2005; Morel et al., 2005; Cappe et al., 2009).

B° ***Physiological***: the electrophysiological studies allowed to record the responses of the modalities during uni- or multisensory stimulations (Molholm et al., 2002; Cappe et al., 2010; Kayser et al., 2010; Brandwein et al., 2011; Franciotti et al., 2011; Senkowski et al., 2011).

C° ***Imaging***: the studies using imaging allowed to show the various levels of activation of a population of neurons during a stimulation (Calvert et al., 2000; Bremmer et al., 2001; Saito et al., 2005; Doehrmann et al., 2010; Gentile et al., 2011; Naumer et al., 2011; Szycik et al., 2012).

Several studies made with fMRI (e.g. Calvert et al., 2001; Doehrmann et al., 2010) demonstrated that unisensory areas could be activated, or at least modified by stimulations of several senses. This phenomenon was described as "early sensory integration" (Giard and Peronnet, 1999; Molholm et al., 2002) but in fact dated from the 70s. Indeed this idea has been suggested after the recording of auditory responses at the level of the primary visual cortex but the technologies of this period limited the possibilities of double checking therefore this type of research was abandoned.

### I.4.5.3 Multisensory neurons

From a neuronal point of view, the cross-modal integration can be defined as a significant difference between the evoked potentials, number of impulses, during a cross-modal stimulation and the evoked potentials during one of the most effective stimulation (Meredith and Stein, 1983; Wallace and Stein, 2007, Stein and Stanford, 2008). These significant differences are revealed by an enhancement or by a depression of the neuronal response. We can add to this definition the fact that a multisensory neuron is a neuron responding to the stimulation of more than one sensory modality or else a neuronal response to one modality altered significantly by the stimulation of another modality (Wallace and Stein, 2007).

Electrophysiological studies on cross-modal integration have been conducted in both non-human primates (e.g. Groh and Sparks, 1996; Wallace et al., 1996; Basso and Wurtz, 1998; Schroeder et al., 2001; Bell, 2005; Ghazanfar and Schroeder, 2006; Smiley et al., 2007) and humans (Giard and Perronet, 1999; Cappe et al., 2010b Senkowski et al., 2011). Moreover other mammals have been used for this type of research like cats (Gordon, 1973; Middlebrooks and Knudsen, 1984; Meredith and Stein, 1986; Wallace et al., 1993; Wallace and Stein, 1994), ferrets (Bizley and King, 2008) (Meredith et al., 2012), rats (Brett-Green et al., 2003; Skaliora, 2004; Menzel, 2005; Rodgers et al., 2008), mice (Cohen et al., 2011) and guinea pigs (Shore, 2005; Shore et al., 2007). We can note that cross-modal integration studies started in the cat mainly because of the easy access to the brain associative areas for electrophysiological records. Therefore from a historical point of view, the most studied structure is the superior colliculus (SC) (Figure 16). At this site converge signals from visual, auditory and somatosensory modalities, which make this area playing a major role in attention, eyes movement generation and, head and pinnae movements.

#### I.4.5.3.1 Superior colliculus

From an anatomical point of view and according to the arrangement of neuronal soma, the colliculi can be differentiated in various layers. The SC is formed by seven layers (from superficial to deep) as following: the stratum zonale (SZ), the stratum griseum superficiale (SGS), the stratum opticum (SO), the stratum griseum intermedium (SGI), the stratum album intermedium (SAI), the stratum griseum profundum (SGP) and the stratum album profundum (SAP). To simplify, these layers can be grouped in two divisions: the superficial (SZ, SGS and SO) and the deep (SGI, SAI, SGP and SAP) layers. The differentiation of these layers is determined according to two criteria: the colliculi afferences and the neuronal responses engendered by stimulation of different sensory modalities. That is why researchers speak about a laminar representation in the CS. According to Wallace and al. (1996) (Figure 16), during a visual stimulation, the responding cells are located in the superficial layers of the SC whereas cells responding to auditory and/or cross-modal stimuli are more in the deep layers. According to Sparks' study (1986) the superficial layers received inputs from the retina and the visual cortex. Furthermore, in non-human primates a retinotopic map was presented by reference? Where cells of each colliculus were activated by stimuli coming from the contralateral visual field. These neurons responded to stationary and moving stimuli; the latter engendering more important responses. In contrast, the deeper layers, referred to "multisensory layers" (Meredith and Stein, 1986; Meredith et al., 1987; McHaffie et al., 2012) received inputs from ascending and descending unisensory afferent sources from cortical and subcortical areas. These inputs originate from sensory modalities and motor areas determining maps of visual, auditory and somatosensory space (Amlôt et al., 2003). The proportion of SC deep layers cells able to respond to cross-modal stimuli are provided by Meredith et al. (1987) and displayed in Figure 17. Importantly, Spark demonstrated in 1986 that SC deep layers neurons respond to visual stimuli but the proportion of such cells decreases while going deeper into the layers. The largest number of neurons discharges during saccadic eye movements. More precisely, 45 % of cells show an increase of responses, while 20 % of cells show a depression. Among these cells showing a depression 80 % respond only to one type of stimulus. According to Bell and al. (2005), the auditory and cross-modal stimuli can influence the sensory and pre-motor activities in the deep layers of the SC and evoke changes in the behavior. Indeed, according to specific criteria and which will be described in the following sub-chapter, stimuli close to the detection threshold showed a facilitation effect for SC multisensory neurons. Authors suggested a formula allowing to calculate the magnitude of the interaction in percentage (Meredith and Stein, 1986, 1987; Wallace et al., 1996):

$$[(CM\text{-}SM_{max})\ x\ 100\ ] / SM_{max} = \%$$

where CM corresponds to impulses evoked by the cross-modal stimulus and SMmax the number of impulses evoked by the best unisensory stimulus. The obtained magnitude may reveal phenomenon of potentialisation (or superadditivity) when multisensory responses exceed the sum of those evoked by unisensory stimulations (Stanford and Stein, 2007) and phenomenon of sub-additivity when cell responses are decreased (Wallace et al., 1993). These principles are described in more details below.

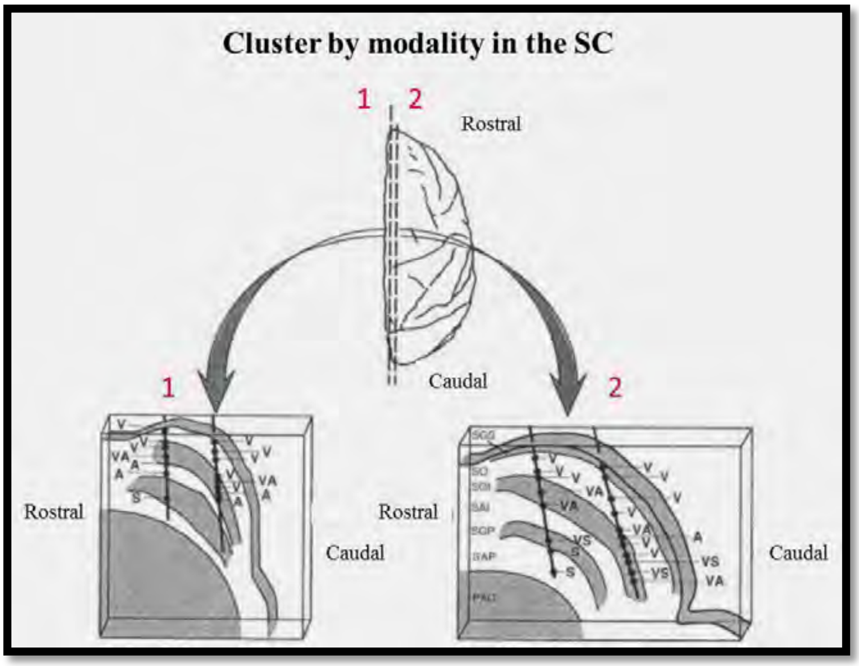


Figure 16: Representation of neurons cluster by modality in the Superior Colliculus: Modified from Wallace et al., 1996.

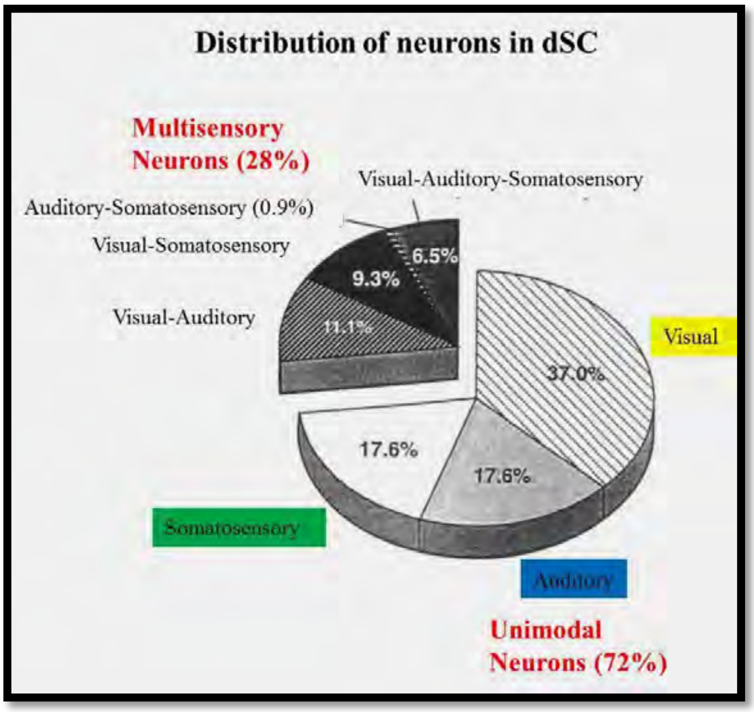


Figure 17: Neurons distribution in dSC (deep layer of superior colliculus). On all neurons (n=108) in deep layers, more than 25% were multisensory. The results demonstrated a predominance of neurons responding to visual stimuli. Modified from Wallace et al., 1996.

### I.4.5.3.2 Multisensory integration principles

First, ***the spatial principle of multisensory integration***: For bimodal neurons, the receptive field of two different modalities very often overlaps and the appropriate stimulation leads to enhanced responses. This is what is called the coincidence spatial principle (Stein, 1998). According to Meredith et al. (1987) it was demonstrated that the more the spatial disparity increases the more bimodal responses decrease.

Second, ***the temporal principle of multisensory integration*** (Figure 18): This principle is related to the synchrony or the asynchrony of stimuli. In particular, the sensitivity of bimodal neurons is dependent on the time between two stimuli. This is about a principle of temporal coincidence (Wallace et al., 1996). A neural response is enhanced during a bimodal stimulation when the time's gap between two stimuli is very short. In contrast, longer time gaps engender normal unisensory responses. However it is important to note that the maximal response is determined by the overlapping of the activity patterns rather than a perfect synchrony (Meredith et al., 1987; Wallace et al., 1996; Kayser et al., 2005; Stein and Stanford, 2008). As a consequence, the larger the temporal disparity between unisensory stimuli is, the fewer enhancements are recorded. Besides, one can wonder how the brain engenders adequate bimodal responses if the stimuli are asynchronous. The answer lies over the duration of neuronal activity induced by the stimulus. Indeed, this neuronal activity will be long enough to allow an overlapping with the neuronal activity resulting from the stimulation of the second modality. According to Meredith et al. (1987), this time window is around 100 msec. The asynchrony between two stimuli may engender three types of responses: an increase, an increase and a decrease, or a decrease. These three types of responses can be found in the same neuron and are dependent on the time gap between the two stimuli. One important aspect in the temporal principle is the overlap of the discharge patterns. For example discharge domains could show 1°) a high discharge rate followed by a fall or 2°) a progressive discharge increase followed by a progressive decrease. This seems to be dependent on the physical properties of the neuron.

To be complete, the time interval between two stimuli of different modalities has an effect on the magnitude of the response, but also on the sign of the integration, namely towards an enhancement or a depression. 4 sub-principles can be established to explain cross-modal integration:

a : maximal levels of response enhancement are generated by overlapping the peak discharge period evoked by each modality.

b : the magnitude of this enhancement decayed monotonically to zero as the peak discharge periods became progressively more temporally disparate.
c : with further increases in temporal disparity, the same stimulus combinations that previously produced enhancement could often produce depression, and
d : these kinds of interactions could frequently be predicted from the discharge trains initiated by each stimulus alone.

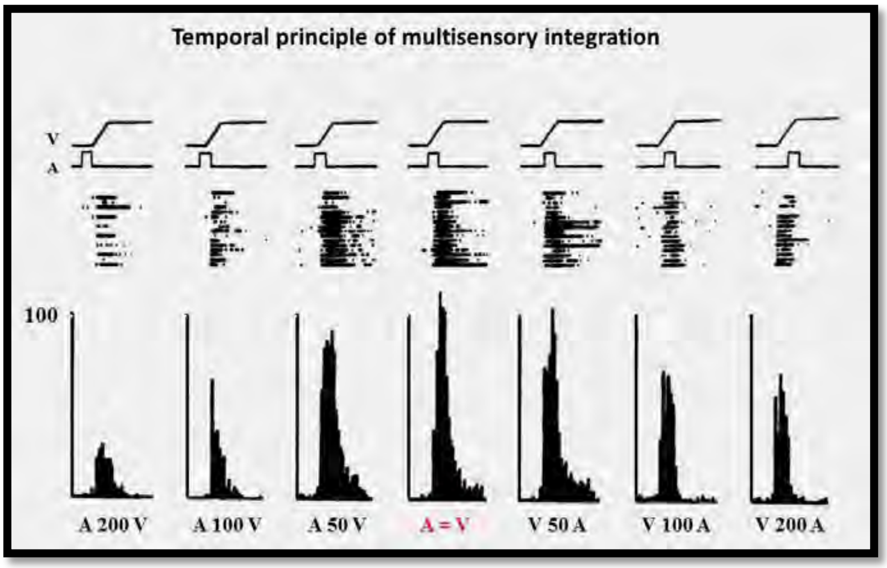


**Figure 18: Decreasing the temporal disparity between 2 stimuli from different sensory modalities can dramatically increase the magnitude of response enhancement. Modified from Meredith et al., 1987.**

Third, the ***Principle of inverse effectiveness***: This principle is linked to stimulus intensity and can be formulated like the closer to threshold the stimuli are, the more the gains produced by the combination will be important (Figure 19; Meredith and Stein, 1986; Stein and Meredith, 1993; Wallace et al., 1996).

According to Carriere et al. (2008), the three principles introduced above do not allow to predict the specificity of a multisensory neuron. Nevertheless, these principles can contribute to determine what type of multisensory integration takes place.

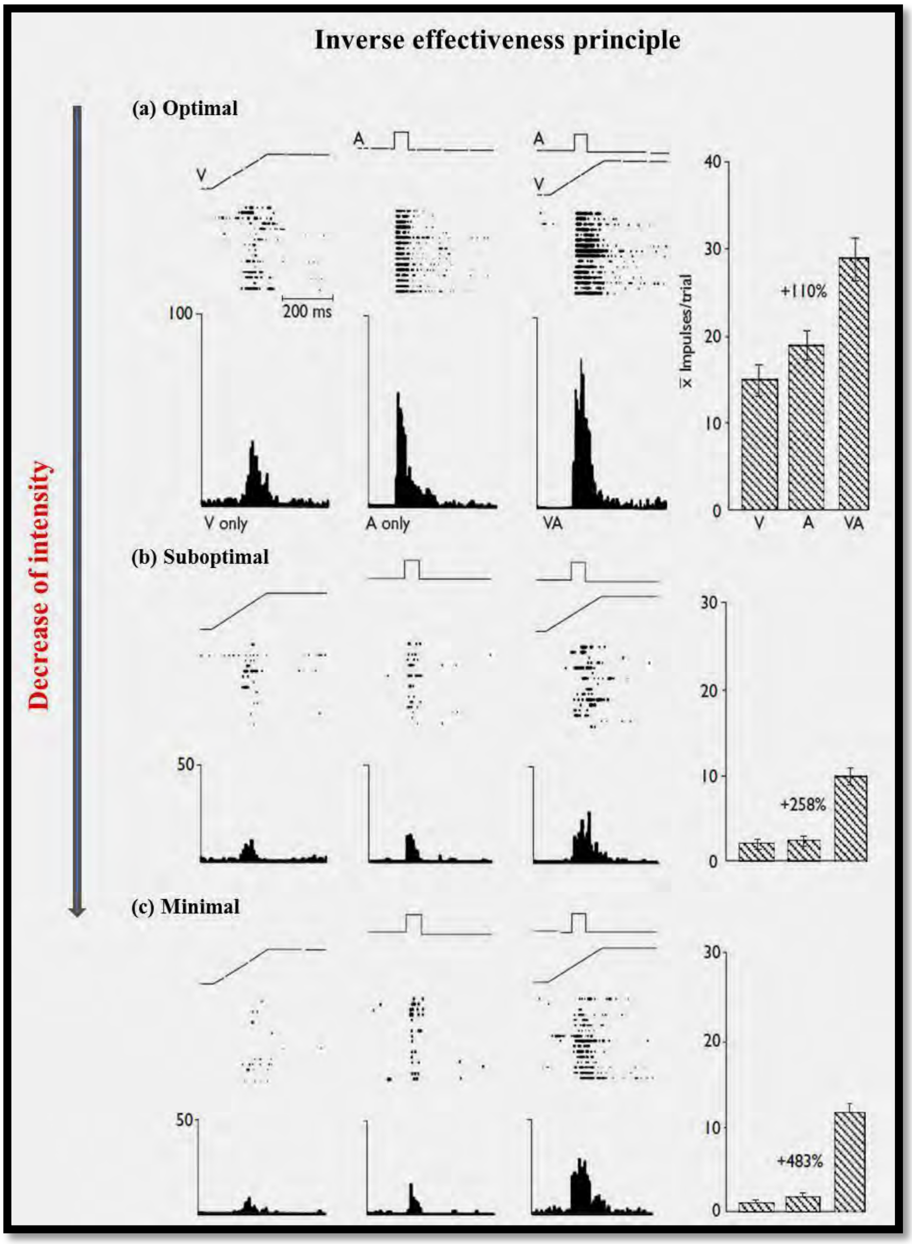


**Figure 19: Inverse effectiveness in multisensory integration. Modified from Stanford and Stein 2007.**

Fourth, ***the receptive field*** of the sensory neuron contributes to multisensory interaction too. Indeed, as stated by Carriere et al. (2008) and Stein and Stanford (2008) the area of sensory space where the multisensory neuron is specificially activated (receptive field) could be modified by another modality to which it responds.

The cross-modal integration intervenes not only in the superior colliculi (SC) but also in other subcortical areas like the basal nuclei.

### 1.4.5.4 The cortical primary sensory areas

For a long time considered as unisensory, evidence are accumulating to demonstrate that cortical primary sensory areas can integrate information of different modalities and treat them in parallel thanks to connections referred as "heteromodal connections". In this case we can speak about early multisensory convergence and we will consider three cortical areas: the auditory, the visual and the somatosensory cortices.

#### I.4.5.4.1 Auditory cortex

The auditory cortex is an area that has been well studied in the multisensory research field. Many other studies showed as well that the auditory cortex is able to process somatensory information (Calvert et al., 1997; Foxe et al., 2002; Murray, 2004), visual cues (Giard and Peronnet, 1999; Foxe et al., 2000; Molholm et al., 2002; King and Walker, 2012), tactile cues (Kayser et al., 2005) and olfactive cues (Cohen et al., 2011). These findings were made in humans, in primates, and also in mice with various methods: electrophysiological recordings (Schroeder and Foxe, 2002; Wallace et al., 2004; Ghazanfar, 2005), functional imaging (Kayser et al., 2005; Pekkola et al., 2005; Kayser et al., 2007b) and magnetoencephalography. From an anatomical point of view, the major multisensory interactions take place at the level of areas surrounding belt and parabelt areas (Figure 18). At first, it seemed that these influences resulted from multisensory neurons from the thalamus projecting on these various areas of the auditory cortex; that is a subcortical origin. These influences could also be due to connections with associative areas such as STS, visual or somatosensory areas. From a physiological point of view, these multisensory integrations allow an improvement of the auditory processing, but also a modulation of the responses from the auditory cortex (Stein and Stanford, 2008). This modulatory effect can be explained by the various visual and somatosenory cortices inputs and originating mainly from layer IV of the auditory cortex (Falchier et al., 2002). This layer is the place where thalamic projections arrive. The studies of Falchier et al. (2010) and Stein and Stanford (2008) demonstrate that various projections from visual areas arrive on the auditory areas. This anatomical study shows that area V2 and prostriata send projections to various auditory areas, among which we find the caudal parabelt of the auditory area (CPB) and the temporoparietal area (Tpt) (Figure 18). Furthermore, according to an older study of Falchier et al. (2002) it would seem that the visual inputs can influence cells at the level of the areas of the belt areas as well as A1. In complement to this study, Kayser et al. (2007) demonstrated by functional imaging that area CPB and Tpt (incorporated into CPB in this study) were highly activated. That was not the case for areas A1 and CM (Kayser et al., 2007). Nevertheless, the study of Kayser et al. (2009a) (Kayser et al., 2009) demonstrate a multisensory interaction at the level of A1. It is however necessary to specify that this interaction takes place only in awake animals.

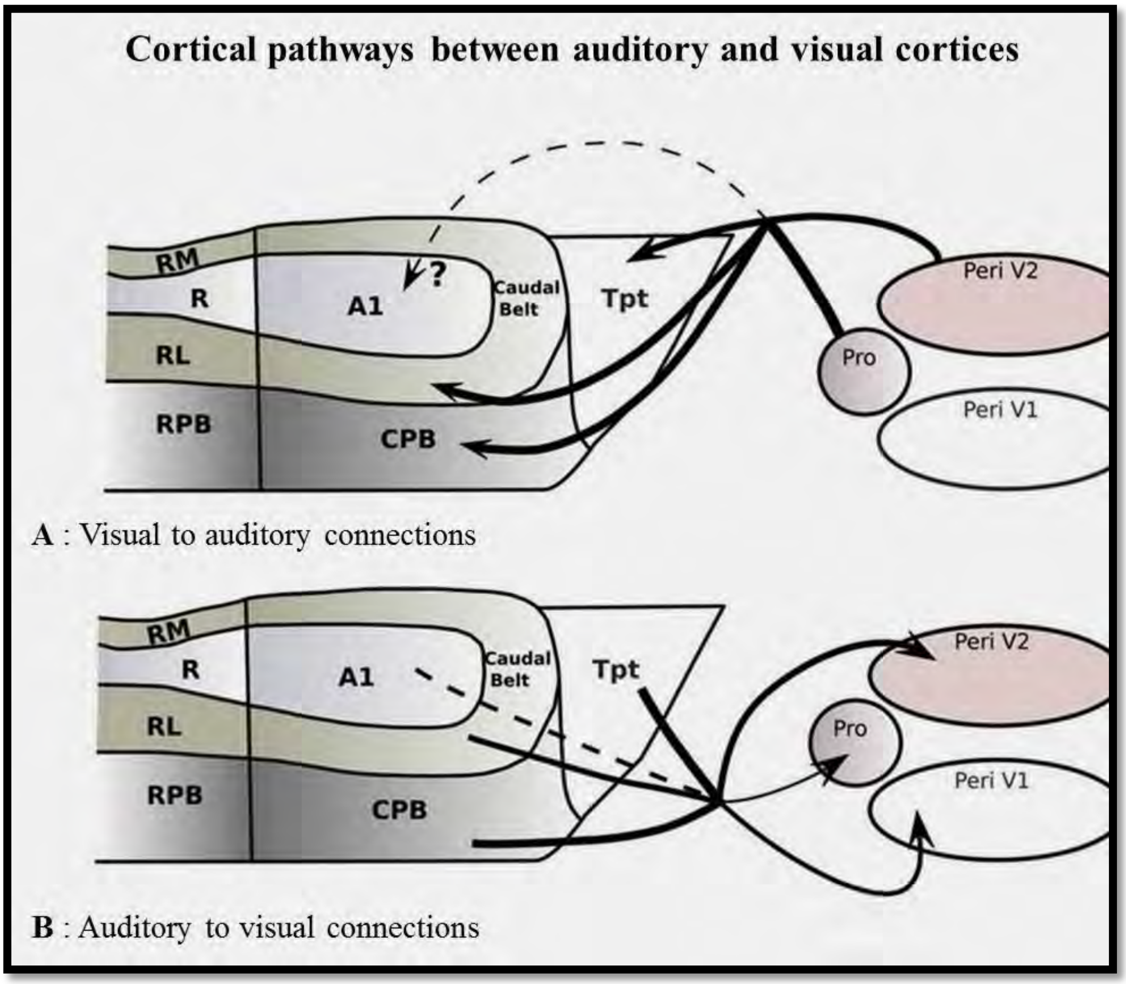


**Figure 20: Representation of different cortical pathways between auditory and visual cortices. From Falchier et al. (2010).**

### I.4.5.4.2 Visual cortex

Similarly to the auditory cortex, the visual cortex is well studied in the multisensory research field. The proofs concerning a non-visual integration at the level of this region were brought by anatomical studies but also with electrophysiological studies (Giard and Peronnet, 1999; Calvert et al., 2001). Previous studies (Morrell, 1972; Fishman and Michael, 1973) have already demonstrated that neurons of the visual cortex could respond to stimuli other than visual. The study of Morrell (1972) demonstrated that in the visual cortex of the cat approximately 41% of neurons showed an activation during an acoustic stimulation. Another interesting point from this study was that these neurons expressed a receptive field which was aligned with the visual receptive field. Consequently there was a spatial specificity of these neurons. However the physiological roles of these activations are unknown. In the study of Fishman and Michael (1973), also conducted in the cat, 38% of neurons responded to both acoustic and visual stimuli. Similarly Morrell (1972) demonstrated that these bimodal neurons expressed a coincidence between visual and auditory receptive fields. In addition, this study suggested that these neurons played a role in spatial localization of stimuli. Another study by Miller and Vogt (1984) , demonstrated auditory projections on the visual cortex of the rat.

More recent studies (Giard and Peronnet, 1999; Falchier et al., 2002; Rockland, 2003; Cappe and Barone, 2005; Martuzzi et al., 2007; Wang et al., 2008) also demonstrated multimodal integration at the level of the visual cortex in both humans primates. In a more general study showing the multisensory interactions at the level of the primary cortices, Martuzzi et al. (2006) demonstrated that the primary cortices responded to the stimulation of other sensory systems via thanks to the BOLD Dynamics technique (Figure 19). Using BOLD dynamics helped to show a correlation between the BOLD peak latency and reaction times. In other words, during bimodal stimulation, shorter RTs are observed (facilitation), and a shift in the BOLD peak latency. Consequently, this study confirms the idea of multisensory convergence.

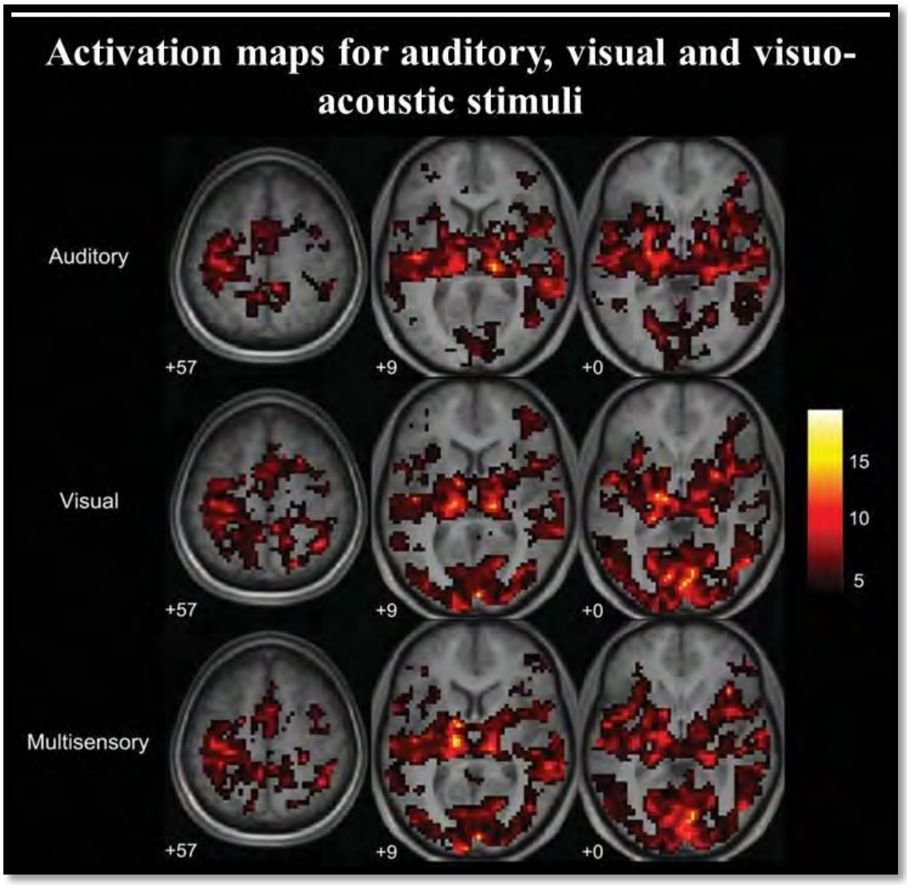


**Figure 21: Representation of an activation map for each type of stimulation. Each stimulus activates the primary sensory cortices. Modified from Martuzzi et al. (2006)**

Numerous studies concerning the audiovisual convergence in the visual cortex result from anatomical studies, such as Falchier et al. (2002), Rockland and Ojima (2003), Cappe and Barone (2005). Rockland and Ojima (2003) study shows direct connections between auditory cortex and V1 and V2 areas. Associative areas of the parietal bone association cortices, such as PG (of area Brodman 7a) and PF, have projections to the calcarine fissure. For reminder, PG and PF, due to their convergence of visual and proprioceptive information, allows the localization of an object relative to the body. Here are the results obtained after neuronal trackers injection in various sites of the auditory cortex of macaque monkeys (Table 3) (*Macaca mulatta*) (Rockland and Ojima, 2003).

**Table 3: Results obtained after tracers injections in the caudolateral and middle lateral belt of macaque monkeys. From Rockland and Ojima (2003).**

| Injection sites | Number of injections | Projections |
|---|---|---|
| Caudolateral belt | 3 injections | In dorsal V2<br>Sparsely to V1 |
| Middle lateral belt | 1 injection | In V2<br>Sparsely to V1 |

This study also reveals that the auditory projections end in layers 1 and 6 of the cortices V1 and V2. This observation follows the conclusions made by Falchier et al. (2002). Consequently this type of connection can be classified "as connection feedback" or "top - down connection".

### I.4.5.4.3 Somatosensory cortex

With regard to the theme of this thesis, it turns out that very few studies investigated the question of the visuo-acoustic integration at the level of the somatosensory cortex. However study in the non-human primate revealed that some somatosensory neurons responded to audiovisual stimuli and tactile stimuli (Zhou and Fuster, 2000; 2004).

### I.4.5.4.4 Premotor cortex

From an anatomical point of view, the premotor cortex (area of Brodmann 6) or "secondary motor cortex" (M2), is situated in the frontal lobe, and it is part of the motor cortex in the large sense. The premotor cortex is clearly distinct from the primary motor cortex (area of Brodmann 4 or M1). More precisely, the area 6 of Brodmann comprises the so-called SMA (Supplementary Motor Area, located in the mesial area 6) and PM (**Premotor Cortex**, located in the lateral area 6). The premotor cortex PM can be divided into two parts: the ventral premotor area (PMv) and the dorsal premotor area (PMd) (Kurata, 1991). These two regions can be subdivided further into two parts, the rostral part and the caudal part thus corresponding to 4 areas: PMv-r, PMv-c, PMd-r and PMd-c (see e.g. Boussaoud et al., 2005). From a physiological point of view, the premotor cortex plays an important role in the control of the movement in response to various external sensory stimuli (Weinrich and Wise, 1982;

Godschalk et al., 1985; Wise et al., 1986). As reported by Roland et al. (Roland et al., 1980): the premotor cortex in man "is activated when a new motor program is established… or when the motor program is changed on the basis of sensory information".

As reported for the non-human primate, the premotor cortex is a site of convergence of visual, auditory, tactile and somoatosensory inputs (Graziano, 1999; Graziano et al., 1999; Graziano, 2001). Nevertheless, 73% of the neurons recorded in PM are synchronized with the movement, that is they have a clear motor role (Weinrich and Wise, 1982). It was defined by Weinrich and Wise (1982) that the time to introduce a movement from a cue of initiation, that is the reaction time, is in the order of 300 msec. In their experimental paradigm, the motor time was 173 msec on average. This information concerning the timing of movements triggered by external stimuli is important in the context of the present thesis: see chapter «Results».

**1° PMv:** The ventral premotor cortex, divided into PMv-r and PMv-c, referred to also as areas F5 and F4 (see Matelli et al., 1989), are electrically micro-excitable. These two areas contain the representations of various parts of the body, mainly the arm, the neck, the hand and mouth movements. The majority of neurons respond to the tactile stimuli (Hamadijida's Ph.D Thesis). However, approximately 40 % of neurons respond to visual stimuli (Graziano et al., 1999). The responses of these bimodal neurons (Fogassi et al., 1996) exhibit a concordance as for their receptive fields (Figure 22). Interestingly, there are even trimodal neurons in PMv, responding to tactile, visual and acoustic stimuli. The auditory receptive field overlaps with the visual and tactile receptive fields. We can also mention that the response in 34 % of these neurons is not influenced by the acoustic intensity, but rather by the distance between the sound source and the head of the monkey: beyond 10 cms, the sound does not produce any response. 34 % of these trimodal neurons are influenced by the sound amplitude but not by the distance. 25 % are influenced by the sound amplitude and by the distance. In summary, these trimodal neurons have the capacity to determine the distance of the sound source. From an electrophysiological point of view, the response latencies to sound are found in a range comparable to the latencies observed in response to visual stimuli, from 45 to 200 msec. A major characteristic of PMv, mainly it rostral part (PMv-r or area F4) is the presence of the so-called "mirrors neurons", reported by Rizzolatti and his colleagues. These neurons discharge in a comparable way when the animal executes a movement or when it observes the movement made by others, such as the experimenter or another monkey (Rizzolatti et al., 1996).

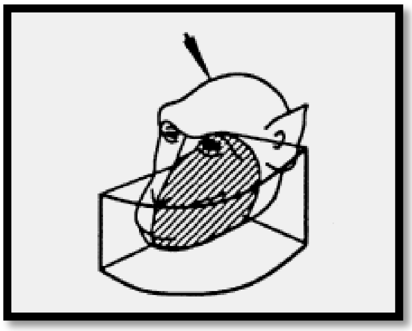


**Figure 22: Representation of the receptive fields of a bimodal neurons in PMv. From Graziano (1999).**

There are important projections reaching PMv originating from the parietal cortex (Matelli et al., 1986), in particular the area 7a. For reminder, the area 7a plays a role in the visuo-motor coordination, due to convergence between vision and proprioception. PMv also receives projections of the anterior intraparietal area (AIP). The neurons in AIP code for the shape, the orientation, as well as the size of objects manipulated by the subject. Consequently, this area plays a major role in the planning of the movements such as grasping of objects and for their manipulation based on visual inputs. We can also mention that PMv receives projections from the prefrontal cortex (PFc) and, of course, there are reciprocal connections between PMv other motor cortical areas (M1, SMA, cingulate motor cortex). Subcortically, the premotor areas receive strong connections from the thalamus, mainly the motor thalamus but also other thalamic nuclei to a lesser extent (see e.g. Morel et al., 2005). These projections are reciprocal, as PMv projects back to the thalamus, giving rise to corticothalamic projections (e.g. Kermadi et al., 1998). According to Markowitsch (1987) (Markowitsch et al., 1987), the thalamo-premotor projections are less numerous on PMv than on PMd. In contrast, the projections of the pulvinar on PMv are denser than on PMd.

**2° PMd:** From a physiological and anatomical point of view, there are similarities between PMd and PMv. Indeed, the PMd is formed by two areas: areas F7 and F2 (Matelli et al., 1989), corresponding respectively to PMd-r and PMd-c (see Hamadjida, Thesis). These areas, also electrically microexcitable (though F7 much less than F2), contain the following main representations: arm and foot. Studies in the 80s demonstrated that PMd plays an important role in visuo-motor tasks. The studies of Weinrich (Weinrich and Wise, 1982; Weinrich et al., 1984) demonstrated that neurons in PMd exhibit a modulation of their discharge during a visual stimulus, when the latter had a motor significance. From the electrophysiological point of view, the latency in response to a visual stimulation is below

250 msec (Godschalk et al., 1981; 1985). The influence of auditory stimuli is less prominent. Indeed, a study of Weinrich (Weinrich and Wise, 1982) showed that only a restricted number of neurons, 2 out of 87, responded to an acoustic stimulation. In summary, there is little auditory inputs to PMd, clearly less than to PMv. Nevertheless, Vaadia (1989) (Vaadia, 1989) demonstrated that there were responses to the auditory stimuli in PMd, however these are present only when the acoustic signal is used to trigger a movement. It is the same for the visual stimuli.

PMd also receives projections originating from parietal areas, such as PO (area parieto-occipital) and MIP (area intraparietal median) (Wise and al., 1997). Here are some reminders concerning these two parietal areas. PO contains neurons with receptive fields sensitive to the orientation and the direction of stimuli. As for MIP, the neurons are sensitive to the visual stimuli and/or to somatosensory stimuli. With respect to the connections with the primary motor area (M1), only PMd-c (F2) receives inputs from M1. These connections are reciprocal, that is PMd-c projects to M1. On the other hand, PMd-r (F7) is not connected with M1 (Ghosh and Gattera, 1995)( Jouffrais, Thesis). In contrast, PMd-c has no connection with the prefrontal cortex, whereas PMd-r is connected with various areas of the prefrontal cortex (Ghosh and Gattera, 1995; Jouffrais, Thesis). As mentioned in the part concerning the connections of PMv, PMd is also connected with the thalamus (see e.g. Morel et al., 2005).

### I.4.6 Audio-visual interactions

The present chapter is focused on audiovisual interactions (the topic of the present thesis), although multisensory interactions are very diverse, such as visuo-tactile (Botvinick and Cohen, 1998; Zhou and Fuster, 2000; Gori et al., 2011), audio-visual interactions (Giard and Peronnet, 1999; Fort et al., 2002; Molholm et al., 2002; Cappe et al., 2010; Doehrmann et al., 2010; Naumer et al., 2011; Gori, 2012; Lanz et al., 2013b) or auditory-somatosensory interactions (Foxe et al., 2002; Murray, 2005; Sperdin, 2009).

We begin here with the presentation of the “pioneering” work by Giard and Peronnet (1999), demonstrating behavioral and electrophysiological data during an audiovisual task of objects recognition in human subjects. The visual stimuli consisted in a circle deformed horizontally ("visual" object A) or vertically ("visual" object B). The auditory stimuli presented a pure tone of 540 Hz ("auditory" object A) or 560 Hz ("auditory" object B). For this protocol, the stimuli were presented either in an unisensory condition (auditory or visual) or in a combined condition, "visual" and " auditory " objects A or the "visual " and " auditory" objects B. Briefly, the subject had to differentiate for every trial which object A or B was presented by pressing one of the two available buttons (a button for objects A and one for objects B). The behavioral results are consistent with data in the literature, namely a shorter reaction time was observed when the modalities were combined. The average reaction times were 562 msec during crossmodal stimulation and, 621 and 631 msec during auditory or visual stimulation alone, respectively (Giard and Peronnet, 1999). Besides these behavioral results, the study was also interested in the attention of the subjects to stimuli. Some subjects were more attentive when the unisensory stimulus was auditory, while others were more aware to a visual stimulus. This attentive bias, derived from the reaction times to responses during unisensory stimulus was referred to as sensory dominance, and two groups were defined: 1) Subjects with a visual dominance (visual TR < auditory TR) and 2) Subjects with an auditory dominance (auditory TR < visual TR). This differentiation in two groups is very interesting for the subsequent part of the study by Giard and Peronnet. Indeed, and additionally to these behavioral results, these authors measured the neural activity using EEGs.

The EEG results showed a neuronal interaction 40 msec after the onset of the crossmodal stimulation. This interaction evoked an early activity during the multisensory integration. The sensory dominance analysis made by Giard and Peronnet (1999) allowed enhancing the very interesting effects of the integration at the level of the visual cortex. This interaction was identified by the differentiation of EPs (evoked potentials) in response to crossmodal stimuli and the sum of EPs in response to the unisensory stimuli: [AV-(A+V)]. Here are the conclusions (Figure 23):

- Human subjects with visual dominance: the early interactions took place between 90 and 105 msec (significant interaction) in these temporal regions. These results suggested an increased activity in the auditory cortex by the addition of the visual stimulus.
- People with auditory dominance. The early interactions took place between 40 and 150 msec in the occipital regions.

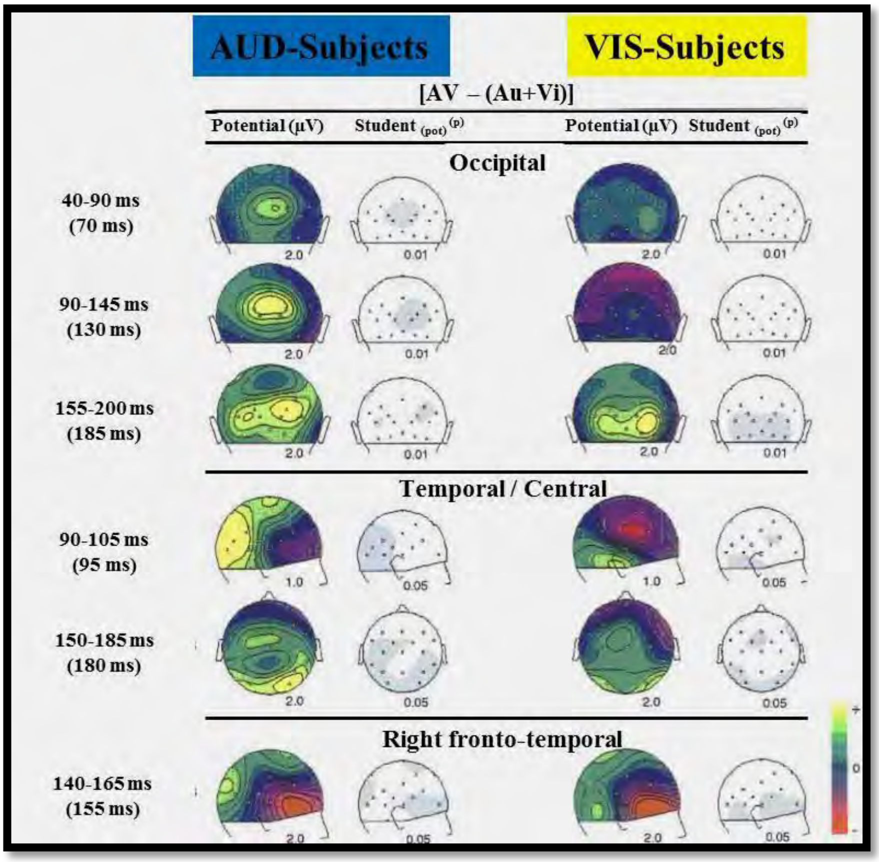


**Figure 23: Modified representation of ERPs topography obtained between 40-200 msec. by Giard and Peronnet (1999). The different maps represent the difference between sensory dominance subjects obtained between the AV condition minus the sum of the unimodal conditions.**

Consequently, the conclusion of these experiments was: "the interactions in the sensory cortex are more important in the cortex of the non-dominant modality» (Giard and Peronnet, 1999; Fort's Thesis, 2002). Other studies, more recent, revealed this early activity as well (Molholm et al., 2002; Talsma et al., 2007; Senkowski et al., 2011). The study of Molholm et al., (2002) aimed at confirming the work of Giard and Peronnet (1999), while simplifying the behavioral task. As Giard and Peronnet (1999), the behavioral results of Molholm et al. demonstrated a significant decrease of the reaction time during crossmodal stimulation. As Giard and Peronnet, and more recently Sun and al. (2009), the study of Molholm et al. (2002) demonstrated an early activity starting from 45 msec after the combined stimulation. This activity also took place at the level of the visual cortex. The authors explained this similarity of early activity in the visual cortex in two ways: a direct or an indirect pathway. Before introducing these two interpretations, we allow to remind briefly that during an auditory stimulation, the auditory cortex is activated after 15-20 msec, to be transmitted further (the auditory processing stream). Therefore, the information (inputs) is transmitted to the visual cortex via two possible pathways:

- Direct pathway: it would be direct connections between the auditory cortices and the visual cortex/cortices, for instance between the areas of the parabelt and V2. These projections are collectively called feed-forward projection or «heteromodal» projections.
- Indirect pathway: it would be an indirect pathway, which involves a relay between the auditory cortex and the visual cortex. In this case, feed-forward projections would reach associative areas (or multisensory convergence areas), such as STS, IPS, and would afterward be transmitted to the visual cortex via feedback projections.

These two postulates of Molholm and al. (2002) were integrated into the van Atteveldt and al. (2013) study that summarizes three alternatives supporting the idea of integration in low-level sensory cortices. The first two are the same as those of Molholm and al. (2002), that is a direct and an indirect pathway. It is obvious that these postulates were also considered by other authors, such as Cappe and al. (2010) and Schroeder and al. (Schroeder et al., 2004). The third alternative lies in interactions of low-level cortices, by feedback projections coming from superior areas (higher-order multisensory cortices). This proposal of Calvert et al. ((Calvert et al., 2001)) corresponds to an indirect pathway as quoted previously, but without feed-forward projections.

According van Atteveldt et al. (2013), the first two proposals are applicable in a complementary way and according to the type of task (detection, identification, discrimination). Complex tasks (such as perception of words or objects identification) would require the indirect pathway, i.e. feedback projections from top level cortical areas (higher-order multisensory cortices) or from multisensory convergence areas. For tasks concerning the localization and/or the detection of stimuli (as in the present thesis) it seems that the involved pathway is the direct one, corresponding to lateral connections (in the § I.4.6) or commonly called heteromodal connections (Cappe et al., 2010), as well as feed-forward connections via the thalamus (in the § I.4.6).

### I.4.7 Hierarchy of integration of cross modal stimuli

This chapter aims at defining the multisensory interactions under a more global aspect, that is how and where the various connections (forward, backward, and laterals) converge (Driver and Noesselt, 2008; Cappe and al., 2009; Klemen and Chambers, 2011). Two dominant models are available in the literature:

1 ° « Parallel stream model »: (Figure 24, left panel). In this model, the multimodal place of integration is situated at the level of the high-order cortical areas. Consequently, every sensory area is specific: a visual input impact on the visual areas. In other words, a crossmodal input does not act on the sensory areas. However, the crossmodal information is returned to the sensory areas via backwards connections, coming from high-order areas.

This model appears a bit "outdated" and is not consistent with what was previously said in this thesis. In particular, the fact that unisensory cortical areas are not any more considered as pure specific area modality. Indeed, this model is challenged as follows: if every crossmodal integration is processed at the level of the superior cortical areas, how come RTs are shorter in response to bimodal stimulation? This question will be treated more detail below.

2° Interconnected model: (Figure 24, right panel). As main difference and according to this second model, the multimodal attributes are integrated already at lower levels (early stage), that is at the level of the sensory areas. Consequently, every level can integrate multimodal information and communicate with the other levels of multisensory processing via connections of various types: forwards, backwards and lateral. The main novelty in this model is the side connection. This type of connections, which puts in connection between the primary sensory areas was put forward in several reports (Lewis and Van Essen, 2000; Rockland and Ojima, 2003; Cappe et al., 2009).

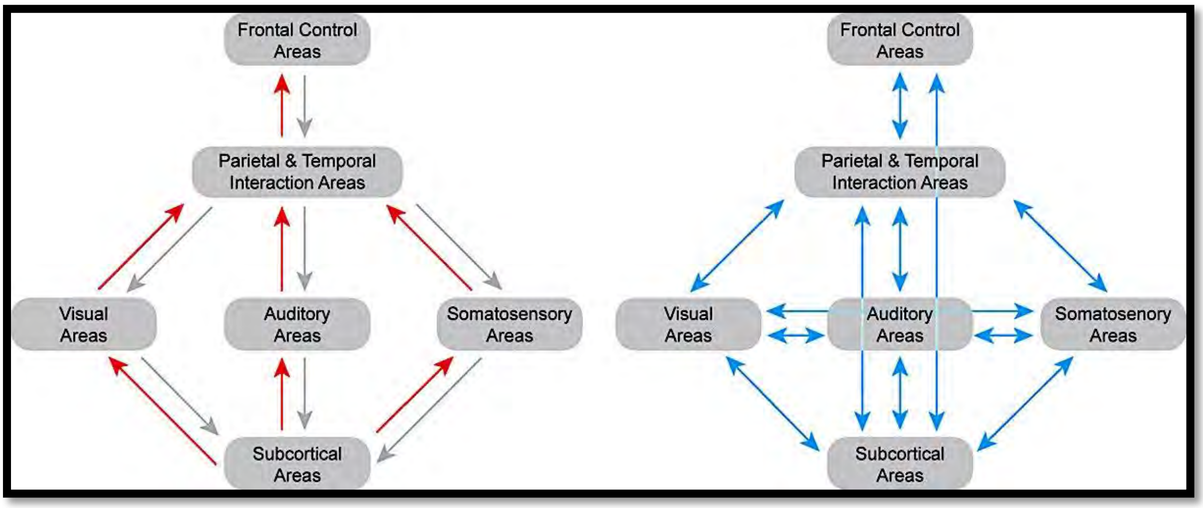


**Figure 24: Comparison of the parallel streams model with interconnected model of multisensory processing. The left panel represents the parallel streams model and the right panel the interconnected model. From Klemen and Chambers (2011).**

## I.5 Objective of the Research

The above general introduction to the present thesis manuscript allowed covering some fundamental aspects concerning the multisensory integration, with emphasis on visuo-acoustic integration. As mentioned throughout this general introduction (in the § I.1, I.2 and I.3), we can understand that each sensory system was developed mainly for the specific type of information it is supposed to process. This modality specificity does not lead to an equal "quality" for the integration of every modality. As demonstrated by Giard and Peronnet (1999), in the course of visuo-acoustic integration, the subjects exhibited a sensory dominance, either an auditory or a visual dominance. This dominance can be demonstrated by the shortest mean of reaction times (RTs) for every modality.

However, in the daily life, it is necessary to integrate these various modalities to process and then to obtain a unified perception of our external environment. This multimodal integration, governed by spatiotemporal relationships (in the § I.4.3), prompted numerous studies (e.g. Giard and Peronnet, 1999; Calvert et al., 2001; Murray et al., 2004), conducted on human subjects, and also on non-human primates or other animal species. These electrophysiological studies allowed to define the various parameters of a multisensory neuron (e.g. super-additivity of SC neurons, Meredith and Stein, 1986), to demonstrate that in specific-modality areas bimodal interactions may occur (e.g. auditory and visuo-acoustic responses in the auditory cortex; Molholm et al., 2002), and to demonstrate that these interactions occurred early during the sensory processing (e.g. ~45 msec post-stimulus; Giard and Peronnet, 1999). Anatomical studies also allowed to determine heteromodal connections between unisensory areas (Cappe et al., 2009b), but also between subcortical structures (e.g. thalamus) and cortical areas (e.g. Morel et al., 2005; Cappe et al., 2009a), possibly contributing as anatomical support for early multimodal interactions.

The initial and general aim of the present Ph.D. thesis was to elucidate some of the electrophysiological bases of the audio-visual integration, in a sensory convergence and sensory-motor integration area, the premotor cortex, in the model of non-human primates (macaque monkeys). It was demonstrated that the visual cortex receives projections/inputs from the auditory cortex and that bimodal neurons respond to auditory stimuli (e.g. Falchier et al., 2002). A recent study also showed that cells in the auditory cortex also respond to visual stimuli (see Celine Cappe’s Ph.D. thesis). In the continuation of this previous work by Céline Cappe, the aim of the present work was to record neuronal activity in the premotor cortex of macaque monkeys during a multisensory-motor integration task. **We hypothesize that the**

**premotor cortex may be a site for rapid (early) convergence of acoustic and visual inputs, leading to faster motor responses (shorter reaction times) triggered by bimodal (visuo-acoustic) stimulation, as compared to motor responses with longer reaction times resulting from unimodal stimulation (visual alone or acoustic alone).** To the best of our knowledge, such investigation on the role of the premotor cortex in a rapid detection of bimodal stimuli is truly original.

To this aim, several preliminary steps are however needed, corresponding to the development of specific methodological skills. Although the behavioural detection protocol was used previously in this laboratory (see Céline Cappe's Ph.D thesis), it was limited by a relatively imprecise control of the visual stimuli. For this reason, the protocol was significantly modified in order to accurately control the intensities of the acoustic and visual stimuli, as well as precisely establish for each monkey or human subject their threshold of perception for each individual modality (in the § II). Indeed, every variable, such as the choice of the auditory, visual, visuo-acoustic stimuli, their intensities, the target, the initiation of the trials by the subject, the software controlling the experiments, were all re-designed in order to improve and validate the entire experimental protocol. A first (though highly time-consuming) step was to train two new macaque monkeys to perform this re-designed detection behavioural protocol and make sure that these monkeys indeed exhibit the expected bimodal behavioural facilitation (shorter RTs in response to visuo-acoustic stimuli than in response to unimodal stimuli). In prevision of a very long lasting investigation in each monkey, a further goal was to develop new technical approaches to implant a chronic headpost fixation device and a chronic recording chamber, with the guarantee that they will stay in place during the several years during which the investigations will be conducted in these 2 monkeys.

Previous anatomical studies (e.g. Morel and al., 2005; Cappe et al., 2009b) demonstrated inputs to the premotor from "sensory" zones of the thalamus, in particular from the pulvinar nucleus, which may represent a very rapid route underlying visuo-motor integration, bypassing separate unimodal information processing in the auditory and visual cortices. The present work represents a first approach in order to validate these anatomically based predictions, by conducting electrophysiological recordings in the premotor cortex (present thesis work) and later on in the thalamus (e.g. pulvinar nucleus). The project in the thalamus would thus be the logical continuation of the present study, to be conducted on the same still alive two monkeys by another investigator.

In parallel to the present project conducted mainly on macaque monkeys, in order to make a bridge with humans, we introduced a feasibility study conducted on few human subjects (in the § II). To this further aim, the same psychophysical protocol, as developed for the non-human primates, was applied to human subjects. The hypothesis is that the multisensory facilitation properties observed in the non-human primates (monkeys; Lanz et al., 2013b) can be reproduced in humans, both on the behavioural and electrophysiological points of view (EEG replacing individual neuronal recordings in the latter case). In other words, the long-term goal is also a matter of translational research. We may compare, for example, non-human primate EEG and behavioural data with human EEG and behavioural data, during the same detection paradigm and, in case of similarity, extrapolate electrophysiology unitary data from the non-human primate to the human.

# II. General Materials and Methods

The general technical aspects, namely materials and methods, used in this thesis will be reported in this chapter. A specific technical refinement, dealing with a new approach to anchor implants on the skull of non-human primates (macaques) will be reported separately, in the form of the first chapter of results in the thesis.

The present general chapter of methods aims at giving an initial and broad outline of the various methodological approaches (subjects, behavior, electrophysiology, surgery) used. More details will be presented in the various chapters of the results, especially in the two articles already published at the time the thesis manuscript was finalized (Lanz et al., 2013 a and b). Consequently, the next sub-chapters will often refer to these two articles for the purpose of not repeating over and over the same information.

All the experiments presented in this thesis were carried out in Professor Eric Rouiller's laboratory, at the department of Medicine, Domain of Physiology and Fribourg Cognition Center, at the University of Fribourg (Switzerland). For non-human primates, all experimental protocols were approved by cantonal and federal Swiss veterinary authorities (veterinary authorizations numbers 173-06, 173-07, 156-04, 156-06, 156-08, 18/10). Furthermore, the experiments were conducted according to both guidelines of the National Institute of Health (Guide for the Care and Use of laboratory Animals, 1996) and of the European Community (Guidelines for Animals Protection and Use for Experimentation). We can also mention the compliance with the ARRIVE guidelines (Animal Research: Reporting *In Vivo* Experiments).

For the new design development of the head fixation device and the chronic recording chamber, collaborations were established with the engineering school (Department of Mecanics) of Fribourg, Medicoat AG (Mägenwil), S+D Scherly (La Roche) and with the Hôpital fribourgeois (HFR) (Department of Radiology).

## II.1 Subjects

### II.1.1 Non-human primates

The topic of this sub-chapter is reported in detail in the article Lanz et al. 2013b (in the § Subjects). Briefly the experiments were conducted on two adult males Macaca *fascicularis*

(Figure 25) coming from our own breeding (a colony of macaques was maintained for breeding in our facility until 2008). One animal is an 11-years-old macaque, weighting around 8.0 kg in 2013 (Mk-LI). The second animal is a 9-years-old macaque, weighting around 8.0 kg in 2013 (Mk-JZ). The animals, grouped by 2 to 5 subjects, were housed in a detention room of 45 $m^3$ (15 $m^3$ until 2010), as previously reported in more detail (Kaeser et al., 2011; Schmidlin et al., 2011). Each monkey was placed under the responsibility of the same experimenter (myself in the present case for the 2 monkeys Mk-LI and Mk-JZ). It is crucial to mention here that before initiating any behavioural training in the laboratory, it was necessary to habituate the monkeys to move voluntarily without intervention of the experimenter from the detention room into a primate chair, allowing the transport of the animal to the laboratory and performing the task in the set-up. This step is highly time consuming (about 3 months) and when properly executed allow the transfer of the animal to the laboratory with stress for the animal and risks for the experimenter. The transfer of the monkeys to the primate chair is illustrated in the form of a video sequence is the article published by Schmidlin et al. (2011).

**Figure 25: Photograph of a *Macaca fascicularis* (Laboratory of Physiology, Fribourg, 2007).**

### II.1.2 Human subjects

In parallel with the studies conducted on the non-human primates, a parallel investigation was conducted on human subjects. Young adults (n=14) aged 20- to 30- years old were enrolled in this study. Ten subjects were enrolled in the behavioural part of the study whereas 4 additional subjects were involved in the combined behavioural/electrophysiological (EEG) protocol. The distribution between genders was equal: 7 females and 7 males. All participants had normal hearing, as confirmed by audiometric threshold evaluation. Furthermore, all participants had normal or corrected-to-normal vision. Human subjects were recruited from the group of friends and colleagues in the laboratory. It is important to mention here that, before the first session, all subjects had no preliminary experience with this protocol. From an ethical point of view, before the first session, informed written and oral consent was obtained from all subjects. An ethical committee approved all procedures.

## II.2 Behavioural Protocol

### II.2.1 Stimuli

This sub-chapter describes the characteristics of the auditory and visual stimuli used for this thesis work, although the same information can be found in the article Lanz et al. 2013b (in the § Stimuli) focused on non-human primate data. For the human subjects, the stimuli used (excepted for the auditory stimulus during one type of session) as well as their calibration were the same as in the monkey experiments. Briefly, a white noise burst of 250 msec. was used as auditory stimulus. Nevertheless, we can mention that the auditory stimulus used during the behavioural sessions in the human subjects was a pure tone burst (1 kHz) of 250 msec. duration. These auditory stimuli were delivered under free-field condition (via two loudspeakers placed laterally with respect to the position of the subject's head). The intensity was chosen according to the protocol and the initial auditory absolute threshold, established previously. In complement, and not mentioned in the article, in the audiometric room used for the experiments, there was a residual unavoidable background noise, presenting a specific spectrum (Figure 26). The spectral properties of the background noise are very important, especially when pure tone bursts were used as stimulus (in our case restricted to the behavioural sessions with human subjects). Indeed, thresholds cannot be lower than the background noise level. Figure 26 illustrates the spectral properties of the background noise present in the audiometric room. As visual stimulus (250 msec. duration), a green light-emitting diode (LED) was used. The choice of a LED was made for two reasons (more details in the related results article). The first one is the opportunity to precisely control the intensity, by changing the voltage of the LED. The second reason is the small size of the LED, forcing the subject to fixate its gaze on the center of the screen.

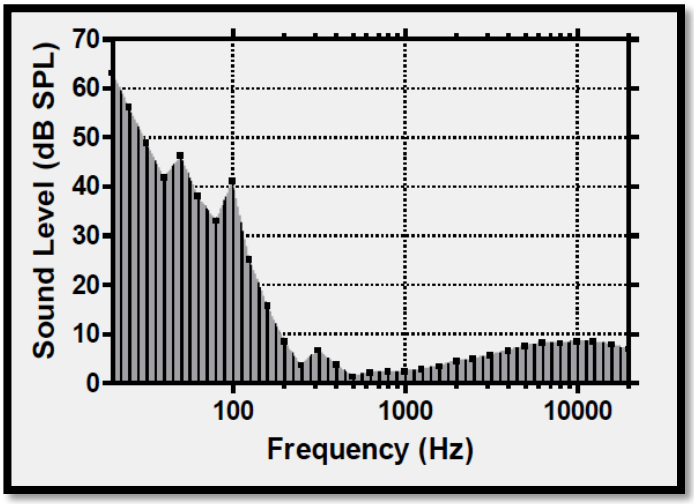
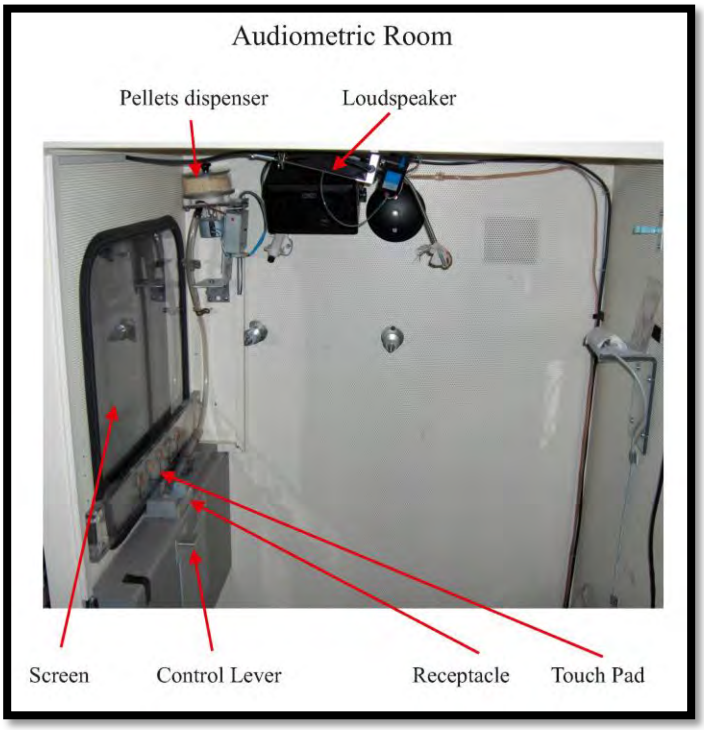


**Figure 26: Representation of the background noise present in the audiometric room.**

### II.2.2 Sensory-motor task

All subjects, the non-human primates and the human subjects, had to carry out a sensory-motor task in a sound-attenuated chamber (Figure 27). To summarize, the subject had to press a lever to initiate a trial, then to release it in order to reach a touch pad in response to a stimulus delivered after a random delay (auditory, visual or visuo-acoustic) (Smith and Olszyk, 1997; Durif et al., 2003; Cappe et al., 2010a). In case of correct response, the animals received a reward in form of food pellets. Wanting to keep exactly the same set-up and protocol for the human subjects, a pellet was also delivered in case of correct response. However, for the human subject, the cue in case of success was the noise of the pellets distributor. This task was used for all the protocols, such as auditory and visual thresholds measurement, but also for the multisensory sessions. More detail information has been reported in the article Lanz et al., 2013b (in the § Sensory-motor task).

**A**



**B**

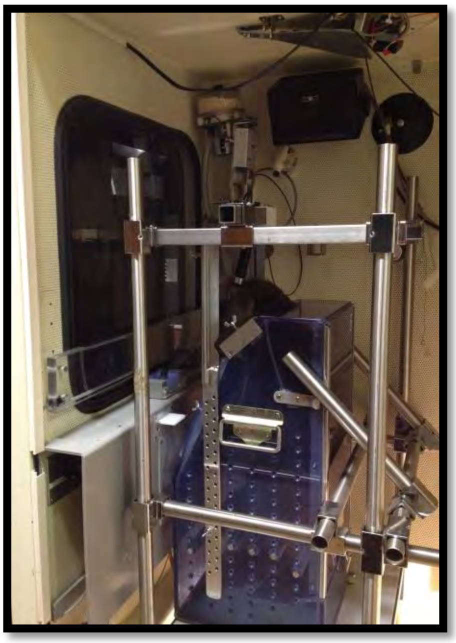


**Figure 27: (A) Photograph of the audiometric set-up equipped for subject's function testing (Laboratory of Physiology, Fribourg, 2007). (B) Photograph of the audiometric set-up with a non-human primate in a primate chair (Laboratory of Physiology, Fribourg, 2013).**

### II.2.3 Auditory and visual threshold

The auditory and visual thresholds were obtained by means of an adaptive staircase method. This method, a psychophysical technique (Levitt, 1971), was a variation of the descending and ascending method of the limits. For the thresholds' search, complementary but essential information must be brought here. Indeed, the period of search for thresholds was not the same between the non-human primates and the human subjects. In the non-human primates, we first established the auditory thresholds. Then, the search for the visual thresholds was carried out during a second time period (Table 4). On the other hand, the auditory and visual thresholds in the human subjects were assessed just before the behavioural multisensory session. Nevertheless, in the course of the electrophysiological recordings, only the auditory thresholds were measured before the EEGs. The chosen visual threshold was an average of the 10 subjects derived from previous behavioural sessions. This procedure was introduced with the aim of decreasing the time spent in the audiometric room and so trying to keep a maximal level of attention.

**Table 4: Information on the different time periods spent for the sensory thresholds' acquisition in the 2 non-human primates.**

| | Auditory | Visual |
|---|---|---|
| **Mk-LI** | 5 months | 4 months |
| **Mk-JZ** | 2 months | 3 months |

To keep coherence in the intensity units, as usually done for the auditory values, the visual thresholds were also expressed in dB. We used this relative unit, because it refers back to a physical quantity without dimension and represents the decimal logarithm of a ratio of two values. The precise description of how intensities were calculated in dB can be found in the article Lanz et al., 2013b (in the § Thresholds assessment).

### II.2.4 Multisensory integration

For multisensory session the same sensory-motor task was used. However during this task the gaze was locked. For non-human primates, a headpost fixation piece was implanted in order to keep the animal's head in a fixed position (see next § II.3). In the human subjects a device similar to that used in optometry was used to restrain head movements. During this multisensory task, the auditory and the visual stimuli were randomly presented individually or in combination. For more detail see the paragraph Multisensory sessions of the article Lanz et al., 2013b.

## II.3 Surgery in non-human primates

As mentioned above, the electrophysiological recordings conducted in non-human primates required a restriction of the monkey's head movements to decrease possible artifacts. To this aim, we modified and developed a head fixation device. The design of this device articulated around a choice of material: the use of titanium screws and an osseo-integration procedure, with the goal to not use orthopedic or dental cement, which is detrimental for the skull. In the same idea, we have developed a new design of chronic recording chamber also using screws and osseo-integration, again without dental or orthopedic cement. All the details concerning these devices, as well as the implantation surgery, post-operative daily care details and results are described in the article Lanz et al., 2013a.

## II.4 Different steps for monkeys behavioural training and CT and MRI acquisition protocol

### II.4.1 Non-human primates behavioural training

This part is intended to describe the training for non-human primates in order for them to perform correctly the multisensory task. This task consisted in responding to three types of stimuli (auditory, visual and visuo-acoustic) individually or in combination. From a general point of view, the various steps required more than one year (variable according to each animal). Therefore the learning was an important and determining phase of the present work.
As a first stage the animal was taught to respond to auditory stimuli with a movement of the hand. Here are the details of the various steps:
1 ° when the animal has been domesticated and comes naturally in the primate's chair (initial stage for every monkey involved in a protocol), we placed it in the experimental room then in the audiometric room without the execution of a specific task. The aim was to win the trust of the animal. This stage lasted few days.
2° the learning phase which was started here required an action from the animal which consisted in a lever manipulation (pressure on a lever). This lever will be used in the final task and will be inducing the trial. First we taught the animal to press the lever in order to receive a reward. This "lever-reward" relation was very important to fix for the continuation of the learning. This training, like all the next phases, firstly took place next to the animal. So the

animal was mimicking our way of activating the lever. Rapidly the animal carried out the task alone in the set-up (consolidation phase) and the researcher supervised the task from the outside via a webcam.

3° the next step  consisted in introducing the notion of lever holding (temporal aspect), when the animal had to keep the lever pressed during a random time. For this the trainer (always next to the animal) was equipped with an electric delivery box allowing the release of a reward at random times. As a consequence the animal learnt how to keep the lever pressed until it received a pellet. Quickly this random time was automatized (thanks to various Matlab and TDT subroutines) and the supervision could be done outside the set-up. This stage required few weeks

4° the next step consisted in introducing a stimulus. The animal did not have to release the lever any more when it received a reward, but it had to release it as soon as it perceived a stimulus. The first stimulus used was an auditory stimulation. At this stage, the animal was free to take the necessary time to respond (no response window or period during which the animal had to respond). The auditory stimuli were chosen among pure tone bursts of various frequencies, noises, different intensities,… .Therefore the animal could in the future respond to any auditory stimulus without an additional learning. After this step, we defined a response window of 800 msec duration. This required several weeks (a few months) until the animal demonstrated a certain stability and reliability in the task execution.

5° afterwards, the last component of the equipment, the touch pad, was introduced. This component allowed us to make sure that the subject well perceived the stimulus. Indeed when the animal perceived the stimulus, it had to release the lever to press a touch pad placed in front of it. This was meant to confirm the perception of the stimulus. A reconditioning from the animal was necessary at this step so that it assimilated the touch pad with the reward. Like all the stages mentioned above, this step was again carried out close to the animal.

6° the next step was the measurement of the auditory thresholds. This stage required a high stability of the animal. The acquisition of such data is described in the publication of Lanz et al., 2013b (in the § Thresholds assessment). The acquisition required several months with the aim of collecting stable thresholds.

As a second stage, the animal was taught to respond to a second modality by using visual stimuli.

7° introducing visual stimuli required to recondition the animal. For this stage we deleted (momentarily) the auditory stimulus which is replaced by a visual stimulus. As during the introduction of the auditory stimulus (step 4°), the animal was free to take the necessary time to respond (no response window or period during which the animal had to respond). The visual stimulus was a flash emitting by a green LED (light-emitting diode). After this step, we defined a response window of 800 msec duration. This required few months until the animal demonstrated a certain stability and reliability in the task execution.

8 ° as soon as the responses to visual stimuli were acquired and stable visual thresholds started to be collected. A description of the measurements can be found in the article of Lanz et al., 2013b (in the § Thresholds assessment).

In the third part of the training the animal was taught to respond to audio-visual stimuli.

9° as what was done with auditory or visual stimuli, visuo-acoustic stimuli were introduced separately. The animal was trained until it demonstrated stability and reliability in its responses.

10° at this stage three different types of stimuli were introduced in the task. The choice of the stimulus type was totally random (automatized procedure) and this step required a longer period than all the steps before and challenged the animal.

11° finally the last component of the present task which was a visual target was introduced to the animal. Such a target aimed at fixing the gaze of the subject (in the center of the screen) could be easily taken as a visual stimulus by the animal so that it required a period of adaptation and a reconditioning. The role of this target could be understood from an electrophysiological point of view when it is known that eyes movements might create artefacts during visual detection tasks. This target appeared when the animal pressed on the lever (in the § Sensory-motor task in Lanz et al., 2013b) and both together initiated the trial.

Importantly these training stages were punctuated with various surgeries aimed at implanting devices necessary for the head fixation and for electrophysiological recordings.

### II.4.2 CT and MRI acquisition protocol

For the design of our various implants (headpost fixation and chronic recording chambers) we have performed different imaging acquisitions like CT scan or MRI (see Lanz et al., 2013a) in the cantonal Hospital of Fribourg (Hôpital fribourgeois). These protocols were performed after agreement with the local veterinary authorities (Fribourg, Switzerland) who allowed the transportation of the animals from the laboratory to the hospital. One can note that a transport authorization was obtained beforehand from the veterinary office for each acquisition. We are briefly going to report the two imaging procedures perform in our monkeys. The animal preparation and the transportation were similar for both acquisitions. First, the animal was sedated in the animal facility and the degree of sedation was chosen according to the protocol (explained below). In case of MRI acquisition the animal legs were shaved (posterior parts) to allow later on the perfusion by intravenous injection once arrived at the Hospital. When the animal was anesthetized, it was then carried in a cage (460x400x290mm; Figure 28) equipped with a mobile wall giving access to the animal when injections were needed. Then the animal was placed in a vehicle for the transport and two people were accompanying it.

**Figure 28: 2 views of the cage of transport (460x400x290) equipped with a mobile wall. Photos from the University of Fribourg, Department of Medicine, Domain of Physiology, Fribourg, Switzerland (2013).**

In the second stage the animal was prepared for the acquisition according to two paths:

- **For CT scans** the protocol was easy. In the animal facility the monkey was sedated with a mixture of Kétamine (Ketasol 100 mg/ml; 2.5 - 5 mg/kg) and Dorbene (Dorbene 1 ml/mg; 0.02-0.06 ml/kg) which is sufficient for a 1 hour sedation. This corresponded to the time needed to conduct the acquisition and to return to the laboratory. The injection was conducted in a primate chair to avoid unnecessary stress. Once arrived at the hospital, the state of sedation was checked and if necessary a second injection of the mixture of Kétamine and Domitor was made. After this control the technician placed the animal inside the scanner in a dorsal position. The acquisition lasted 5 to 10 minutes. In general, CT scan acquisitions consisted in different anatomical sequences with standard algorithms (soft or bone) and GSI imagery (for acquisition when the animal was implanted) performed in a GE Discovery HD 750 (Figure 29).

**Figure 29: GE Discovery CT HD 750 scanner. Image modified from http://www.ctmedicalscanners.com/ge-ct-scanners/ge-discovery-ct750-hd-ct-scanner/.**

- **For MRI**, the protocol was more complex. The transport to the MRI facility was performed under sedation with Ketamine (Ketasol ; 100 mg/ml ; 2.5 – 5 mg/kg) and benzodiazepine (Midazolam chlorhydrate; 0.1 – 0.2 mg/kg) mixed in the same syringe and injected in i.m.. As for the CT scan the injection was made in a primate chair. At the MRI facility the animal is placed inside the scanner in a ventral position with the head in extension hold in place by a MRI compatible set-up (in Plexiglas) (Figure 30). During the whole procedure the animal was anesthetized by a continuous i.v. injection of propofol / Ringer-lactate solution mixture (0.1 – 0.4 mg/kg/min) plus ketamine (0.0625 mg/kg/min) using long sterile tubing from outside the MRI room. An automatic syringe controlled the flow. The vital parameters of the animal (ECG, $O_2$ saturation) were continuously monitored with a special captor. Oxygen supply was also provided. The animal was surrounded by heat-insulating materials in order to avoid hypothermia. In general MRI acquisitions consisted in different anatomical sequences (3DT1, T2, DTI, FLAIR, SWAN, etc.) performed in a GE 3 tesla scanner. A standard 32 channels head coil was used for the acquisition.

**Figure 30: 2 different views of the animal placed in ventral position with the head in extension hold in place by a MRI compatible set-up (in Plexiglas). Photos from the University of Fribourg, Department of Medicine, Domain of Physiology, Fribourg, Switzerland (2013).**

In both cases, the return of the animal in the laboratory facility was made under permanent control to monitor the awakening phase.

## II.5 Electrophysiology

### II.5.1 Single-unit recordings in non-human primates

The electrophysiology recording sessions were performed in non-human primates during the cross-modal task. Before the beginning of electrophysiological recordings, the animal had to reach a plateau of performance in the behavioural data (see above). Neuronal extracellular activity was recorded in the right premotor cortex (PM) of both monkeys through the implanted chronic recording chamber (see article Lanz et al., 2013a for more detail). The choice of the stimuli intensities and the data analysis are explained in the paragraph Electrophysiology of the article Lanz et al., 2013b.

### II.5.2 Electroencephalography (EEG) in the human subjects

Beforehand, it is important to mention that this part of the study is only a pilot study, a kind of feasibility study carried on a limited number of human subjects (n=4). Indeed, this study aimed at advancing the stability and the replication of the method used in the non-human primates. However, it was obvious that this study in parallel aims, in the future, at creating a bridge between the non-human primates and human subjects.

The EEGs were performed in human subjected enrolled in the cross-modal task. Before electrophysiological recordings acquisition, the human subject's auditory threshold was determined (the visual threshold was the average value derived from the 10 subjects engaged in the behavioural session). The EEG recordings were performed with a cap containing 65 active electrodes regularly distributed over the scalp (Figure 31: actiCAP, Brain product GmbH, Gilching, Germay). The EEGs recordings required an impedance of 25 kΩ on the active electrodes, obtained by placing an electrolyte-gel in between the electrode and the skin (ABRALYT *HiCl* 10%, Abrasive Electrolyte-Gel; EasyCap GmbH, Steingrabenstrasse 14, 82211 Herrsching, Germany). The Global Field Power (GFP) was derived from the entire brain's surface.

**Figure 31: Photograph of a 65 electrodes cap used for EEG recordings. From the website of Brain product GmbH**

The evoked potentials (EPs) signals were amplified (BrainAmp DC, Brain product GmbH, Gilching, Germany, filtered (High Pass and Low Pass), collected, displayed online and stored on a SSD drive using a 64-channel EEG system (BrainVision Recorder, Brain product GmbH, Gilching, Germany).

The sensory-motor task was generated and controlled by a customized workstation, elaborated form RpvdsEX software (Tucker-Davis Techonologies, US) and an eye tracking system (ISCAN Inc., USA). However the electrophysiological data were recorded with the software BrainVision (BrainVision Recorder / Analyzer, Brain product GmbH, Gilching, Germany).

These data, analysed offline, were then exported into the Cartool software, developed by Denis Brunet (Geneva University Hospital and Medical School, Geneva, Switzerland) (Brunet et al., 2011). For the analysis (offline), various filters were used. The EPs signal was filtered between 1 Hz (high pass) and 40 Hz (low pass). In case of contamination with 50 Hz electric noise, we could use a 50 Hz notch. EPs were derived from more than 200 averaged sweeps. For every stimulus delivered, the recording time window started 100 msec pre-stimulus and terminated 500 msec post-stimulus (total duration: 600 msec). For each signal and for defined pre-stimulus delay, every mean signal was recorded, then subtracted from this signal for all time points. This procedure, commonly named «Baseline Correction», allowed making sure that the effects observed during post-stimulus period were not present during the pre-stimulus phase. In the present experimental protocol, the baseline correction used is in a time window of 100 msec. before the stimulation.

## II.6 Timeline in non-human primates

To summarize the time course of the various consecutive experimental steps, a timeline for each monkey is shown in the next figure (Figure 32). This figure is also shown in the article Lanz et al., 2013a.

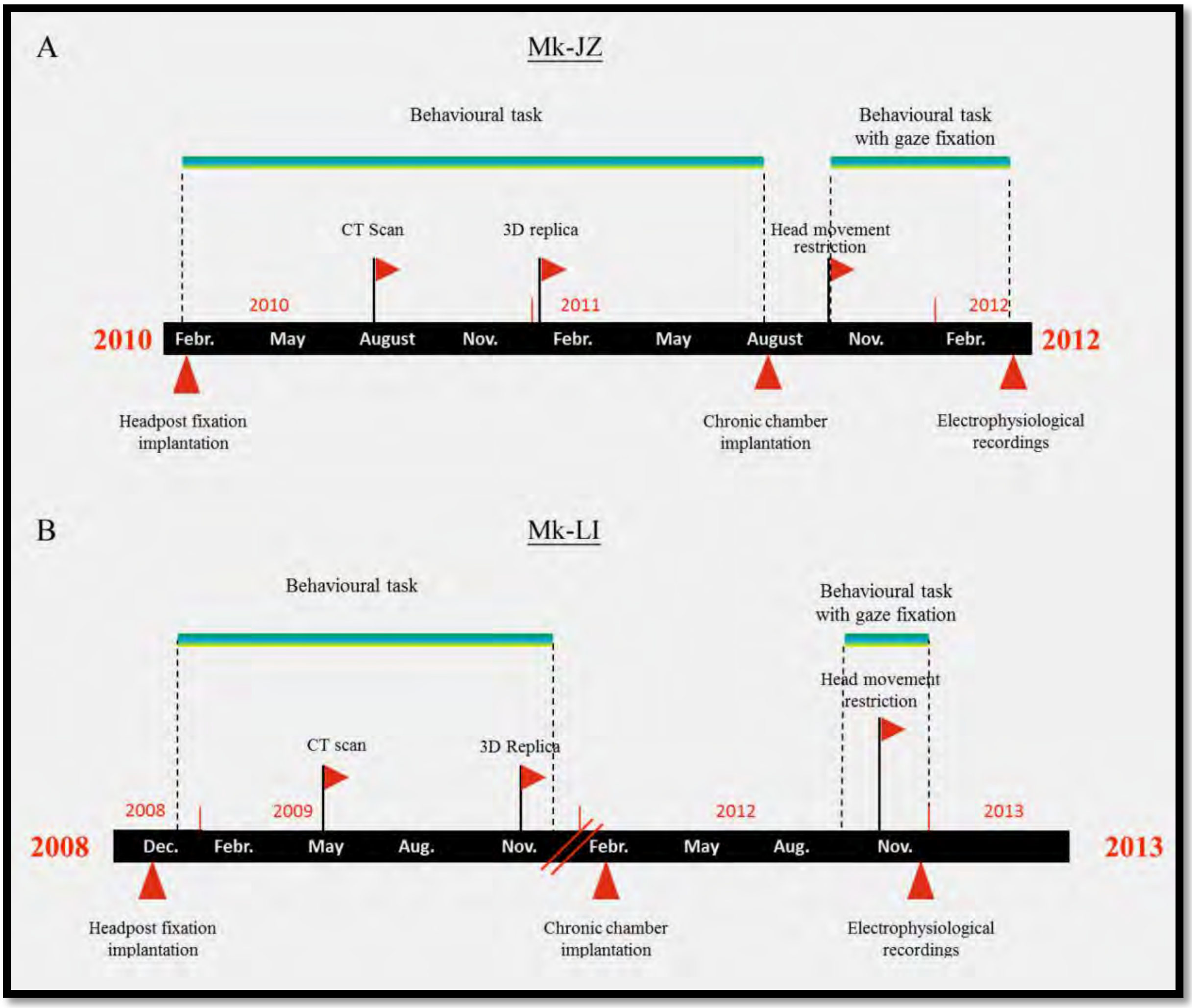


**Figure 32: Representation of the timeline for both monkeys. (A) Timeline for Mk-JZ and (B) for Mk-LI. Taken from the article Lanz et al., 2013a.**

## II.7 Article in methodology

The present thesis work required first to develop new approaches in order to conduct behavioural and electrophysiological investigations in non-human primates over a very long period of time (several years). In the past, according to our experience in the laboratory, when dental or orthopedic cement was used to anchor head fixation devices or chronic recording chambers, the bone skull suffered very badly from the presence of the cement, leading to a strong degradation of the bone health condition. As a result, the implants were lost, conducting to the interruption of the experiment, with the frustrating loss of the enormous time invested before to train the monkeys. Indeed, after the deterioration of the bone skull, it was nearly impossible to re-implant the monkeys. For this reason, our goal was to refine the implantation method in order to ensure a fixation mode which lasts several years. The hypothesis was that if cement is omitted in the fixation procedure, one may obtain a more stable anchoring, lasting several years. However, such an approach requires implanting the animal far earlier, so that the bone can grow above the implanted material. A further hypothesis was that such osseo-integration can be enhanced using an appropriate coating, attracting the bone rather than repulsing it as the cement does. This part of the thesis work thus follows the principle of the 3Rs in the context of animal experimentation ("*Refinement*" in the present case).

A precise and comprehensive survey of this whole methodological development, with the related results, can be found in the article (Lanz et al., 2013a). More specifically, it describes the development of a headpost fixation device in titanium (with the aim of fixing the gaze), the new design of chronic recording chambers in tekapeek, as well as the surgery necessary for the various implantations and different results. In addition, several procedures dealing with the maintenance of the implants (cleaning of the chronic chamber, cleaning of the skin around the implants, etc) are illustrated in the form of video sequences, accessible as described in the article Lanz et al., 2013a.

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Basic Neuroscience

# Refined methodology for implantation of a head fixation device and chronic recording chambers in non-human primates[☆]

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## HIGHLIGHTS

- A head fixation device and a chronic recording chamber can be implanted without using dental resin or orthopedic cement.
- Complete osseous-integration of implant can be obtained thanks to a hydroxyapatite coating.
- A perfect matching of the implants with individual skull surface can be ensured with a plastic replicate of the skull (3D printing).
- Implanting surgeries can be greatly facilitated by the use of personalized implants and 3D printing.
- Outstanding longevity of the implants used: 4 years for head fixation device and 1.5 years for chronic recording chamber.

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## ABSTRACT

The present study was aimed at developing a new strategy to design and anchor custom-fitted implants, consisting of a head fixation device and a chronic recording chamber, on the skull of adult macaque monkeys. This was done without the use of dental resin or orthopedic cement, as these modes of fixation exert a detrimental effect on the bone. The implants were made of titanium or tekapeek and anchored to the skull with titanium screws. Two adult macaque monkeys were initially implanted with the head fixation device several months previous to electrophysiological investigation, to allow optimal osseous-integration, including growth of the bone above the implant's footplate. In a second step, the chronic recording chamber was implanted above the brain region of interest. The present study proposes two original approaches for both implants. First, based on a CT scan of the monkey, a plastic replicate of the skull was obtained in the form of a 3D print, used to accurately shape and position the two implants. This would ensure a perfect match with the skull surface. Second, the part of the implants in contact with the bone was coated with hydroxyapatite, presenting chemical similarity to natural bone, thus promoting excellent osseous-integration. The longevity of the implants used here was 4 years for the head fixation device and 1.5 years for the chronic chamber. There were no adverse events and daily care was easy. This is clear evidence that the present implanting strategy was successful and provokes less discomfort to the animals.

## 1. Introduction

In the field of neurosciences, the macaque is a model of choice (scientifically and ethically justified; see Weatherall report, 2006). This monkey is highly adapted for neuronal investigations due to its large similarity to the human brain from an anatomical and a functional point of view. In modern neurosciences, there is a large range of approaches to investigate brain function, also applicable, to some extent, to non-human primates: functional brain imaging (fMRI), electroencephalography (EEG), positron emission tomography (PET), transcranial magnetic stimulation (TMS), single

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*Abbreviations:* HA, hydroxyapatite; CT, computed tomography; MRI, magnetic resonance imaging; VPS, vacuum plasma spraying.

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neuron recording, etc. The quality of the resulting data depends on the level of interferences caused by artifacts, which may be produced, for instance, by muscular contractions related to head and/or eyes movements or mastication. A further challenge in this type of research lies in the fact that the animal has to be awake, and the head must be kept fixed. Indeed, any head movement would create recording artifacts. Furthermore, in the case of electrophysiological recordings, there is a risk that, in a non-head fixed system, the recording electrodes move and cause brain injuries. That is why it is preferable that the animal's head be immobilized when it is performing behavioral tasks. To this aim, until recently, numerous laboratories used a head fixation device anchored to the skull with dental acrylic cement (Fuchs and Luschei, 1970; Lisberger and Westbrook, 1985; Guo and Li, 1997; Kermadi et al., 1997, 1998; Liu and Rouiller, 1999; Churchland and Lisberger, 2000) or orthopedic cement (Durif et al., 2003; Peeters et al., 2009; Kaeser et al., 2010, 2011). Such an approach allowed creation of a firm point of fixation, but the interface between the dental resin or the orthopedic cement and the bone was not optimal. It was observed that the cement adhered to the bone in a superficial way without integration between the two components (bone and cement). This represented a considerable risk of fracture. Although variable from one animal to another, the presence of cement (dental or orthopedic) exerted a detrimental impact on the bone in the mid- and long-term run. In particular, the risk of infection, inflammation, growth of granulation tissue and softening of the bone was increased. These effects were often initiated by the high temperature generated when the cement was applied to the bone surface, and as a consequence increased the risk of head fixation device losses of over time.

In line with recent reports (Adams et al., 2007, 2011; McAndrew et al., 2012), the goal of the present study was to introduce a refined method to anchor a biocompatible head fixation device and chronic recording chambers on macaque's skull, without the use of dental resin or orthopedic cement. This aim was achieved here by taking advantage of newly developed materials and coatings which are used for orthopedic surgery. These are generally assimilated by the bone instead of being rejected by it. However, for a perfect integration between implants and bone, a perfect match of the shape of the implants (head fixation device or recording chamber) with the skull surface of each individual monkey is required. A 3D replicate of the skull of the living monkey was obtained based on CT and MRI data. This replicate was used to accurately guide the positioning of the implants on the skull as well as to derive their shape so that they would perfectly match the contour of the skull at the calculated target position.

## 2. Methods

### 2.1. Subjects

The present experiments were conducted on two adult *Macaca fascicularis*, originating from our own breeding colony. At the time of headpost fixation (see Fig. 3), one animal (Mk-LI) was 9-years-old and weighed about 8.0 kg, whereas the second animal (Mk-JZ) was 7-years-old and weighed about 8.0 kg. The body weight was checked daily. In case of a 10% loss of weight, the experiment would be interrupted until weight was regained (an interruption criterion that was not met in the course of the present study). Between daily experimental sessions the animals shared living quarters with other monkeys (groups of 2 to 5 animals) in an enclosure of 45 $m^3$ (15 $m^3$ until 2010; see e.g. (Kaeser et al., 2011)). They could freely move and had free access to water. The experiments were conducted according to the guidelines of the National Institute of Health (Guide for the Care and Use of laboratory Animals, 1996), of the European Community (Guidelines for Animals Protection and Use for Experimentation) and the ARRIVE guidelines (http://www.nc3rs.org.uk) (Animal Research: Reporting In Vivo Experiments), as well as the Swiss veterinary authorities (cantonal and federal) who approved the experimental procedures.

### 2.2. 3D replicate (print) of the monkey's skull

The first stage was to obtain such a 3D replica of the living monkey's skull. The acquisition of the skull morphology involved using a computed tomography scan (CT scan) (Department of radiology at Hôpital Fribourgeois [HFR]). The obtained CT scan was processed with the Osirix software (64 bits) in order to fabricate a 3D reconstruction of the skull. This model was transferred to the Engineering School of Fribourg for final processing. The final 3D print was performed with the following equipment: 3D printer, 3D uPrint Plus which uses Fused Deposition Modeling (FDM) Technology to build 3D replica with ABS*plus* thermoplastics. The principle of the 3D replica of the skull is illustrated in the supplementary video sequence #1 (http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l1.html). The present 3D replication took approximately 25 h. It was then polished, including removal of unwanted plastic parts by overnight treatment in a chemical bath. Because the 3D replica was based on CT data, the skull surface and the bone thickness was a 1:1 representation of the monkey's skull. Although the thickness of the skull could be determined by the CT images, the 3D model was used instead during surgery and was advantageous.

### 2.3. Head fixation device

Similar to other recent studies (Adams et al., 2007, 2011; McAndrew et al., 2012) the aim here was to develop a stable and solid implant without using dental or orthopedic cement. In this study the head fixation device initially developed by Adams et al. (2007, 2011) was chosen as a base and was modified according to experimental needs. The material used to elaborate the head fixation device was titanium, which has been used for more than 30 years in the medical industry. Titanium presents the advantage of being, along with gold and platinum, one of the most biocompatible metals, and is resistant to body fluids (Rubo de Rezende and Johansson, 1993). Titanium demonstrates high corrosion resistance and the highest strength-to-weight ratio of any known metal. One of the most important advantages associated with the use of titanium was that bone adheres well to it and yields good osseous-integration (Brånemark et al., 1969; Albrektsson and Albrektsson, 1987; Rubo de Rezende and Johansson, 1993; Augat et al., 1995; Betelak et al., 2001). The head fixation devices were manufactured (Ateliers Clément S.A. CH – 1731 Ependes) from a pure titanium cube (CP, Grade 2) as mono-blocks, allowing excellent osseous-integration (note however that Grade 5 would be recommended if one wanted to reduce artifacts for subsequent MRI). Because the head fixation device needed no welding, a break at the weld line between the post and the footplate was prevented (Adams et al., 2007).

The head fixation device used in the present study is illustrated in Fig. 1A. From a mechanical point of view, it could be divided into two different parts. The base of the implant presented a "K-shaped" footplate designed for attachment to the most rostral part of the skull (Fig. 1B) with 12 or 16 bone-titanium screws (cortex screws Ø 2.7 mm, self-tapping; SYNTHES®; length of 6 or 8 mm), depending on the weight of the animal and the size of the skull. The precise shape of the base of the implant may be refined using the 3D print of the monkey's skull, as explained in the section "recording chamber" (see also supplementary video sequence #2 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l2.html). The upper part of the head fixation device, which is the only visible

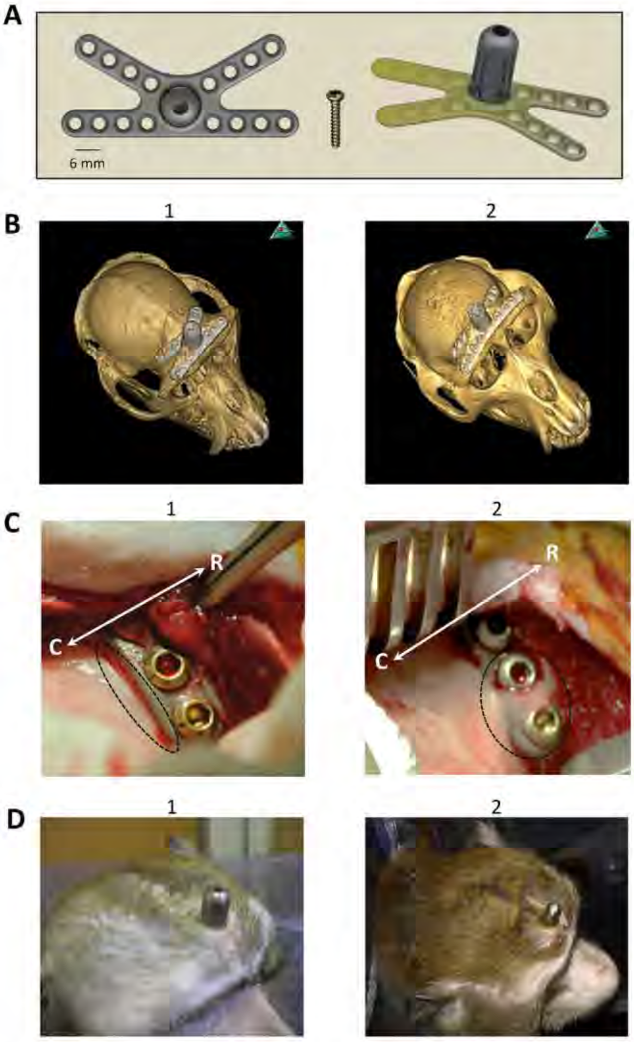

**Fig. 1.** (A) View of the head fixation device (design derived from Adams et al., 2007). (B) Head fixation device fixed on the monkey's skull (B1: monkey Mk-JZ and B2: monkey Mk-LI). (C) Osseo-integration of the head fixation device was observed during the surgery for chronic recording chamber implantation. The incision made along the skull midline (Rostro-Caudal (R-C)) allowed observation of an osseo-integration along the footplate for MK-JZ (C1) and above the footplate for MK-LI (C2). (D) Head fixation device, after 1 month (D1) and 3 months (D2) after implantation (in Mk-LI).

part after implantation (Fig. 1 D1 & D2), is a vertical cylinder of 10 mm in diameter and 20 mm high, with rounded edges starting at 3 mm from the top. The length of the cylinder was shorter than the height of the wire mesh of transfer cages used to channel the monkeys into the primate chairs (see (Schmidlin et al., 2011)), so as to avoid any accident in the cage. An 8 mm deep hole with an internal thread was designed at the top of the cylinder. This was to allow the device to be fixed to the experimental set-up, thus ensuring a firm head fixation. When the animal was not in the experimental set-up, the opening on top of the cylinder was closed with a headless screw. The head fixation device placed and fixed on the skull of the monkey is shown in Figs. 1B1 and B2 (see also supplementary video sequence #3 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l3.html).

In the present study, the head fixation device (Fig. 1) was implanted on monkeys of about 8 kg. For smaller monkeys, a comparable head fixation device could be used, but modified with shorter footplates accommodating only 12 screws.

### *2.4. Coating process*

In line with recent reports (Adams et al., 2007, 2011; McAndrew et al., 2012), the aim was to develop a stable and solid implant without using dental or orthopedic cement. Important to this study was that the base of the head fixation device (which would be in contact with the bone) was coated with of a naturally occurring mineral form of calcium apatite known as: Hydroxyapatite ($Ca_5(PO_4)_3(OH)$) (phosphate minerals groups) abbreviated as HA or HAP. This material is widely used to coat implants, to provoke a strong connection to the host bone. The main applications are coatings for orthopedic hip implants for the cementless implantation technique. HA is the preferential material for this application due to its chemical similarity with natural bone, allowing bone to bond directly to HA coated surfaces. The poor mechanical properties of synthetic calcium-phosphates hindered the use of this material for load bearing implants. As a result natural HA-coatings on mechanically stable substrates have become widely used. Vacuum plasma spraying (VPS) has been established as the most suitable technique for industrial coating production. This innovative coating technique in the field of the electrophysiological research allowed a better anchoring of the implant to the skull, as well as faster adherence. The advantages of this coating were demonstrated earlier on a canine model (Cook et al., 1992) and on human patients (Jaffe et al., 2007).

Despite the successful application of plasma sprayed coatings in the biomedical field, between 0.5 and 3% of the hip endoprostheses failed due to bacterial infection (Harris and Sledge, 1990). For the present implantation of a head fixation device and a chronic recording chamber the incidence of implant infection was expected to be even higher, as the implants were transcutaneous and it would be more difficult to keep the environment sterile for animal surgery. To generate an antibacterial effect in the HA coating, the integration of silver (Ag) was a promising procedure. Ag is well known for its antibacterial properties against all bacteria strains. As HA has a high exchange rate with metal-ions, an ion exchange process was used to incorporate Ag into the HA spray powder. The obtained powder (HA–Ag) was then used for the plasma spraying process to form a HA–Ag coating on the implants, comparable to the pure HA-coating.

To coat the present implants, this newly developed HA–Ag coating complex was applied for the first time for in vivo applications. Several in vitro experiments showed that the coating releases Ag+ ions, which was effective against bacterial colonization on the implant surface. As the Ag content in these coatings was very small, the antibacterial effect addressed only the risk of short term implant infection after surgery. Indeed, Ag is also toxic to bone cells, as well as acting against bacteria. However, the bacteria proved to be more sensitive to Ag than the bone cells. In addition, a majority of Ag ions solubilized very fast. They mainly affected the bacteria present on the implant during surgery. Several days later, when the bone cells started growing on the surface of the implant, the dilution of Ag ions was much lower compared with the amount during the first hours. The areas of the implant, which were not meant to be coated, were masked with Polyimide tape and then covered by metal masks. HA–Ag coatings were produced by Medicoat AG (Switzerland) by VPS type MC60. Because the implant fixation must withstand high forces, strong tensile bond strength of the coating bond to the substrate must be guaranteed. Therefore a titanium bond coating was applied before the HA-Ag coating using the same procedure. The Ag concentration in the coating was measured by inductive coupled plasma (ICP) at EMPA, St.Gallen (Switzerland). The Ag content was detected to be 1500 ppm (Fig. 1A, right panel).

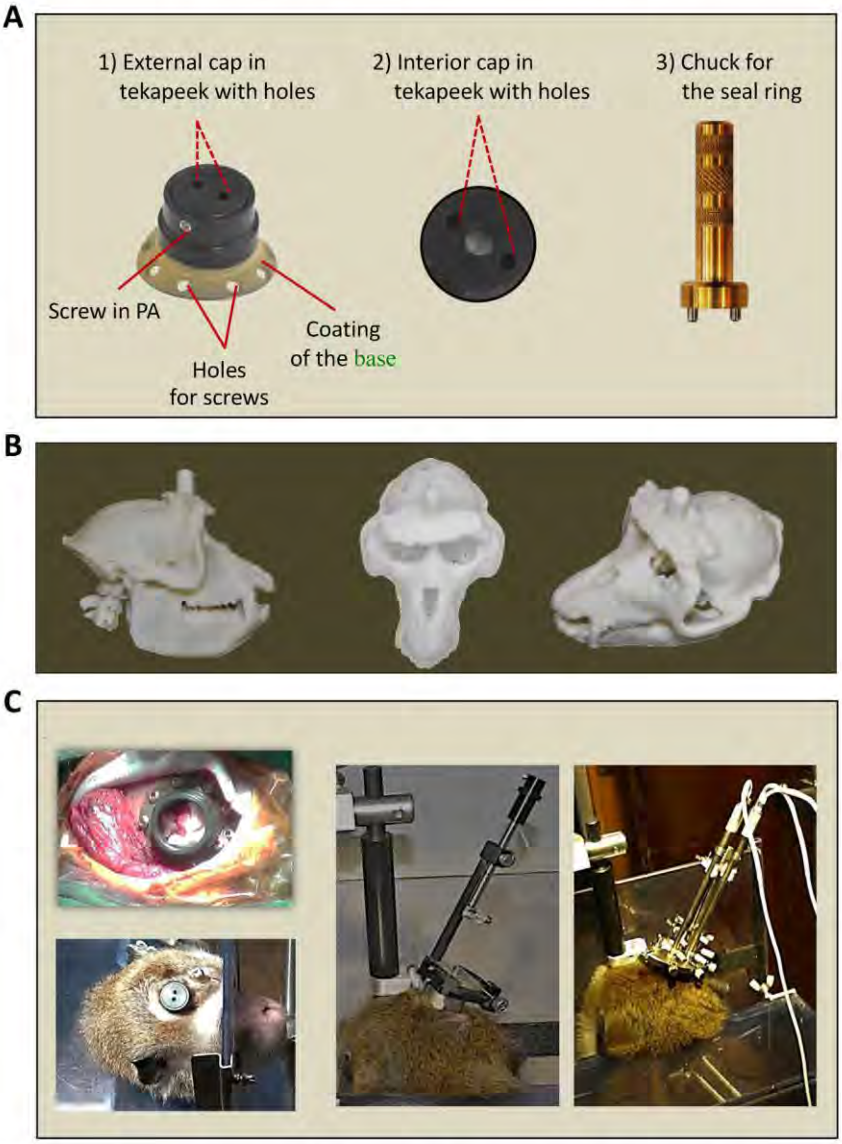

**Fig. 2.** (A) On the left, general view of the chronic recording chamber built to access the premotor cortex. The bottom edge in yellow corresponds to the coating with hydroxyapatite. In the middle, an interior cap ensured the sealing of the chronic recording chamber and isolated the dura mater, when no electrophysiological recording session takes place. The rightmost panel shows the screwdriver used to manipulate the internal cap inside the chronic recording chamber (see supplementary video sequence #7 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l7.html). (B) View of the 3D print replicate of the monkey's skull in plastic (see text; Mk-LI). (C) On the left, the chronic recording chamber is shown when it was implanted during the surgery (in Mk-LI) and as it appears several months after implantation (in Mk-JZ). In the middle and on the right (Mk-JZ), the pictures show the system used to fix the head of the monkey, together with the adaptation on the chronic recording chamber of a Narishige® single electrode drive system (middle) or a NAN® multiple electrode driving system (right).

### 2.5. Recording chamber

In parallel to the head fixation device, a similar idea was employed to design new chronic recording chambers. With these new chambers electrophysiological data could be derived from behaving monkeys with their head fixed. The aim here was to increase the animal's comfort (again no dental or orthopedic cement to fix the chronic chamber on the skull, as recently proposed by Adams et al., 2011), while reducing the daily care of the chamber and infection risks. To ensure optimal anchoring of the chronic chamber on the scalp, its shape was adapted to the 3D replicate (print) of the corresponding monkey's skull. This 3D print allowed definition of the exact position and the shape as well as the best fit of the chronic recording chamber on the skull.

In the present study the chronic chamber has a cylindrical shape (Fig. 2 A) and is made of tekapeek- an industrial plastic with high temperature, chemical, electrical and radiation resistance (similar to metals such as titanium). Tekapeek has the advantage of guarding the option to perform subsequent MRI investigations with minimal artifacts. Furthermore, tekapeek is lighter than metal, thus reducing the weight of the chamber placed on the monkey's skull. The milling of the chamber is illustrated in the supplementary video sequence #4 (http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l4.html). The chronic chamber is comprised of a base (28 mm in diameter), which adheres to the bone, with a cylinder on top (24 mm in diameter and 9 mm in height) giving access to the dura and offering the possibility to fix an electrode driving system (Fig. 2C). The base

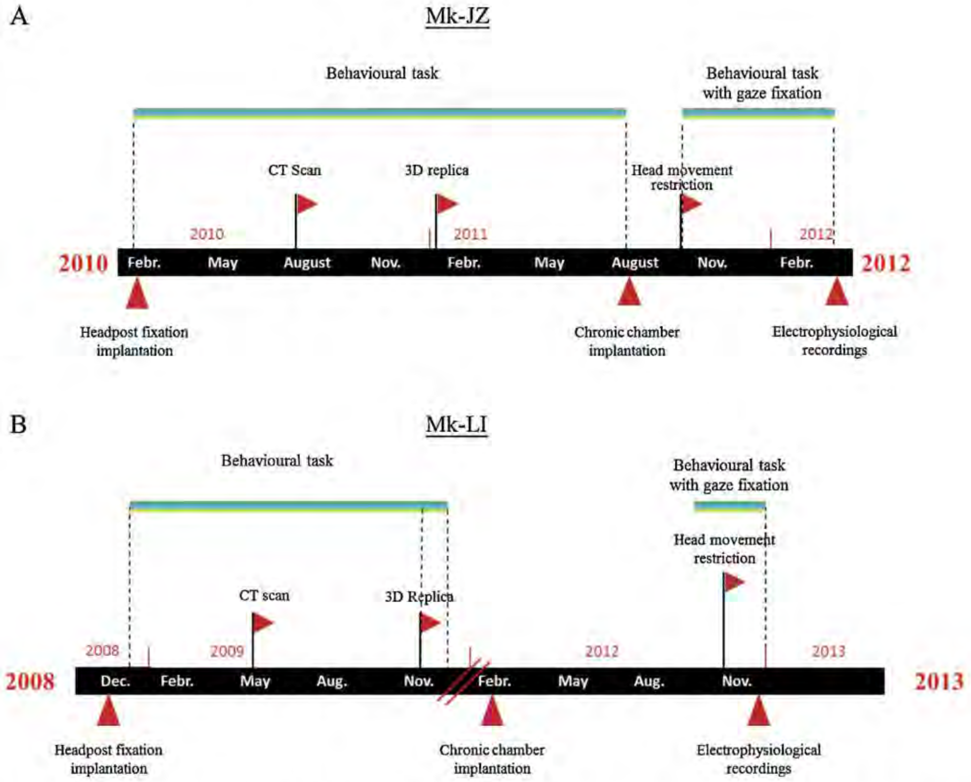

**Fig. 3.** Timeline of the implantation procedures, behavioral and electrophysiological recordings the two subjects underwent during the overall experimental protocol.

of the recording chamber was coated with HA (Fig. 2A1), as was done for the head fixation device. Briefly, the base of the recording chamber was coated with a titanium bond layer between the substrate and the HA coating. The titanium was very reactive and bonded very well to different materials (for more detail, see "Head fixation device" section above).

The exact position of the chronic chamber on the skull was defined by superimposing a MRI scan of the monkey's brain (providing anatomical position of sulci and cortical gyri for example) with the corresponding CT scan (providing an accurate replica of the skull surface) performed in the same animal. Then the shape and contour of its base was precisely adjusted to the bone surface, as it appeared on the 3D print of the skull. The base of the chronic chamber is comprised of 7 holes used to position fixation screws (Fig. 2A1). The cylinder was larger at the bottom than at the top forming a shoulder on which the electrode driving system rested (Fig. 2C). The internal diameter of the cylinder was 21 mm, which corresponded to the size of the grid guiding the electrodes held by the electrode driving system.

When the animal was not in the experimental set-up, the chronic recording chamber was covered with a cap. Instead of using a standard cap placed on top of the cylinder, a cap was designed which could be placed inside the cylinder. The advantage here is that the size of the implant is reduced. To ensure appropriate sealing, the cylinder is closed first with a sealing ring (Fig. 2A2), which is screwed into the cylinder using an ad-hoc screwdriver (Fig. 2A3). The sealing ring was used mainly to cover a round piece of silastic placed on top of the dura. The sealing ring is 5.2 mm thick with a diameter of 20 mm, and a central opening of 4.7 mm (Fig. 2A2). The central opening prevents a pressure increase on the silastic joint when the sealing ring is screwed/unscrewed. To allow the screwing/unscrewing of the sealing ring two small 3 mm holes were made. Finally, an external cap is positioned on top of the cylinder (Fig. 2A1), ensuring hermetic closure of the chamber. The external cap is a 7.8 mm thick disk with a diameter of 20 mm, comprised of two holes on the top, and a threaded hole on the side. The hole on the side needs to be aligned with a corresponding hole in the side wall of the cylinder, in order to close the chronic chamber by means of a headless polyamide screw (M5 × 3). The cap can be removed by means of a modified crowbar. A comprehensive view of the chronic recording chamber was available in the supplementary video sequence #5 (http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l5.html).

### 2.6. Implantation surgery

First the animal was sedated by an intramuscular injection of a mixture of ketamine (Ketanarkon® 10 mg/kg body weight), benzodiazepine (Midazolam 0.1 mg/kg) and methadone (0.2 mg/kg). This sedation allowed the preparation of the monkey for the surgical intervention, involving shaving the skull and preparation for an intravenous injection of Propofol (diisopropylphénol), coupled with gas anesthesia (Sevoflurane; see below). In this step, the analgesics Carprofen (Rimadyl®, Pfizer, 4 mg/kg) and broad spectrum antibiotics (Albipen®, Intervet, 30 mg/kg) were injected subcutaneously. In addition, to reduce edema, dexamethasone (Decadron 0.3 mg/kg, mixed 1:1 with saline) was injected i.m. Finally, an injection of atropine (0.05 mg/kg i.m.) was given to reduce bronchial secretion.

In the operating room, under sterile conditions, the animal was intubated, allowing ventilation with a 50%/50% mixture of $O_2$ and air, containing 2.5% Sevoflurane to ensure anesthesia. This was complemented by continuous i.v. perfusion of Propofol (Fresenius 0.1 mg/kg/min). At potentially painful steps of the surgery (e.g. craniotomy), an i.v. flow of opioid (Fentanyl 0.1 μg/kg/min) was used. In addition, during the entire surgery, the animal received a continuous i.v. perfusion with lactate-ringer at a rate of 5 ml/kg/h.

During the entire surgical procedure, physiological parameters were continuously monitored (e.g. body temperature, $O_2$ saturation, heart rate, respiration rate, exhaled $CO_2$). At the beginning of the procedure, the skin of the head was cleaned and disinfected with an iodized solution (Betadine or Povidone-iodine). An incision of the skin along the skull midline of about 10 cm was made with a scalpel. The muscles were then reclined from the skull to expose the bone surface. The head fixation device was then positioned on the skull at its foreseen position (as rostral as possible: see Fig. 1B). At this step, if necessary, in order to ensure a perfect fit on the skull surface and taking advantage of the flexibility of the titanium, the footplates of the head fixation device could be slightly adjusted with sterile tools. Before anchoring the head fixation device on the skull, the bone (periosteum) was abraded extensively. The insertion of the screws was done according to the protocol by Adams et al. (2007). Namely the first screw to be implanted was situated on the left-hand side of the leg of the arch; the second was placed on the last hole of the right posterior leg. For insertion of each screw, a power drill was used to make, small pilot holes of a smaller diameter. Furthermore, to avoid bone damage resulting from a temperature increase, a flow of saline (0.9%) from a large syringe was used for cooling during the drilling. An increase in temperature could cause bone softening near the holes. The screws were carefully inserted by hand, without exerting too much pressure, in order to minimize risks of cracking or damaging the bone. The length of screws was chosen according to the CT scan performed a few weeks before the operation and the 3D printing of the skull (see above).

Once the head fixation device was anchored to the skull, first the muscles then skin were stitched to the midline. A small opening was left on the posterior part of the cylinder of the head fixation device to act as a natural catheter, allowing for possible leakage of secretions, and to inject antibiotics, if needed. Several months later, a similar surgical procedure was conducted to anchor the chronic recording chamber on the surface of the skull (see supplementary video sequence #6 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l6.html and Fig. 2C- top left panel).

### 2.7. Daily care for the head fixation device

After the implantation of the head fixation device, the wounds and scars were cleansed daily with an antiseptic iodized solution (Betadine ® 500 ml, Mundipharma Medical Company). The animal was examined for possible inflammation and appearance of infection as well as observation of general behavior. An anti-inflammatory and antibacterial cream (Panalog ® ad US. Vet., Novartis) was applied around the implant. Furthermore the animal received antibiotics and analgesic for at least 10 days. The big advantage with this type of implant was that after about 1 month, daily care was no longer necessary. However, a bi-weekly cleaning with Betadine was done, as well as a daily routine check of the animal.

### 2.8. Daily care for the chronic recording chamber

The chronic recording chamber's daily care required more time and was more frequent than that of the head fixation device. Just after surgery the wounds and scars were cleansed daily with an antiseptic iodized solution (Betadine® 500 ml). During the following ten days, the animal received injections of antibiotics (Albipen®) as well as analgesics (Rimadyl®). At the end of every daily care, a cream (Panalog® 15 ml, Novartis) was applied around the chronic chamber. Similar to head fixation device, this treatment was pursued during one month post-implantation. Afterwards cleaning the external part of the chronic recording chamber was no longer performed on a daily basis, but a superficial cleaning with Betadine® was required about twice a week.

The inside of the chronic recording chamber required a lot more attention and care (see supplementary video sequence #7 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l7.html). The chamber was cleansed at least once every three days using sterilized material (gaze band, surgical forceps, silastic joints, two different caps). During the recording period, cleaning was performed at every recording session. The inside of the chronic chamber was cleaned using Betadine. Before closing the chamber, the silastic joint (Silicone sheeting) (LP 500-9, Manufactured by LPI) was coated with a layer of antibiotic cream (Fucithalmic Vet®, 3 mg/g; Dechra). Once the daily recording sessions had begun, the dura mater was scraped once a week, in order to limit tissue growth and dura thickening, which would block the penetration of recording electrodes.

### 2.9. Timeline

To clarify the time course of events the two subjects were to follow during the experimental protocol, a timeline was established and displayed, in Fig. 3. This timeline indicates the starting point of the procedures (headpost fixation implantation, CT scan, 3D replica, chronic chamber implantation) and the behavioral tasks/first electrophysiological recordings accomplished. At the time of the publication the protocols were still on-going.

## 3. Results

### 3.1. Head fixation device

To ensure a high and stable bone integration of the head fixation device, the monkeys were implanted very early, well before the behavioral training was started. Mk-LI was implanted on November 12th 2008, whereas Mk-JZ was implanted on February 19th 2010. As shown in Fig. 1D, after respectively 1 month (1 D1) and 3 months (1 D2) post-implantation, only the cylinder of the head fixation device was visible on the rostral part of the monkey's head. The skin retracted and adopted a position fitting the circumference of the cylinder of the head fixation device. There was no infection or inflammation around the implant and therefore only minimal care was necessary at 2 weeks intervals in the form of cleaning around the implant with Betadine®.

After a period of behavioral training, the monkeys underwent the implantation of a chronic recording chamber. This surgery provided the opportunity to check the integration of the head fixation device to the bone, as it appears after 1 year in Mk-JZ and after 3.5 years in Mk-LI (Fig. 1C). The implant was perfectly adapted to the topography of the skull, as expected after the final adjustment of the footplates during the first surgery. The osseous integration was not the same for both animals, which is in line with the different time intervals. In the first monkey (Mk-JZ; Fig. 1C1), after one year, it was observed that an osseous layer had settled along the footplate edge. In the second animal (Mk-LI; Fig. 1C2), after three and a half years, there was a clear bone growth over the footplate and in between two adjacent titanium screws. From a behavioral point of view, the animal presented no discomfort in relation to the head fixation device during the behavioral training. Note that, during the entire training period, the head of the animal was not fixed in the experimental set-up, to avoid mechanical constraints, which could disrupt bone growth around the footplates. The use of the head fixation device to fix the monkey's head using a rigid arm anchored to the experimental set-up is shown in Fig. 2C.

**Fig. 4.** Pictures showing the building up of another chronic recording chamber to access a different area (thalamus) after completing the investigations in the premotor cortex (see text). On the left, the picture shows a simulation of the new chronic recording chamber before it was fabricated. On the right the picture shows the real chamber in tekapeek and placed on the 3D replicate printing of the monkey's skull (Mk-LI).

### 3.2. *Recording chamber*

The chronic recording chamber, illustrated in Fig. 2, was implanted in both monkeys (August 23rd 2011 in Mk-JZ and February 9th 2012 in Mk-LI), with the aim to access the premotor cortex in the right hemisphere (Fig. 2C). As the shape of the chronic chamber was well adapted to the 3D print of the corresponding monkey's skull (Fig. 4) the implantation during surgery was straightforward and the final position easy to achieve. Indeed, only the targeted position on the skull provided a perfect match between the base of the chronic chamber and the contour of the skull. A preliminary positioning of the chronic chamber was performed to determine the region of the bone to be removed. The bone was then marked with a sterile pencil around the circumference of the inside of the chronic chamber. The contour of the cylindrical piece of bone was cautiously removed using a drill, exposing the dura mater. This was immediately photographed in order to register the position of blood vessels to be avoided in subsequent electrode penetrations. The positioning of the chronic recording chamber on the skull immediately after screw fixation is shown for MK-LI in Fig. 2C -top left panel. Depending on the monkey's size and the position of the chronic chamber, a part of the temporal muscle had to be removed, especially if the chronic chamber was located laterally. First the muscles were sutured and then the skin, all around the chronic recording chamber. Complete scarring took place over a few weeks (Fig. 2C). The skin around the chronic chamber (see supplementary video sequence #7 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l7.html) required only minimal, bi-weekly, cleansing with Betadine®, as was the case for the head fixation device. As illustrated in Fig. 2C, the chronic recording chamber allowed fixation of different electrode driving systems like the Narishige® system (Narishige International limited, Japan, Fig. 2C) and the NAN® system (NAN Instrument, LTD, Israel, Fig. 2C).

The present approach eliminates the use of dental acrylic resin or orthopedic cement, and gives the option to remove a chronic chamber at a later time point when electrophysiological investigations in the corresponding brain region are complete. In this fashion another brain region could be targeted for a subsequent step. It is true that a chamber fixed with acrylic cement could possibly be removed, but the underlying bone might be in bad condition and certainly more traumatized (soft bone, presence of infection, bone thickness not suitable for re-implantation, etc.), as compared to the present approach. Such a strategy would allow the use of the same trained monkey to extend the investigations to additional brain regions. In the present case, once the electrophysiological recordings are completed in the premotor cortex, the first chronic recording chamber implanted will be removed and replaced by a second one, designed to reach the thalamus (Fig. 4). The second chamber is basically the same as the first one: cylindrical shaped with a bottom edge large enough to ensure a perfect seal with the bone. In addition, the edge will be extended laterally and rostrally in order to cover the skull area where the first chamber had been implanted (Fig. 4). A round protrusion of the tekapeek will be made to fit perfectly into the bone hole drilled for the first chamber. This second chamber will be positioned more caudally and near the midline to allow vertical penetrations to reach the thalamus.

## 4. Discussion

The present report provides evidence that both the head fixation device and the chronic recording chamber can be implanted on a monkey's skull for a long period (4 years for the head fixation device), without the adverse events observed in the past with dental resin or orthopedic cement, in terms of inflammation, infection or rejection. To demonstrate the benefit of the present approach, a complementary retrospective analysis (Table 1) was made from data collected in our laboratory on subjects with head post fixation fixed with different cements (dental resin or orthopedic cement). These data demonstrate that the use of cement is associated with a much shorter duration of implants and an increase in infections and/or loss of the device, when compared with the two monkeys included in the present study. Therefore this new protocol guarantees an excellent osseous-integration of the implants. This is due to: (1) a coating of the implants with HA (Figs. 1 and 2), thus potentiating the integration offered by the titanium itself and (2) a 3D print replicate of the skull of the living animal for precise design and adjustments to individual skull shape. The 3D print step represents a crucial improvement in allowing production of truly custom-fitted implants that perfectly fit the monkey's skull. The longevity of the implants demonstrated here on two monkeys (up to 4 years for the head fixation device) is substantial progress and should be compatible with various behavioral and electrophysiological protocols conducted in non-human primates. As illustrated in the supplementary video material, the two implants are well tolerated by the monkeys, even during the cleaning procedures. Due to the elimination of dental acrylic resin or orthopedic cement, the implants have a limited mass above the monkey's head, reducing the probability that the animal hits its head against obstacles in its environment (e.g. enclosure). Another advantage of avoiding dental resin or orthopedic cement is a considerable time gain, about one hour, during surgical implantation of the head fixation device or the chronic recording chamber. In addition, surgery undertaken to anchor the implants is much easier, as the difficult process of

**Table 1**
Retrospective survey of head post implants fixed to the skull with dental resin or orthopedic cement in 11 monkeys, involved in electrophysiological protocols (1994–2006). The table lists for each monkey the duration of implantation (before the device had to be removed or was lost), the occurrence of infections and the devices losses.

| | Subject ($n = 11$) | Implantation duration (months) | Infections | Loss of device |
|---|---|---|---|---|
| | MK-1 | 10 | | |
| | MK-2 | 12 | | |
| | MK-3 | 7 | | X (2x) |
| | MK-4 | 6 | | |
| | MK-5 | 11 | | X |
| | MK-6 | 3 | | |
| | MK-7 | 12 | X | X (2x) |
| | MK-8 | 6 | | |
| | MK-9 | 8 | | |
| | MK-10 | 6 | | X (2x) |
| | MK-11 | 9 | X | X |
| Average | | 8 | 18% | 45% |

skillful application, in successive small amounts, of dental resin or orthopedic cement, is skipped. Thus the present work supports the idea of elimination of dental acrylic resin or orthopedic cement, as previously suggested by Adams et al. (2007, 2011) or McAndrew et al. (2012).

Another valuable contribution of the present study consisted in confirming the suitability of the "K-shape" base of the head fixation device, which allowed the elimination of dental acrylic resin or orthopedic cement, as initially proposed by Adams et al. (2007). The longevity of the implanted device was observed to be at least 17 months by these aforementioned authors. This was extended to 3 years and 8 months in a more recent report (Adams et al., 2011). In the present study, the follow up is more than 4 years. No incidents were seen, despite the fact that it was used to fix the head of two strong adult male macaque monkeys (8 kg body weight) in behavioral and electrophysiological experiments. The clean and discrete appearance of the head fixation on the monkey's head is also confirmed in the present study (see Fig. 6 of Adams et al., 2007;Fig. 1D in the present report). Experience shows that it is crucial to implant the head fixation device early enough in the protocol, so that the osseous-integration can occur during several months, before the monkey's head is fixed in the set-up. At least 3 months is recommended, but longer may be safer. This relatively long delay can be anticipated to some extent, as the head fixation device can be successfully implanted in juvenile monkeys (Adams et al., 2007). In the present study, in contrast to the work of Adams et al. (2007), a further improvement was introduced in the form of coating the head fixation device with HA in order to enhance the osseous-integration. Although titanium is compatible with MRI investigations in spite of some artifacts, one could also envisage, in the future, building the head fixation device from tekapeek, as the chronic recording chamber, to minimize artifacts. The "K-shape" head fixation device (Adams et al., 2007, 2011; present study) is clearly less bulky and less uncomfortable for the monkeys than the head ring approach. (Isoda et al., 2005).

The design of a custom-fitted chronic recording chamber to a living monkey is new, as such an attempt was only recently reported (McAndrew et al., 2012). An important difference however is that these authors imported the CT reconstruction of the monkey's skull into a 3D CAD (computer-aided design) program where the implant was designed at the target location on the skull. The present study went one step further. The skull derived from the CT scan was printed out in 3D (Fig. 2B and Fig. 4) in order to obtain a true replicate of the living monkey's skull, similar to what would have been obtained if the monkey had been sacrificed. The replicate of the monkey's skull can be used to test and determine, with high precision, several options concerning size and position of chronic recording chambers, to reach a given brain region. It is also possible to easily check for possible conflicts between several chronic chambers to be implanted either simultaneously or one after the other. When the approach and the final position have been chosen, the chronic recording chamber can be fabricated by the machinist using the contour which was determined by means of the 3D skull replicate print. In their report (McAndrew et al., 2012), the authors mentioned that the longevity of the device remained to be seen, especially after reporting that the implant became loose after 6 months, requiring re-implantation. Furthermore, due to a gap between the chronic chamber's edge and the skull, there was skin and hair growth into the implant (McAndrew et al., 2012). Such undesired event did not occur in the present study, even after a year following implantation of the chronic recording chamber (Mk-JZ). It is very likely that a perfect seal was obtained due to the 3D print replicate of the skull. Using this 3D print, the relatively large and flexible edge at the bottom of the chamber could be formed to adhere very tightly to the skull with the use of titanium screws. As a result, pressure exerted by the screws all around the implant produced a perfect seal (Fig. 2). As evidenced by the cleaning procedure, the inside of the chronic recording chamber is totally impermeable to fluid with respect to the outside. This is an important requirement to minimize risks of infection inside the chronic chamber. The present study differs in several aspects from the recent report of Adams et al. (2011). First, in their report, Adams et al. (2011) used an acrylic free titanium chronic recording chamber whereas here the chamber was machined from Tekapeek. The latter presenting the advantage to be lighter and to reduce artifacts in MRI investigations. Furthermore, Adams et al. (2011) used HA as a paste to seal the chamber to the skull during the surgery; by contrast, in the present study, HA was coated onto the implant itself before implantation. Another important difference is that the titanium chamber used by Adams et al. (2011) was comprised of 5–6 feet, each with one hole to insert a screw to anchor the chamber to the skull. In our case (Fig. 2), the feet were replaced by a continuous thin edge all around the base of the chamber (Fig. 2) which included the holes to insert the screws ($n = 7$). When tightened, the screw pressure ensured a perfect match of the chamber's base onto the skull surface due to of the relative flexibility of the Tekapeek and its thin continuous edge. The present study demonstrates that Tekapeek can replace titanium to fabricate implants (at least the chronic recording chamber, but possibly also the head fixation device) and exhibits good osseous-integration properties due to the hydroxyapatite coating. The use of Tekapeek is especially favorable for MRI investigation, as artifacts are minimized. Along this line, a further improvement may consist in replacing titanium screws by ceramic screws.

The steps newly introduced in the present study, such as coating the implants with HA and the 3D print replicate of the skull, increases the cost of the experiments, but remains a worthwhile investment considering the longevity of the implants, the ethical and commercial values of the non-human primates, as well as the long time period invested in this type of experiments – mainly training the monkeys to perform difficult behavioral tasks. Indeed, the rejection of an implant (head fixation device and/or chronic recording chamber) by the bone is dramatic in any case, as it can ruin a long and expensive behavioral and electrophysiological experiment. It is extremely difficult to re-implant a second time at the same location.

In summary, the present study contributes to refining recent proposed techniques (Adams et al., 2007, 2011; McAndrew et al., 2012) to optimizing the anchoring of a head fixation device and a chronic recording chamber on the skull of macaque monkeys. In our opinion it should include a coating with HA and a 3D print replicate of the skull. As such, the present study is well in line with the 3Rs initiative to improve the conditions of laboratory animals. Indeed, the longevity of the implants as well as the minimal discomfort offered by custom-fitted implants contribute to substantially reducing the stress for the monkeys, decreasing the risks of infection and reducing the number of animals used in this type of experiment. The possibility to remove and replace implants is a very positive aspect because the same animal can be used over a longer time period and allows investigation of remote brain regions (e.g. premotor cortex and thalamus).

## Conflict of interest

HA is provided by Medicoat AG (Switzerland).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jneumeth.2013.07.015.

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## Conclusion

The work, reported in the article Lanz et al., 2013a, extended and refined previous studies conducted by Adams et al. (2007 and 2011) in order to optimize the anchoring of various implants in the field of the non-human primates' chronic experiments. We thought that the use of the hydroxyapatite in the device coating was necessary for the optimization of the research with our experimental models. This in spite of the constraint time: indeed, several months (3 months) were necessary between the surgery and the first head fixation (in the case of the head fixation device). The advantages of these implants were: 1. The longevity of the anchoring (no loss of device); 2. The use of personalized implants, that was custom-fitted implants; 3. A decrease of infection occurrences; 4. The possibility to remove and replace an implant; 5. The reduction of the number of animals used in this type of experiments (3R initiative, in this case "*Reduce*").

# III. Results

## III.1 Non-human results

The aim of the present thesis work, following the development of new methodological approaches, was to record neuronal activity in the premotor cortex of non-human primates (*Macaca fascicularis*) during a multisensory-motor integration task. With these electrophysiological recordings, we aim at testing the hypothesis according to which the premotor cortex (PM) is a site of early convergence of auditory and visual inputs. Consequently, the PM may initiate faster motor responses when triggered by multimodal (in our case visuo-acoustic) stimulation, as compared to unimodal stimulation. In the past, in our laboratory (Céline Cappe's Ph.D thesis), it was demonstrated in the non-human primates that during a visuo-acoustic integration, the bimodal reaction times (RTs) was shorter than the unimodal RTs. Secondly, based on single-unit electrophysiological recordings, it was shown that in a specific-modality cortex (in this case, the auditory cortex), some neurons were able to respond to auditory or/and to bimodal (=multimodal neurons) stimuli. Other recent studies (Kayser et al., 2009; Falchier et al., 2010) confirmed this finding, as well as in other unisensory cortex, such as the visual cortex (e.g. Wang et al., 2008).

For this study, we focused our interest to a polysensory, sensorimotor cortical area, the premotor cortex (PM), where different inputs from various modalities converge (auditory, visual, and somatosensory). The results of these investigations obtained in two monkeys can be found in the article (Lanz et al., 2013b). Briefly it describes the different behavioural assessments of the multisensory integration process in monkeys, such as reaction times, the percentage of errors and errors progression with practice. During a second step, single unit recordings were derived from PM in the same two monkeys executing the behavioural paradigm.

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# Multisensory integration in non-human primates during a sensory-motor task

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Daily our central nervous system receives inputs via several sensory modalities, processes them and integrates information in order to produce a suitable behavior. The amazing part is that such a multisensory integration brings all information into a unified percept. An approach to start investigating this property is to show that perception is better and faster when multimodal stimuli are used as compared to unimodal stimuli. This forms the first part of the present study conducted in a non-human primate's model ($n = 2$) engaged in a detection sensory-motor task where visual and auditory stimuli were displayed individually or simultaneously. The measured parameters were the reaction time (RT) between stimulus and onset of arm movement, successes and errors percentages, as well as the evolution as a function of time of these parameters with training. As expected, RTs were shorter when the subjects were exposed to combined stimuli. The gains for both subjects were around 20 and 40 ms, as compared with the auditory and visual stimulus alone, respectively. Moreover the number of correct responses increased in response to bimodal stimuli. We interpreted such multisensory advantage through redundant signal effect which decreases perceptual ambiguity, increases speed of stimulus detection, and improves performance accuracy. The second part of the study presents single-unit recordings derived from the premotor cortex (PM) of the same subjects during the sensory-motor task. Response patterns to sensory/multisensory stimulation are documented and specific type proportions are reported. Characterization of bimodal neurons indicates a mechanism of audio-visual integration possibly through a decrease of inhibition. Nevertheless the neural processing leading to faster motor response from PM as a polysensory association cortical area remains still unclear.

**Keywords: sensory-motor, detection task, non-human primate, facilitatory effect, electrophysiology**

## INTRODUCTION

Traditionally sensory modalities like vision, hearing, touch, pain, chemical senses, and others have been investigated mostly individually. However, the number of studies showing cross-modal integration (Calvert et al., 2000; Calvert, 2001a; Driver and Noesselt, 2008; Ho et al., 2009) increased in the past decade and demonstrated that multisensory integration provided clear advantages in terms of subject survival. In other words, estimates from different modalities were more reliable than isolated estimates and contributed to form more meaningful representations of the environment. Behaviorally, integrating information across sensory modalities improved the speed of detection and reduced perceptual ambiguity (Stein et al., 1996; Rowland et al., 2007; Wallace and Stein, 2007). Interestingly, some authors claimed that multisensory integration was an acquired ability (McIntosh et al., 1998; Stein and Stanford, 2008; Brandwein et al., 2011). Electrophysiologically, multisensory processing was described through bimodal neurons activities (Allman et al., 2009) and those were reported almost exclusively in studies of the superior colliculus (SC; Stein and Meredith, 1993). Other studies confirmed similar findings in the cortex (Wallace et al., 1992; Martuzzi et al., 2007) and especially in the auditory cortex of non-human (Kayser et al., 2009; Falchier et al., 2010) and human primates (Foxe et al., 2002), and also in the visual cortex in non-human (Rockland and Ojima, 2003; Wang et al., 2008) and human primates (Giard and Peronnet, 1999; Calvert et al., 2001b). Very few data were available in the motor cortex and less in the premotor cortex (PM; corresponding to Brodmann's area 6 or M2) yet considered as another candidate for polymodal integration because of the convergence of visual, auditory, and somatosensory inputs (Graziano, 2001). It is this area that we decided to investigate in the present study while presenting auditory (A) stimuli, visual (V) stimuli, or a combination of both modalities (visuo-acoustic, VA) in a non-human primate model, in the context of a detection task.

Behaviorally speaking, two adult macaque monkeys were trained to detect A, V, or VA stimuli and respond to them with a stereotyped reaching arm movement. This detection task was comparable to previous studies conducted in human subjects (Giard and Peronnet, 1999; Molholm et al., 2002; Gori et al., 2011) and in macaques (Cappe et al., 2010). However, for the first time stimuli levels used with monkeys were based on individually determined perceptual thresholds in order to precisely determine the auditory

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**Abbreviations:** RT, reaction time; PM, premotor cortex; PSTH, peristimulus time histogram; SD, standard deviation.

versus visual stimuli intensities required to modify the multisensory gain in motor response latencies. Performances were assessed by measuring the reaction times (RTs), calculating the percentage of correct responses and reporting the errors progression. As expected, RTs were shorter when movements were triggered by cross-modal stimulation than by unimodal stimulation. This behavioral facilitation usually known as redundant signals effect (RSE) might be tested through two different models: (1) The race model (Raab, 1962) which assumed that shorter RTs in a multisensory context were due to the sensory modality which first detected the cue; and (2) the co-activation model (Miller, 1982) which explained that a parallel processing of unimodal channels interacted somewhere in the sensory information processing system.

Electrophysiologically speaking, single units were recorded from a chronic recording chamber anchored above the PM in the same two monkeys executing the behavioral paradigm. The aim was to characterize bimodal neurons in PM possibly responsible for rapid audio-visual integration leading to a fast motor response. This hypothesis was built on the basis of previous studies (Graziano et al., 1997) which reported that aside from a majority of neurons from ventral PM responding to tactile stimuli about 40% were active in response to visual stimuli. Graziano et al. (1999) characterized trimodal neurons too which were active when a supplementary acoustic stimulation was delivered. In addition studies in the dorsal PM from Weinrich and Wise (1982) and Weinrich et al. (1984) demonstrated that some neurons modulated their discharge rates during a visual stimulation. Therefore it became clear that PM which played an important role in the preparation and control of voluntary movements (e.g., Wise and Kurata, 1989; Wise et al., 1997; Luppino and Rizzolatti, 2000) had to be investigated in the context of multisensory integration in order to characterize its contribution to generate a unified percept used to generate quick behavioral responses Therefore the present study is an attempt to link single-unit activities in PM to behavioral performance in order to better understand the neural representations that guide motor behavior.

## MATERIALS AND METHODS

### SUBJECTS

Two adult non-human primates (*Macaca fascicularis*; Mk-LI and Mk-JZ) were enrolled in the present study. Mk-LI was 9 years old and Mk-JZ was 7 years old at the time of the onset of electrophysiological recordings. The monkeys Mk-LI and Mk-JZ are distinct from the two monkeys used in a previous study from this laboratory based on an earlier, less elaborate version of the psychophysical paradigm (Cappe et al., 2010), with some important differences as outlined in the discussion. The monkeys' weight was monitored daily and both weighed around 8 kg. When a 10% loss of weight was measured, experiments were interrupted until they recovered their previous weight. Such event did not occur in the course of the present study. Between experimental sessions, the animals shared with other monkeys (groups of two to five animals) a detention room of 45 $m^3$ (15 $m^3$ until 2010; see e.g., Kaeser et al., 2011; Schmidlin et al., 2011), in which they could freely move and had free access to water. They were never deprived of food but the daily intake was adjusted to the performance in order to not loose motivation. The days without tests, the animals were fed by the animals' caretakers. Otoscopic examination was carried out regularly to verify that the external ear canal and the tympanic membrane were intact and free of infection. The experiments were conducted according to both guidelines of the National Institute of Health: *Guide for the Care and Use of laboratory Animals* (1996), and of the European Community: *Guidelines for Animals Protection and Use for Experimentation.* Furthermore, the cantonal and federal Swiss veterinary authorities approved the experimental procedures (veterinary authorizations 173-06, 173-07, 156-06, 156-08, 18/10).

### STIMULI

The subjects were trained to perform a detection sensory-motor task with visual and auditory stimuli delivered individually or simultaneously. The tests were carried out in a modified double-walled electrically shielded sound-proof room (compact model, type AB200, Eckel Industries of Canada).

Sounds were delivered under free-field conditions through two loudspeakers (RTO, Hi-Fi 2 Way Speaker System, model HF-10) positioned at equal distance of the monkeys' heads (∼20 cm), on the left and on the right, and at the same height. The auditory stimulus was a white-noise burst of 250 ms duration generated digitally by RPvdsEx software (Tucker-Davis Technologies System 3, USA) and later converted to analog format by a real-time processor (RP2.1 or RX6, Tucker-Davis Technologies, USA). The calibration of the system was performed with a sound level meter (Brüel and Kjaer, 2231) using a microphone (Brüel and Kjaer, 4189, pre-polarized, 1/2″) placed at the normal location of the center of the head. Calibration was based on a calculated reference voltage generated at 94 dB SPL at 1 kHz.

Visual stimuli were delivered by a green light-emitting diode (LED 1.9 mm in diameter, Kingbright) positioned in front of the subject, at eyes' height and at a distance of 23.5 cm. The diode was on during 250 ms and the apparent intensity was digitally controlled through RPvdsEx software (Tucker-Davis Technologies System 3, USA) which supplied current pulses of specific frequencies. The conversion into analog format was provided by a real-time processor (RX6, Tucker-Davis Technologies, USA). This visual stimulation appeared on a black background screen displaying repeatedly (every trial) a white round centered target (2 cm in diameter) to lock the gaze during the experiments. The calibration of the system was performed with a CCD camera (Prosilica ccd camera, Prosilica GE) placed at the normal location of the eyes and expressed in Lux (lx) which corresponds to the luminous flow received by unit area. This calibrated value was related to the current pulses frequency used for supplying the diode therefore allowing us for the rest of the paper to refer to Hertz units instead of Lux.

### SENSORY-MOTOR TASK

During a first phase of training, which lasted several months (approximately 8–10 months), the subjects were taught a sensory-motor task where they had to release a lever, then press a touch pad in response to either visual (V), or acoustic (A), or VA stimuli. This training was based on a positive reinforcement protocol described previously (Durif et al., 2003; Cappe et al., 2010) and modified from Smith and Olszyk (1997) (**Figure 1**). Briefly all the recording sessions were performed in a sound-attenuated chamber and the trials were initiated by the animal when pressing a

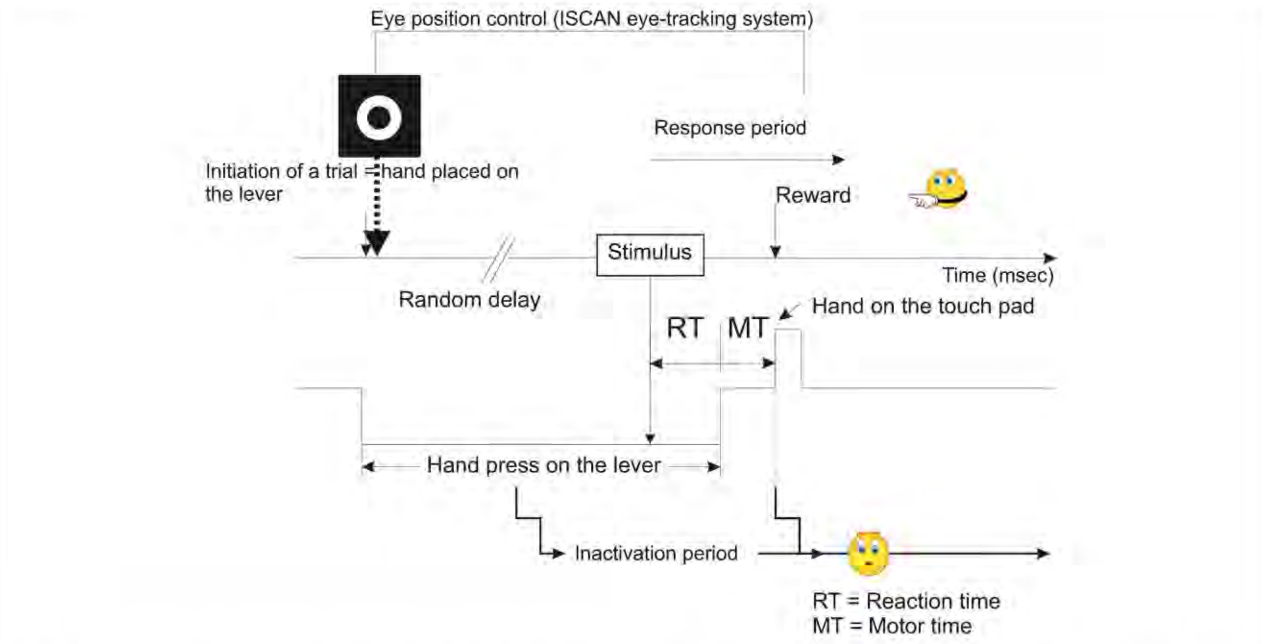

**FIGURE 1 | Schematic representation illustrating the temporal course of a typical trial of the multisensory and sensorimotor integration task used in the present study (modified from Smith and Olszyk, 1997).** See also Movie S1 in Supplementary Material.

lever with the left hand. At this step, a target was displayed on the screen facing the animal, and although the head was unrestrained, gaze fixation was encouraged to be maintained up to the end of the motor period (in anticipation to electrophysiological recordings where neurons may be responding to gaze direction too). Following this initiation time, a random delay was set ranging from 1 to 4 s in order to minimize anticipation of stimulus onset. The delay ended with the presentation of a stimulus (visual, acoustic, or VA) and the subject was requested to touch a pad positioned above the lever with the left hand. A correct response was rewarded by one banana-flavored pellet (Dustless Precision Pellets® Primate, Grain-Based, Bio-Serv, NJ, USA). If the subject released the lever in absence of stimulus or in anticipation (RT $<150$ ms), corresponding to a false alarm, a 3-s time out was generated during which it was impossible for the subject to initiate a new trial, and of course to receive a reward. When the motor response occurred after the stimulus with a RT larger than 800 ms, then the trial was considered as erroneous (lack of detection, as in absence of motor response to a true stimulus). The behavioral task was entirely controlled and monitored using a customized workstation, elaborated from RPvdsEx software (Tucker-Davis Technologies, USA) and running on real-time processor devices (RP2.1 or RX6, Tucker-Davis Technologies, USA). The eye-tracking system (ISCAN Inc., USA) was also incorporated into our workstation.

### THRESHOLDS ASSESSMENT

Both auditory and visual thresholds were obtained by using an adaptive staircase method. This well-known psychophysical technique (Levitt, 1971) is a variation of the method of limits involving both ascending and descending limits and where the test is continued until several reversals are accomplished. Usually threshold is the average of at least six or eight reversal points. The configuration used in the present study was (1) an initial stimulus level having a high probability of a positive response, (2) a decrease of the stimulus level by half after a positive response (or an increase by half after a negative response) therefore the significant initial step chosen (**Table 1**) and, (3) an average of the last eight reversals. Those steps of the protocol are illustrated in Movie S1 in Supplementary Material (http://www.unifr.ch/neuro/rouiller/research/gerardmat1.php) where the monkey is handling a lever only (training paradigm). For the experiments, the minimum step size was fixed at 2 dB SPL for acoustic stimuli and 2 Hz for visual stimuli (diode pulse rate frequency) in order to not produce thresholds under the precision reached by the calibration of our equipment (for loudspeakers as well as LEDs). Common reported errors of habituation and anticipation have been respectively minimized thanks to (1) a random delay before stimulus onset and (2) a 3-s time out plus no reward whenever the subject prematurely reported detecting the stimulus before its occurrence.

Both auditory (binaural) and visual thresholds were expressed in decibels. Therefore visual thresholds had to be converted from Hertz into decibels according to the formula: $\mathrm{dB} = 20\log_{10}\left(\frac{P_2}{P_1}\right)$ where $P_1 = 18.98$ Hz (lowest visual level detected by 10 healthy human subjects from our laboratory) and $P_2$ the tested level.

The threshold testing was completely automatized with RPvdsEx environment (Tucker-Davis Technologies, USA) and with several subroutines elaborated with MATLAB®(The Mathworks, Inc., USA) and Labview (National Instruments™, USA).

**Table 1 | Parameters used according to the type of threshold search procedure.**

| | Visual | Acoustic |
|---|---|---|
| Initial intensity | 1000 Hz (79 dB) | 50 dB SPL |
| Steps | 100 Hz (33 dB) | 10 dB SPL |
| Step division | Division by 2 after each inversion | |
| Min. step | 2 Hz (−45 dB) | 2 dB SPL |
| Inversions | 13 Inversions | |
| Threshold | Last four peaks and four valleys average | |

### MULTISENSORY SESSIONS

For multisensory sessions, the sensory-motor task was similar to the one developed for determining thresholds. The gaze was still locked but in addition the eye position was monitored using an ISCAN eye-tracking system (RK-426 Pupil/Cornal Reflection Tracking System, ISCAN®, Inc., USA) to ensure that the subject did not move his eyes from the beginning of the trial till the detection of the stimulus. During this cross-modal task, auditory and visual stimuli were presented individually or in combination (see Movie S1 in Supplementary Material). The random distribution of these three conditions was provided by the customized workstation and more specifically by the RPvdsEx software (Tucker-Davis Technologies, USA). Therefore a daily session consisted of at least 200 trials randomly distributed into acoustic, visual, and VA conditions. At these daily sessions, the tested intensities were fixed based on the threshold values obtained previously plus 10 dB. Levels of performance were considered as stable when the daily sessions contained generally less than 15% of erroneous trials (false detection or anticipation of motor response). At that step, a head fixation device was anchored to the skull (see below). Monkeys were then re-trained over a period of 1 month to execute the entire auditory-visual-motor task with the head restrained in order to re-establish a stable level of performance. The recordings (psychophysics) went on afterward over several months followed finally by electrophysiological investigations. The daily recording sessions generated data such as RTs and percentage of correct performance with identification of different errors, such as execution or detection errors.

### SURGERY

As mentioned above, when the subjects reached a daily stable level of performance, a first surgery aimed at implanting a head fixation device (for details about the methods and the device see (Lanz et al., 2013). Briefly, this device was used to restrain the monkey's head movements in order to allow eye position monitoring and therefore was anchored to the skull. No dental or orthopedic cement was used but only screws and an osseous integration facilitation procedure. A second surgery was performed after several months in order to implant a chronic recording chamber in tekapeek, allowing daily single-unit recordings in the PM (again more detail on the method and the device can be found in Lanz et al., 2013).

### ELECTROPHYSIOLOGY

Electrophysiological recording sessions were performed when the monkeys were engaged in the protocol of multisensory-motor detection task and exhibited a stable level of behavioral performance (see details in the Section "Multisensory Sessions" above). Neuronal extracellular activities were recorded with tungsten microelectrodes (5–7 MΩ impedance from FHC, Bowdoinham, ME, USA), advanced perpendicularly with respect to the dura through the chronic recording chamber into the PM. The electrode driving system used was from Narishige®(Narishige International limited, Japan). For both monkeys, the activities recorded were derived from single neurons in the right PM (contralateral to the arm used to execute the motor task). At the exploratory stage, auditory and visual stimuli were fixed at 30 and 90 dB above threshold, respectively, and when a putative interesting neuron was identified (stable activity over approximately 100 trials), a new acquisition was started again with stimuli at different levels of lower intensities. Recording sessions took place on a daily basis during a period of several months (2–3 months) and concurrently behavioral data were stored (as described in the Section "Multisensory Sessions" section). Neurons were discriminated using the principal component feature space spike sorting software (SpikePac from Tucker-Davis Technologies, USA) which allowed us to select and sort out data during acquisition but also to perform playback of stored data for dynamic visualization of neural activities. These data were then exported into MATLAB®(The Mathworks, Inc., USA) to perform off-line analysis. A subroutine was designed in order to sort out data into three matrices (A, V, and VA) and to build-up dot rasters and peri-stimulus time histograms (PSTHs) where every trial's activity is aligned against the onset of the stimulation. Neuronal responses were transformed into spikes per second. For the analysis, three periods of the same duration (200 ms) were defined: a reference period of "spontaneous" activity before delivery of the stimuli, an activity period during the presentation of the stimuli (A or V or VA), and a post-stimulation period following the latter. The neuron baseline activity was defined as the mean of activities recorded during the reference period. For each condition (A, V, or VA), comparisons were carried out between the discharge means of the reference period and the activity period and, between the reference period and the post-stimulation period with a two-sample $t$-test. Another comparison with the same test was performed between activity periods of different conditions and between post-stimulation periods. A significant response of the neuron was identified when $p < 0.05$. A standard deviation (SD) was calculated and helped us to visually assess when the evoked activity was overshooting the mean baseline activity plus 2 SDs (excitation; see e.g., Wannier et al., 2002; Durif et al., 2003) or was below the mean baseline activity minus 1 SD (inhibition).

## RESULTS

### ABSOLUTE SENSORY THRESHOLDS

Auditory and visual behavioral thresholds were first evaluated independently in our two subjects (Mk-LI and Mk-JZ) with psychophysical tools (adaptive staircase method) using respectively white-noise bursts or focused flashing lights. Mean thresholds and SDs were calculated from 15 sessions over 5 months of training (Mk-LI) and 2 months of training (Mk-JZ) for each sensory modality. Performing a Mann–Whitney test confirmed that no significant difference was observed between the two animals for visual thresholds ($p = 0.06$, mean threshold = 24.6 dB SPL in Mk-LI and

23.6 dB SPL in Mk-JZ). However a difference for auditory thresholds was observed ($p = 0.04$, mean threshold = 7.8 dB in Mk-LI and 6.6 dB in Mk-JZ).

### REACTION TIMES: MEAN VALUES AND TIME COURSES

For both monkeys, following the initial training, a period of stable behavioral performance was selected and data were extracted from sessions comprising at least 200 trials. Data were usually collected daily, 5 days per week. The distribution of RTs in response to visual, auditory or VA stimulations is displayed in **Figure 2**. These data were obtained over a period of respectively 12 (Mk-JZ) and 10 (Mk-LI) months before the initiation of the electrophysiological recording period. All data were obtained at 10 dB above sensory thresholds (see paragraph above), corresponding to 17 dB SPL for the auditory stimulation and 34 dB (representing 950 Hz in diode current pulses) for the visual stimulation. The results showed that mean auditory RTs [450 ms ($n = 1{,}391$) for Mk-LI and 377 ms ($n = 1{,}091$) for Mk-JZ] were significantly different (Mann–Whitney test, $p < 0.0001$) from visual RTs [424 ms ($n = 1{,}648$) for Mk-LI and 359 ms ($n = 1{,}292$) for Mk-JZ]. VA RTs [409 ms ($n = 1{,}883$) for Mk-LI and 340 ms ($n = 1{,}091$) for Mk-JZ] were significantly shorter than auditory RTs ($p < 0.0001$) and visual RTs ($p < 0.0001$; **Figure 2**) in both monkeys. Mean auditory RTs were about 20 ms longer than visual RTs. RTs resulting from VA stimulation were shorter than the fastest unimodal stimulation (visual in the present case). In addition, subject Mk-JZ showed clearly shorter RTs (approximately 70 ms less) than subject Mk-LI.

The progression and variability of mean RTs with time for both subjects Mk-LI and Mk-JZ are depicted in **Figure 3**, during the time window preceding the electrophysiological recording. In this Figure, data from Mk-LI were collected over 32 sessions and showed in most cases the same general pattern as described in **Figure 2**, namely mean VA RTs shorter than mean visual RTs, themselves shorter than mean auditory RTs. In **Figure 3**, data from Mk-JZ were obtained over 23 sessions and showed the same pattern. In both monkeys, the RT values were considered as stable enough to initiate the electrophysiological recordings.

### TIME COURSE OF ERRORS

During the time window of psychophysical data collection, the proportion of erroneous trials was generally below 15%. Errors recorded during the sensory-motor detection task are reported in **Figure 4A** for subject Mk-LI and **Figure 4B** for subject Mk-JZ, respectively). Errors have been divided into "execution" errors when the animal was deviating from the protocol and "not detected" errors when an expected response to a stimulus did not happen. The distribution over time and the color code for errors occurring during visual, auditory or VA stimulation was the same as the one used in the previous section. As far as the "execution" errors were concerned for subject Mk-LI (**Figure 4A**), differences were significant across the sessions and within the three sensory modalities [$F(2, 93) = 79.35, p < 0.001$]. The "not detected" errors also showed significant differences across the sessions and within the three sensory modalities [$F(2, 93) = 3.26, p = 0.04$], whenever they were all three available.

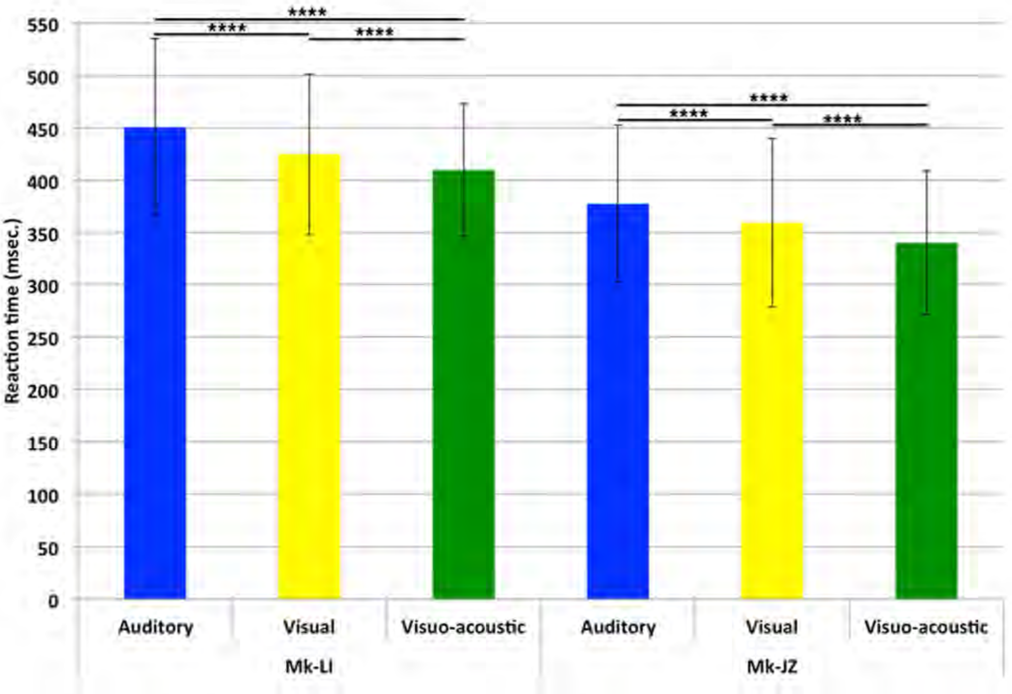

**FIGURE 2 | Mean reaction times and their SDs obtained at 10 dB above unisensory thresholds.** Data derived from several months of daily sessions in Mk-LI (12 months) and in Mk-JZ (10 months). ****$p < 0.0001$ (Mann–Whitney test).

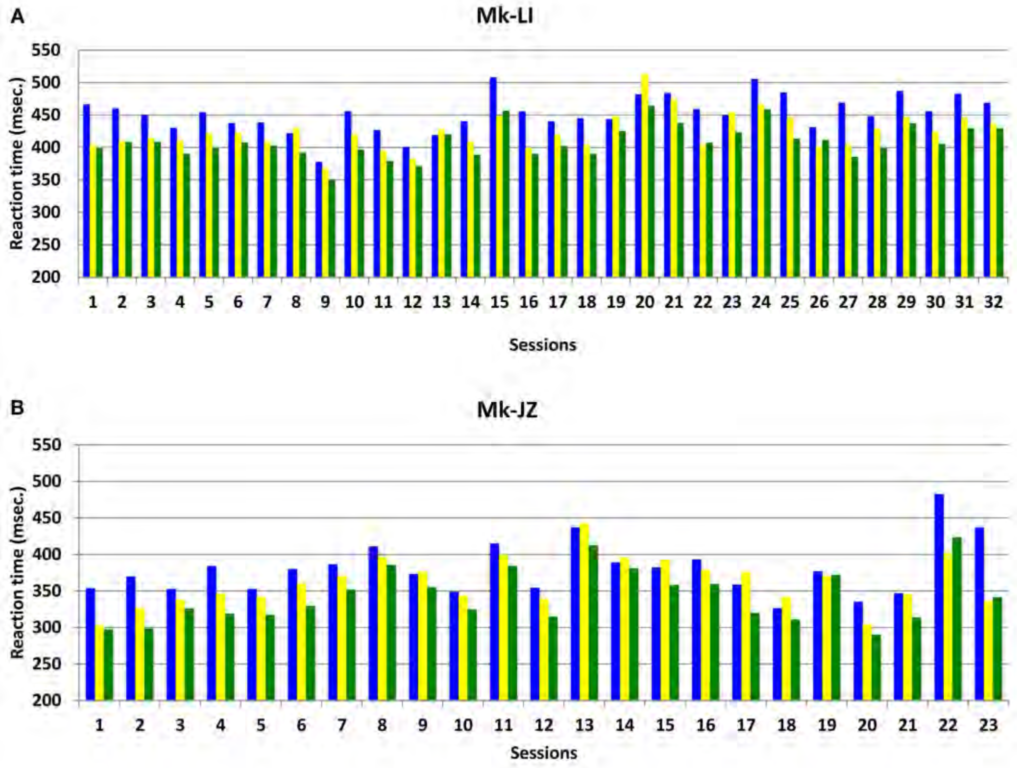

**FIGURE 3 | Mean reaction time progression over time (daily sessions) for subjects Mk-LI (A) and Mk-JZ (B) with intensities 10 dB above unisensory thresholds.** The data were collected from a restricted time window (32 daily sessions in Mk-LI and 23 daily sessions in Mk-JZ), preceding the electrophysiological data collection. RTs in response to auditory stimuli are in blue, to visual stimuli in yellow and to both modalities delivered simultaneously in green.

In **Figure 4B**, the "execution" errors made by subject Mk-JZ showed significant differences across the sessions and within the three sensory modalities [$F(2, 66) = 25.0$, $p < 0.001$]. The "not detected" errors also showed significant differences across the sessions and within the three sensory modalities [$F(2, 66) = 3.61$, $p = 0.03$]. From a general point of view, both graphs (**Figures 4A,B**) showed that the two subjects did mainly execution errors rather than detection errors and that the errors were randomly distributed over time. As shown in **Figures 4C,D** ($n = 32$ and 23 respectively), and as expected for multisensory facilitation, the percentage of errors was lowest in the VA condition, as compared to the A and V unisensory conditions. Furthermore, there were fewer errors in the V condition than in the A condition.

### STIMULI INTENSITY EFFECTS

The variations of RTs as a function of stimuli intensities are reported in **Figure 5**. The first pool of data ($n \approx 1{,}000$) collected for each animal was obtained at 10 dB above unisensory thresholds which corresponded to 17 dB SPL for auditory stimuli and 34 dB for visual stimuli. In Mk-JZ, three other supra-threshold auditory intensities have been tested (15, 30, and 47 dB SPL) combined with 44 and 48 dB for visual stimuli. The differences between uni- versus multi-sensory RTs for each subject have been tested with a non-parametric $t$-test (Mann–Whitney test) and were significantly different between all stimulation conditions ($p < 0.0001$). In Mk-LI, two supra-threshold intensity conditions are shown (**Figure 5**), also exhibiting statistically significant RTs differences, except for A versus V at A = 17 dB SPL and V = 34 dB. As a second step, the effect of stimulus intensity on RTs has been tested for each sensory modality through a one-way ANOVA for Mk-JZ and a $t$-test for Mk-LI. It resulted that an increase of the auditory intensity decreased significantly the response time ($p < 0.0001$) but not between 15 and 17 dB (not significant); an increase of the visual intensity decreased significantly the RT between 34 and 48 dB ($p < 0.0001$), as well as between 44 and 48 dB ($p < 0.0001$). However no difference was demonstrated between 34 and 44 dB. Concerning the combined stimuli, we obtained a significant difference ($P < 0.0001$ and $P < 0.01$ between VA = 15 dB SPL; 44 dB and VA = 30 dB SPL; 44 dB, which demonstrated that RTs decreased when intensities of stimuli increased. For Mk-LI the increase of intensity decreased significantly the RT ($P < 0.001$) for all conditions (A, V, and VA).

To determine if the RSE are consistent with the race model or the co-activation model, we applied for every parameter a cumulative distribution function and a Miller's race model inequality (Miller, 1982), respectively. The purpose of this equation is to test whether the probability for a RT during a cross-modal stimulation is higher than the summation of both uni-sensory RT.

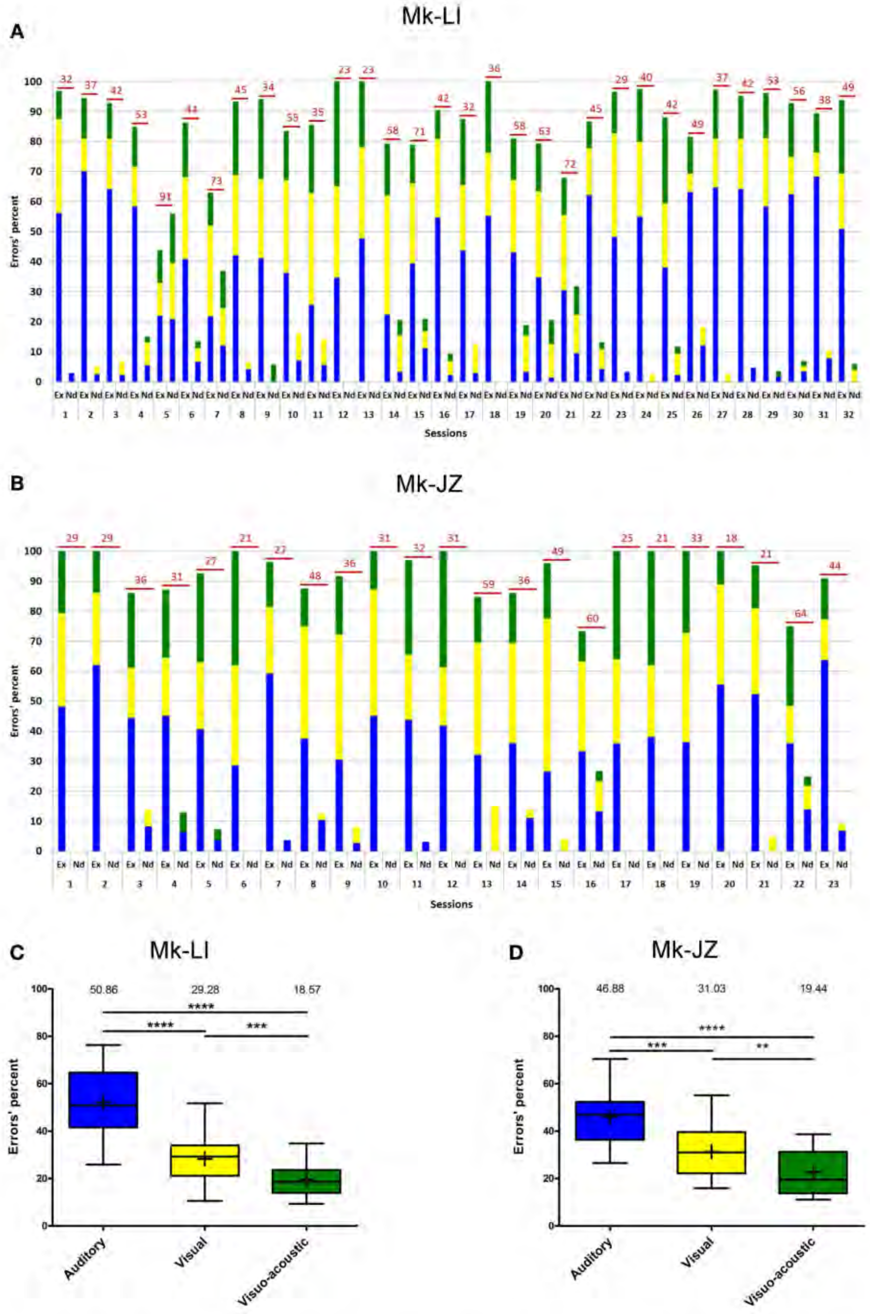

**FIGURE 4 | Errors' progression for both subjects (same time window as in Figure 3). (A)** for Mk-LI and **(B)** for Mk-JZ. The total number of errors per session was indicated on top of each bars. "Ex" stood for Execution errors; "Nd" for Not detected errors. The percentages of errors displayed in **(A,B)** were distributed in the form of box and whisker plots **(C,D)**. The end of the whiskers represented maxima and minima. The Mann–Whitney test showed *p*-values results as ** when $p < 0.01$, *** when $p < 0.001$ and **** when $p < 0.0001$.

As a first step, the cumulative distributions are displayed in **Figures 6A,B,E,F,I,J**, respectively for each monkey and each parameter tested (as in **Figure 5**). The more the curve is shifted to the left, the shorter is the RT, which means that we have a higher probability at a given RT latency. For all parameters, there is a leftward shift for cross-modal stimulation (AV) (corresponding to green curves in **Figures 6A,B,E,F,I,J**), as compared to the A or V curves.

In complement, an analysis of the inequality of Miller was tested for every parameter (**Figures 6C,D,G,H,K,L**). This

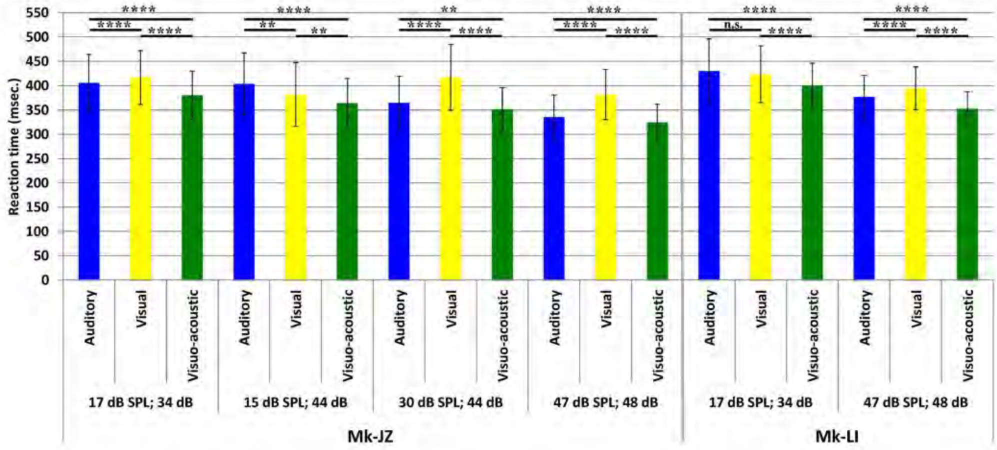

**FIGURE 5 | Mean reaction times (with their SDs) obtained for different combinations of supra-threshold intensities and for both monkeys (Mk-JZ and Mk-LI).** n.s.: $p \geq 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$ (Mann–Whitney test). The data were derived from the electrophysiological daily sessions' collection.

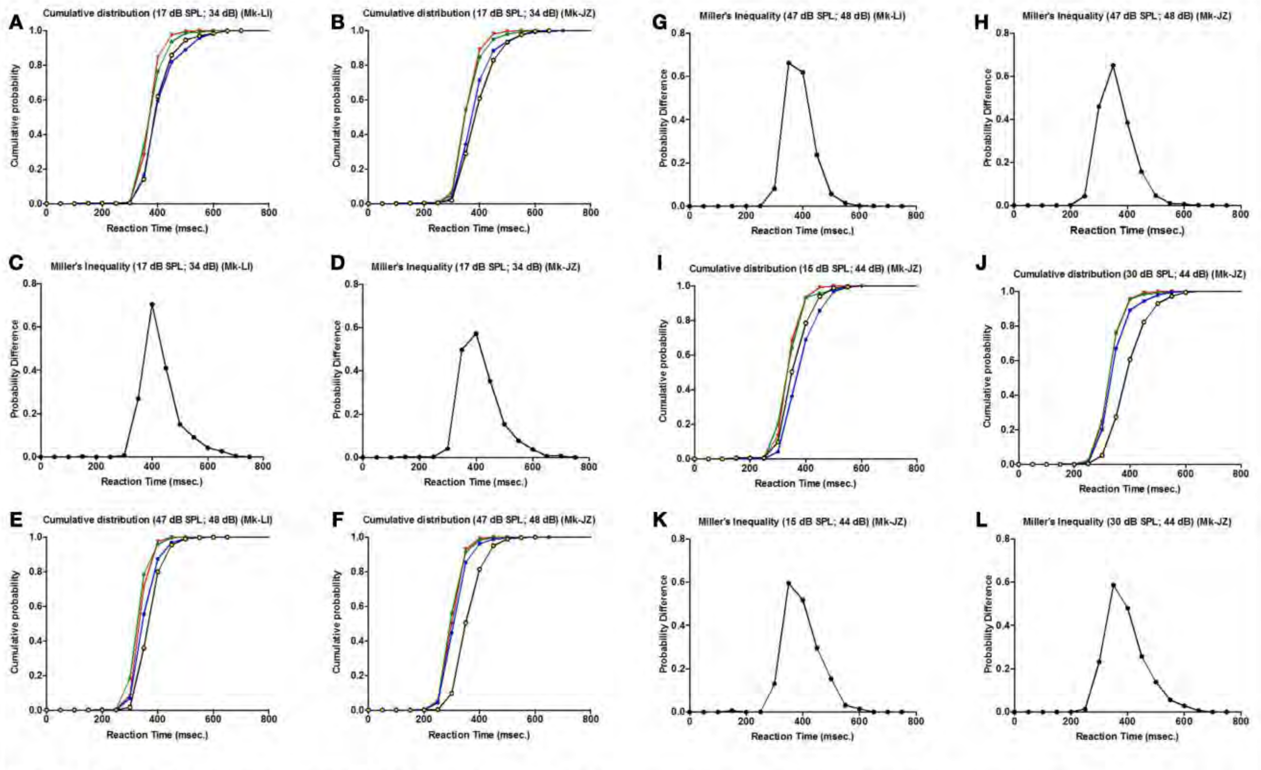

**FIGURE 6 | Cumulative distribution function for different intensities for both monkeys (Mk-LI and Mk-JZ). (A,B,E,F,I,J)** are for the cumulative distribution functions for Mk-LI **(A,E)** and for Mk-JZ **(B,F,I,J)**. **(C,D,G,H,K,L)** are for the Miller's Inequality for Mk-LI **(C,G)** and for Mk-JZ **(D,H,K,L)**. In the cumulative distribution graphics, the blue curve represent the auditory condition, the yellow/black curve the visual condition and the green curve the visuo-acoustic condition. The red curve is the model predicted by Miller's race model inequality (Miller, 1982).

inequality is defined as: $P[\mathrm{RT(VA)}] < [P(\mathrm{RT(A)}] + P[\mathrm{RT(V)}] - [P(\mathrm{RT(A)}] \times P[\mathrm{RT(V)}]$ where $P(\mathrm{RT})$ is the cumulative probability density function of RT. This standard analysis (see Murray et al., 2005) consisted in calculating the probability distribution for each condition minus their joint probability. This model represented the upper limit that would be explainable by probability summation. In the panels quoted above, we could observe violations of the race model (i.e., positive values for the probability difference) for all parameters tested, which invoked neural response interactions. Furthermore, we could notice a decrease of the probability differences' values when the intensities increased (in **Figures 6G,K,L**).

### BIMODAL GAIN

Similarly to the calculation reported by Stein and Meredith (1993), the multisensory gain was plotted in **Figure 7**, corresponding to the decrease of the mean RT in percent in the VA condition, as compared to the faster mean RT in unisensory conditions. At 10 dB above threshold, subject Mk-JZ showed a bimodal gain ranging from 5 to 6% whereas, in subject Mk-LI, the bimodal gain ranged from 3.5 to 6%. At intensities higher than 10 dB above thresholds (conditions 3–5 in **Figure 7**), a lower gain was observed than at 10 dB above threshold for Mk-JZ (ranged from 4.5 to 3%) but not for Mk-LI exhibiting a gain of 6.5% at high intensities. More generally, the maximal gains were around 5 to 6% and the principle of inverse effectiveness was largely verified in Mk-JZ, but less so in Mk-LI (**Figure 7**).

### ELECTROPHYSIOLOGICAL RESULTS

The goal here was to test the hypothesis that the sensory modality influences the coding of the motor response during its preparation and planning within and/or after the RT period.

A total of 132 neurons, pooled between Mk-JZ and Mk-LI, were recorded from the PM while the subjects were performing the VA detection task. Electrodes tracks were directed toward PM, mainly its dorsal division (PMd) and were distant from each other by 0.5 mm along the rostrocaudal and mediolateral axes. A surface map of the electrodes coordinates was constructed for each monkey. **Figure 8** shows a representative set of PM neurons and categories were defined according to the discharge patterns. The top panel entitled "Response patterns" corresponded to responses obtained during the period of stimulation (250 ms duration). All the responses of these types were significantly different from the 200 ms-reference period according to the two-sample $t$-tests performed for each single unit. The four types of response patterns were:

- Onset: when a strong and sharp excitation happened after the onset of the stimulus. It corresponded visually to an overshoot over the average activity of reference plus 2 SDs,
- Sustained: when an enhanced activity was observed all along the stimulation period,
- Late: when a strong and sharp excitation arose on the second half of the stimulation period (∼100 ms and later).
- Inhibition: when a significant decrease of the activity happened during the stimulus, corresponding visually to a decrease of activity below 1 SD subtracted from the average activity of reference.

The bottom panel entitled "Modulation patterns" considered significant variations of neuronal activity with respect to the period of reference observed during the post-stimulation period. The two-sample $t$-tests performed over a period of 200 ms were

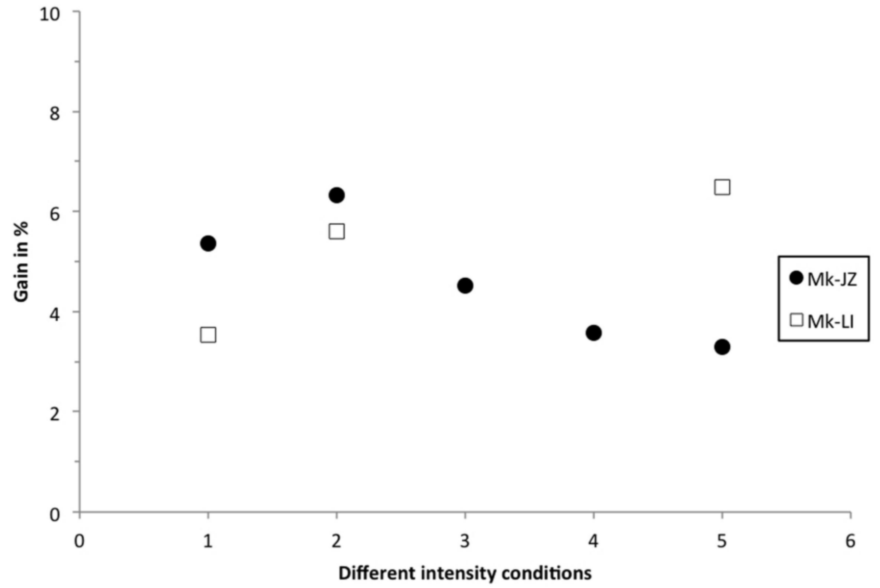

**FIGURE 7 | Multisensory gain expressed in percent of the mean RT in visuo-acoustic conditions as compared with the mean RT of the fastest unisensory condition**. Filled circles display the values for Mk-JZ as a function of the stimulus conditions shown in **Figure 2** [Condition 1 = 17 dB SPL (auditory), 34 dB (visual)] and **Figure 5** [Condition 2 = 17 dB SPL (auditory), 34 dB (visual)]; Condition 3 = 15 dB SPL (auditory), 44 dB (visual); Condition 4 = 30 dB SPL (auditory), 44 dB (visual); Condition 5 = 47 dB SPL (auditory), 48 dB (visual)]. Opened squares display the values for Mk-LI as a function of the same stimulus conditions.

significant against the reference period. In this category three types of responses were identified:

- Offset: when a short period of activity happened immediately after the end of the stimulus,
- Post-sustained: when an enhanced activity was observed all along the post-stimulus period,
- Inhibition: when a decrease of activity was observed after the stimulation period.

In addition to this categorization we determined the percentage per modality of the neurons expressing a significant change in their activity during or after the period of stimulation (**Table 2**). One could notice that the percentages of modulated neurons (change of activity post-stimuli) were roughly two times higher than the number of neurons responding during the stimuli. These percentages were stable across sensory modalities. Concerning the stimulus responding neurons the table shows a slightly higher but not significant ($\chi^2 = 3.29$; df = 2; $p = 0.19$) number of multisensory neurons than unisensory neurons.

Considering further the pattern of activity presented above (**Figure 8**), proportions of unimodal and multimodal neurons in PM are depicted in **Figure 9**. The indicated percentages resulted from a calculation against the total number of neurons expressing an activity within a modality and a pattern. The main activity enhancements corresponded to the "sustained" (35%) and "post-sustained" (72%) types whereas, the inhibition (between 12 and 46%) represented a minority among the recordings. However the inhibition was more frequent in response patterns compared to modulation patterns. One could also note that the combined modality expressed the least percentage of inhibition, especially for response patterns where it was statistically significant: $\chi^2 = 7.19$; df = 2; $p = 0.03$. In general, this side of **Figure 9** showed significant differences in the distribution ($\chi^2 = 25.27$; df = 6; $p = 0.0003$). By contrast, modulation pattern distributions were comparable across the three modalities as shown by a $\chi^2 = 4.68$; df = 4 and $p = 0.32$ (no significant differences detected).

It is noteworthy that a neuron could express several patterns during and after the stimulation. For example, a neuron responding to an auditory stimulus could present a "Response pattern" formed by an "Onset" followed by a "sustained" activity or, a

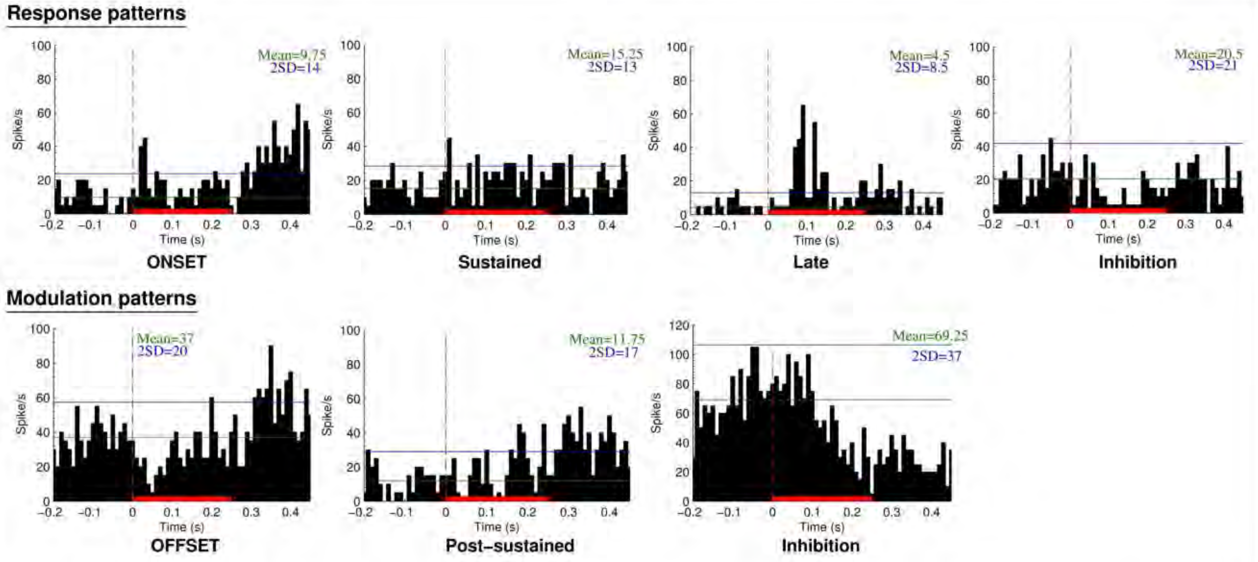

**FIGURE 8 | Neuronal categories obtained from recordings in PM during supra-threshold auditory, visual, or visuo-acoustic stimulations.** The top panel considers the responses during the stimulation period whereas the bottom panel is based on the modulation patterns during the post-stimulation period. The peristimulus histograms (PSTH) display the number of spikes over 40 trials as a function of time before, during (red solid bar), and after the presentation of the stimulus. The vertical red line at time zero is the onset of stimulus presentation. Bin width is 10 ms. The horizontal green line represents the mean discharge rate of reference derived from the period preceding the presentation of the stimulus. The blue horizontal line is the mean discharge rate of reference plus 2 SDs, considered as the limit of confidence for an excitatory response.

**Table 2 | Proportion of neurons exhibiting a change in their activity as a function of the sensory modality and the epoch.**

| | **Acoustic** | | **Visual** | | **Visuo-acoustic** | |
|---|---|---|---|---|---|---|
| | **Stimulus** | **Post-stimulus** | **Stimulus** | **Post-stimulus** | **Stimulus** | **Post-stimulus** |
| Percent | 25 ($n = 33$) | 60.6 ($n = 80$) | 21.2 ($n = 28$) | 57.6 ($n = 76$) | 34.1 ($n = 45$) | 56.1 ($n = 74$) |

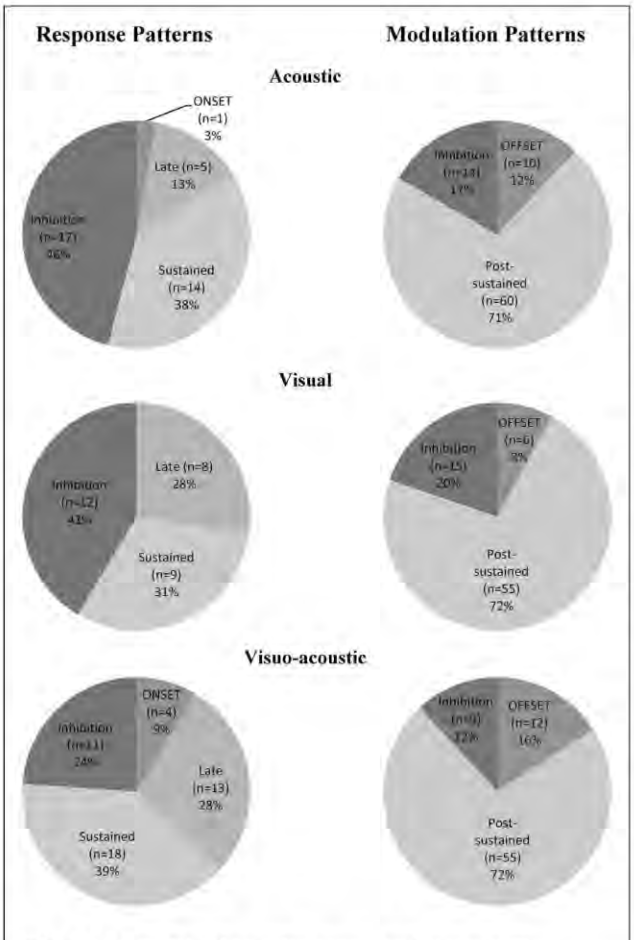

**FIGURE 9 | Proportion of acoustic-, visual-, and visuo-acoustic-responding cells from PM according to the categorization presented in Figure 8**. Numbers represent percentages over the entire cell population from the two monkeys together. Movie S1 in Supplementary Material: illustration of the sensory-motor detection task execution.

"Modulation pattern" formed by an "Offset" followed by a "post-sustained" activity. Such neurons were reported in **Table 3** and grouped per sensory modality.

**Table 3 | Proportion of neurons expressing two different patterns per sensory modality.**

| | Acoustic | Visual | Visuo-acoustic |
|---|---|---|---|
| Percent | 18.8 (*n* = 18) | 17.6 (*n* = 16) | 27.7 (*n* = 26) |

## DISCUSSION

As compared to a previous study from this laboratory (Cappe et al., 2010) based on two monkeys, the present report extends to two more macaque monkeys the observation that RTs in response to combined acoustic and visual stimuli are significantly shorter than RTs in response to separate, individual presentation of acoustic or visual stimuli (**Figures 2** and **5**). In line with the study of Cappe et al. (2010), the bimodal gain (RT decrease) expressed in percent of the RT obtained in response to unimodal stimulation is in the order of 5% (**Figure 7**). The present study demonstrates that the bimodal gain (reduction of RTs in the VA condition) is robust, as it was maintained in spite of several months of training, followed by a time window of several months during which the psychophysical data were collected daily (**Figure 2**), as well as during a subsequent short period of acquisition preceding the electrophysiological investigations (**Figure 3**). The significant decrease of RTs in response to bimodal stimulation (as compared to the shorter unimodal RTs) was observed for a range of intensities going from 10 dB above threshold up to about 40 dB above threshold, whereas in the study of Cappe et al. (2010) this bimodal effect disappeared mostly at 30 dB above the acoustic threshold, as well as for higher intensities. The loss of bimodal facilitation identified by RTs decrease at strong intensities is in line with the well-established principle of inverse effectiveness (Stein and Meredith, 1993; Holmes, 2009). For this reason, intensities higher than 40 dB above acoustic or visual thresholds were not considered in the present psychophysical study. A parallel between the principle of inverse effectiveness observed at behavioral level (see e.g., Mk-JZ in **Figure 7**) and the activity of single neurons is not straightforward. Indeed, the absolute behavioral threshold measured for a given sensory modality is not directly linked to the threshold of individual neurons, as reflected by its discharge rate. For this reason, in our electrophysiological investigations in PM, it was necessary to use in most cases stimuli intensities higher than the behavioral thresholds, in order to detect an influence of the stimulation paradigm on the neuronal activity.

The design of the present study exhibits some substantial differences as compared to the experimental paradigm of Cappe et al. (2010). First of all, the latter authors used as visual stimulus a flash light covering spatially a large portion of the monitor facing the subject. In contrast, in the present paradigm, the visual stimulus was restricted to a spatially limited LED, requiring a precise control of gaze toward this visual target. Even more important, the authors (Cappe et al., 2010) acknowledged that the visual thresholds were not accurately determined. As far as the auditory threshold is concerned, in Cappe et al. (2010), it was derived from the behavioral task itself by extrapolating the intensity generating 75% of correct trials. As a result, in one monkey (MK2 in Cappe et al., 2010) the auditory threshold was only approximated, between 10 and 20 dB SPL. The present study is clearly different, with unimodal thresholds precisely determined in dB for each sensory modality, using an automatized paradigm distinct from the multisensory detection task, based on a preceding, systematic, and high resolution adaptive staircase method, implemented here for the first time in macaques. The two monkeys enrolled in the present study exhibited fairly close threshold values, although it was significantly different for the visual threshold, but not the auditory one. The auditory thresholds (6.6 dB SPL in Mk-JZ and 7.8 dB in Mk-LI) observed here are consistent with the thresholds (1–8 dB SPL range) obtained from other macaque species (Stebbins et al., 1966; Stebbins, 1973, 1975; Pfingst et al., 1978; Smith and Olszyk, 1997) with different methods and with the monkey MK1 from Cappe et al. (2010).

A further difference with the study of Cappe et al. (2010), in which the controlled variation of intensities was focused on the acoustic stimulus, the present study tested different intensity levels well defined with respect to both the acoustic threshold and the visual threshold (**Figure 5**). In the study of Cappe et al. (2010), for acoustic intensities close to threshold (10 dB above), the acoustic RTs were longer than visual RTs, but this was the other way around at all other acoustic intensities tested, with auditory RTs significantly shorter than visual RTs (see **Figure 3** in Cappe et al., 2010). In the present study, visual RTs were also shorter than auditory RTs when stimuli were presented at 10 dB above the respective unimodal thresholds (**Figure 2** of the present study). At intensities higher than 10 dB above thresholds, the auditory RTs tended to be shorter than visual RTs (**Figure 5**), but this was less systematic than in Cappe et al. (2010). This slight difference may be explained by the different visual stimulus used or by an inter-individual difference across monkeys. Finally, as an extension of the early study of Cappe et al. (2010), the present study provides quantitative data regarding the occurrence of errors in the behavioral task, with clear demonstration of a reduction of errors in the VA conditions.

Briefly, a comparison with the study of Molholm et al. (2002), conducted in human beings could be made. Indeed, the protocol is also based on a detection sensory-motor task, and not discrimination. However, it is necessary to mention that the auditory and visual intensities were larger than the absolute sensory thresholds. The results in humans also showed RTs facilitation during multimodal stimulations. Nevertheless the differences between the unisensory RTs and the multisensory RTs (between 42 and 50 ms) were larger in humans than in monkeys in our study. In Molholm's study the violation of the race model demonstrated that the observed facilitatory effect did not result from the auditory and visual neural integrative processes alone.

In a general manner, we observed that RTs were facilitated (shortened) when the two modalities (A and V) were delivered simultaneously, as compared to RTs obtained after unimodal stimulation (A or V). The RSE is demonstrated by the cross-modal RTs exceeding the predictions established by the probability summation. To determine if our RSE are the resultant of the race model or the co-activation model we applied for every condition the Miller's inequality. Due to the results consistent with a model violation (as shown in **Figure 6**), we could conclude that, in case of multisensory stimulation, a neural response interaction occurred before the monkey's movement generation.

Several anatomical studies provide possible mechanisms and locations for early interactions between distinct sensory modalities, as representing putative convergence of information at the origin of these RSE. At cortical level, there are connections between different sensory areas, referred to as heteromodal connections. For example, the parietal VIP area in the monkey receives inputs from the auditory, visual, somatosensory, and vestibular systems, as well as from polysensory areas (Duhamel et al., 1998; Schroeder and Foxe, 2002). Consequently, the neurons of VIP express multimodal responses. Recently it was demonstrated that cortical areas considered as unisensory may have direct connections with other unisensory areas (Schroeder et al., 2001; Cappe et al., 2007; Kayser et al., 2007). In particular, it was demonstrated that visual cortical areas are reciprocally connected with auditory cortical areas (Falchier et al., 2002). These studies are consistent with multimodal interactions which can take place at relatively low levels of the chain of cortical information processing. However, in order to generate very fast motor response to multisensory stimuli (faster than unimodal stimulation), even earlier convergence of sensory information is likely, at subcortical level. For instance, there is evidence for rapid multisensory integration at the level of the superior colliculus (Meredith and Stein, 1986; Cuppini, 2010) a midbrain structure providing access to the motor system (Sparks, 1986; Rezvani and Corneil, 2008; Sommer and Wurtz, 2008). A recent anatomical study (Cappe et al., 2009) also provided indirect evidence in favor of low level, early multisensory integration in the thalamus. Besides its classical role of relaying sensory information to the cerebral cortex with reciprocal modulating feed-back corticothalamic projections, the thalamus is also playing a role in a driving, feed-forward projection system, representing an anatomical support for rapid and secure transthalamic transmission of information from a low level (unimodal) cortical area to another unimodal cortical area (see for review, Rouiller and Welker, 2000; Sherman and Guillery, 2002, 2006; Cappe et al., 2009). These feed-forward transthalamic loops involve corticothalamic projections terminating with giants endings, consistent with fast and secure synaptic transmission, which may favor faster and more reliable motor response to bimodal stimuli, as compared to unimodal ones. As hypothesized by Cappe et al. (2009), the pulvinar nucleus of the thalamus (mainly its medial nucleus PuM) receives projections from different sensory cortical areas, and then is in position to send rapidly the unified multisensory information to the motor system, via its thalamocortical projection to the PM. Furthermore, PuM receives projections from the superior colliculus. These anatomical data need to be confronted with neurophysiological investigations, by means of single neuronal recordings or EEG in animals (Meredith and Stein, 1986; Romanski, 2007; Bizley and King, 2008; Cohen et al., 2011; Perrault et al., 2011), and also by fMRI and EEG in human subjects (Foxe et al., 2000; Molholm et al., 2002; Doehrmann et al., 2010; Senkowski et al., 2011). Some of these functional data showed neuronal activity in cortical areas (auditory, somatosensory, visual area, etc.) in connection with multisensory integration. The present behavioral data and their electrophysiological counterparts in PM represent an attempt along this line to elucidate the pathways involved in early multisensory and sensorimotor integration. Along this line, the same two monkeys (Mk-JZ and Mk-LI), after completion of the neuronal recordings in PM, will be implanted with another chronic recording chamber to permit access to the pulvinar nucleus of the thalamus, in order to test its possible contribution to such a multisensory detection task.

The electrophysiological data in PM (**Figures 8** and **9**) are in line with previous studies reporting single neurons' responses to auditory or visual stimuli (Graziano, 1999, 2001; Graziano et al., 1999) in this motor cortical area, in the context of the control of a motor act triggered by a sensory stimulus. However, in previous studies in PM, the sensory stimulus was generally used so that it represents a cue-signal from which the subject has to select an appropriate motor response in a conditional behavioral task with delay, the latter being followed by a go-signal prompting the motor response itself. In particular, variations of the cue-signal within a given modality (different colors, different positions, etc.) instruct distinct motor acts (e.g., movements in different directions). With that respect, the present study is clearly different as two distinct unimodal stimuli triggered the same motor response, without any delay up to a go-signal before the subject has to respond behaviorally to the stimulus. The present paradigm is a simple detection task with fast stereotyped motor response irrespective of the stimulus property, whereas conditional tasks with delay involve a discrimination of different stimuli and their interpretation for conversion into the appropriate motor act among a palette of possible motor responses. To our knowledge, the present study is the first investigation in PM based on such detection behavioral paradigm, comparing unimodal versus bimodal stimulations. Not surprisingly then, the present electrophysiological data derived from PM (**Figures 8** and **9**) in the context of the detection task appear different as compared to PM data derived from a conditional task with delay in which various visual cue signals (left and/or right positioned LEDs) instructed distinct motor responses in the form of unimanual or bimanual reach and grasp movements (Kermadi et al., 2000). In the latter study, the responses to the cue signals were relatively frequent in PMd (in about 40% of the neurons) and they were strong (see e.g., **Figure 5A** of Kermadi et al., 2000). In the present study (detection task), the responses to the sensory stimuli in PM are clearly less frequent and not as prominent as those observed by Kermadi et al. (2000). This difference may be interpreted in the sense that in the conditional task with delay, the monkey had to pay more attention to the cue-signal, as it was crucial to select a specific motor response among three possibilities. In the present detection task, the sensory stimulus does not represent a basis to select a motor response, as only one motor act is requested and therefore the pertinence of the sensory stimulus is limited to the time at which the motor act has to take place. For this reason, less prominent and less frequent "sensory" responses in PMd can be expected in the present detection task, as compared to conditional tasks with delay comprising behaviorally more meaningful sensory instructions. Actually, the sensory stimuli delivered in the present detection task are more comparable to the go-signal of the conditional tasks with delay. In the studies of Kermadi et al. (1998, 2000), "sensory" responses to the go-signal were fairly rare (not only in PMd, but also in M1 and SMA), thus in line with the low occurrence of responses to the visual and/or acoustic stimuli in the present detection paradigm.

To some extent, the response types in PM illustrated in **Figure 8** in the present detection task of visual and/or auditory stimuli is reminiscent of the responses observed in the ventrolateral prefrontal cortex, when faces and/or vocalization stimuli were presented to awake behaving monkeys (Romanski, 2007; see her **Figure 4**). However, again, neurons exhibiting multisensory integration were clearly more frequent in the ventrolateral prefrontal cortex (50% of bimodal neurons; Romanski, 2007) than found in PM for the present detection task (34.1%). The difference between prefrontal cortex and PM may be explained by the well-known and prominent role of the former in associating distinct sensory modalities, especially vision and hearing, as well as by substantial differences related to the stimuli properties. In the study of Romanski (2007), the stimuli were vocalizations and faces, clearly more meaningful on the cognitive point of view than the simple noise bursts and LED stimuli delivered in the present behavioral paradigm.

In conclusion, our quantitative behavioral data, based on well controlled stimulation conditions, demonstrated in adult macaque monkeys that the detection of acoustic and visual stimuli presented simultaneously is faster and more reliable than when either stimulus is presented alone, in line with previously reported human data. In the PM, neuronal activity recorded during this detection task exhibited in parallel a statistically significant difference in the distribution of response patterns to the stimuli across the three modalities (visual alone, acoustic alone, and bimodal), in the sense of a decrease of inhibitory responses in the bimodal condition, as compared to unimodal condition. However, at that step, there is not yet demonstration of a causality relationship between the change of response patterns' distribution and the behavioral effect observed in the bimodal condition, as compared to unimodal stimulation. The present study confirmed that non-human primates are high standard model in multisensory research, especially because of the possibilities of direct transfer of knowledge to humans. This was supported at different levels by similar results (behavioral, for example) compared to those obtained in humans or in other primate species. The present preliminary steps toward the ambitious goal of gaining access to the knowledge of multisensory integration should pave the way to revisit some neurological diseases [Alzheimer disease, specific language impairment (SLI) or attention syndrome deficit (ASD)] which have been shown to interfere with usual illusions (e.g., McGurk effect) when mismatching auditory and visual cues are presented. The outcome of such studies might elucidate the underlying mechanisms of unified percepts and therefore opening up new paths in clinical research regarding some still pending medical challenges.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fnhum.2013.00799/abstract

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## Conclusion

The present study, reported in the article Lanz et al., 2013b, yielded from a behavioural point of view in non-human primates results consistent with the literature (Rowland et al., 2007; Wallace et al., 2007, Cappe et al., 2010). The common conclusions are that the bimodal stimulation improved the detection speed (decrease of RTs) and the performance (decrease of errors). The second part of this work relates to the single unit recordings derived from the premotor cortex (PM). From these electrophysiological data, we were able to create a classification of PM neurons according to the response patterns and the modulation patterns when the monkeys performed the multisensory behavioural task. These data are consistent with a convergence of auditory and visual information in PM at neuronal level, in the context of the present multisensory paradigm. However, the precise temporal issue, namely the decrease of RT in the bimodal condition, remains to be investigated in more detail at neuronal level, in combination with recordings and interventions in other brain regions (also sub-cortically), believed to be part with PM to the neural network involved in such multisensory integration process.

## III.2 Human results

### III.2.1 Introduction

As reminder (see Audiovisual integration in the Introduction), multisensory studies are not restricted to the animal models. Indeed, very numerous studies on the broadcasting (audiovisual) interactions (e.g. Giard and Peronnet, 1999; Molholm et al., 2002, Cappe et al., 2010a, Senkoski et al., 2011; Gori et al., 2012), visuo-tactile (Gori et al., 2011) and auditory-somatosensory (Murray et al., 2005; Sperdin et al., 2009) interactions were conducted on human subjects. In this Ph.D thesis, we aim at extending knowledge on audiovisual integration, derived from the pioneer studies of Giard and Peronnet, (1999) and Molholm et al. (2002) which brought important behavioural and electrophysiological result. Briefly, these studies demonstrated that, during a multi-modal stimulation (auditory and visual), reaction times (RTs) were significantly shorter than unisensory RTs. These results are in line with data derived from animals (Stein and Meredith, 1993; Cappe et al., 2010b). This behavioural facilitatory effect during a multi-modal stimulation is commonly namely redundant signal effect (RSE) (Raab, 1962, Miller 1982). The redundant signal effect, when proven by an evaluation of the summation of probability, is indicative of a neuronal interaction occurring early in the information processing. The behavioural analyses do not allow demonstrating mechanisms as well as identifying the brain regions where these neuronal interactions take place, electrophysiological studies are necessary. These electrophysiological studies (Giard and Peronnet, 1999; Molholm et al., 2002, Talsma et al. 2007, Sun et al., 2009; Senkowski et al., 2011) have demonstrated that this neuronal interaction occurred early after the stimulus and that various brain regions were involved. First neuronal interaction occurred after 45 - 50 msec (Giard and Peronnet, 1999; Cappe et al., 2010a; Senkowski et al., 2011) in the posterior scalp.

The present human data represent a counterpart of the study conducted on non-human primates and reported in the article corresponding to the previous chapter of results. As mentioned above in the chapter "Material and Methods", these human data were derived from a feasibility study aiming at establishing in the future a link between the non-human primates and human subjects based on the same experimental protocol of multisensory integration (Chapter “Perspectives”).

As in the above article reporting on non-human primate data, this chapter will be divided in two parts:

1. Behavioural assessment of multisensory integration: The reaction times (in the § III.2.3.1), the analysis of the stimuli intensity effects (in the § III.2.3.2), as well as the bimodal gain (in the § III.2.3.3) are the behavioural parameters presented in these paragraphs and derived from a study conducted under my supervision with two students in biomedical sciences in the course of their bachelor work. The behavioural results were obtained from a population of 10 subjects.
2. Electrophysiological assessment of multisensory integration (in the § III.2.3.4): The EEG investigation was conducted in 4 human subjects, all members of the laboratory, who performed the same multisensory task as that considered for the behavioural data. The present electrophysiological part represents a pilot study at the level of the present Ph.D. thesis, which may be extended to a larger population of human subjects (n=20) in a future step.

### III.2.2 Material and Methods:

The methodological description for this experimental part was provided in the chapter II (General Materials and Methods). Consequently, we here only remind essential essential aspects.

The study was conducted on a total population of 14 human subjects (aged 20- to 30-years old), distributed according to two different assessments of multisensory integration (in the § II.1.2). Ten subjects were enrolled in the behavioural assessment and 4 other subjects in the electrophysiological assessment. The distribution between genders was equal: 7 females and 7 males. The multisensory integration evaluations were based on a positive reinforcement protocol (Durif et al., 2003; Lanz et al., 2013b), requiring a sensory-motor task (in the § II.2.2 and II.2.4). For four subjects enrolled in the electrophysiological recordings, an EEG cap containing 65 active electrodes regularly distributed over the scalp (in the § II.5.2) was used.

To be able to quantify the multisensory effect, two parameters were evaluated according to the protocol in which the human subjects were enrolled: 1) Reaction times (RTs), as well as the stimuli intensity effects on RTs during the behavioural assessment obtained in three conditions: at 10, 20 and 30 dB above the sensory thresholds (obtained for each individual modality); 2) The global field power (GFP) and the event-related potentials

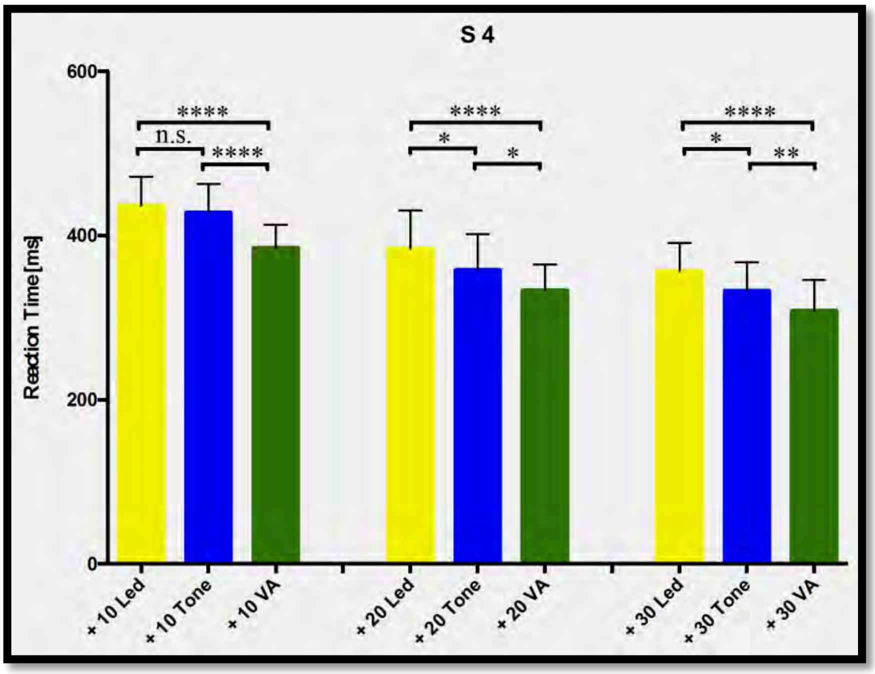
(ERPs) topography during the electrophysiological assessment at 30 dB above the sensory thresholds.

## III.2.3 Results

### III.2.3.1 Reaction Times (RTs): multisensory facilitation

For all human subjects (n=10), in which the sensory (auditory and visual) thresholds were determined individually, the data were obtained from behavioural tests performed in three distinct conditions: at 10, 20 and 30 dB above the threshold obtained for each individual modality. For each intensity condition, the block of data consisted of 90 random trials. Data were collected for each subject during a single test session. The distribution of RTs in response to visual, auditory or visuo-acoustic stimulation, and for each condition, is displayed in Figure 33, as obtained from 3 representative individual human subjects (S4, S6 and S8). The seven other subjects are displayed in the part Appendix (Appendix 1).

**A**



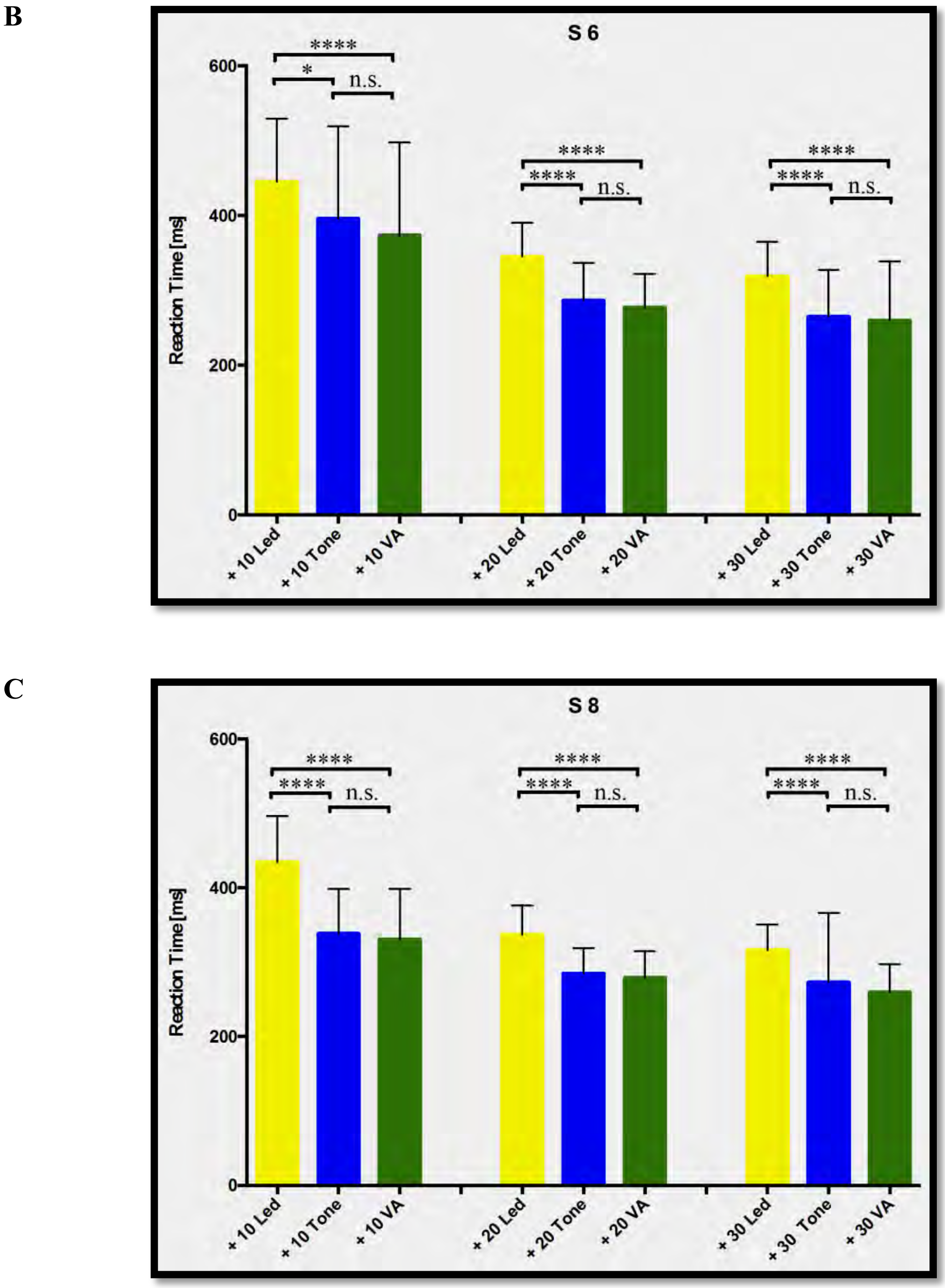


**Figure 33: Mean reaction times and their SDs obtained at 10, 20 and 30 dB above the unimodal thresholds. Data from 3 individual human subjects (S4, S6 and S8). n.s.: p≥0.05 ; *:p<0.05 ; **: p<0.01 ; ****: p<0.0001 (Mann-Whitney Test).**

Generally, the graphs of Figure 33 show that, as expected, there is a decrease in RT when the intensity of stimulation increased (10, 20 and 30 dB above the thresholds) for the 3 conditions (V, A and AV). We also noticed that the mean visual RTs (yellow bars) were longer than mean auditory RTs (blue bars), themselves longer than the mean visuo-acoustic

RTs (green bars). The mean visuo-acoustic RTs were shorter than the fastest mean RTs obtained in response to unimodal stimulation (in this case the auditory stimulus), although this difference was statistically significant for the subject S4 only.

Below, the description of the data obtained for the three individual human subjects illustrated in Figure is presented.

**First individual subject (Figure 33 A): S4**

1° **At 10 dB** above the unimodal thresholds: the mean visual RT (437 msec) was not significantly different (Mann-Whitney Test; $p \geq 0.05$) from the mean auditory RT (428 msec). The mean visuo-acoustic RT (385 msec) was significantly shorter ($p < 0.05$) than the RTs obtained for each of the two unisensory stimulations. The mean RT in response to the visuo-acoustic stimulation was significantly shorter (43 msec) than the fastest mean RT obtained in response to unimodal stimulation (auditory in the present case).

2° **At 20 dB** above the unimodal thresholds: the mean auditory RT (358 msec) was significantly shorter ($p < 0.05$) than the mean visual RT (384 msec). The mean visuo-acoustic RT (333 msec) was significantly shorter ($p < 0.05$) than the mean RTs in response to each of the two unisensory stimulations. The difference between the auditory and the visual mean RTs was about 26 msec and the mean RT in response to the visuo-acoustic stimulation was shorter (25 msec) than the fastest mean RT obtained in response to unimodal stimulation (auditory in the present case).

3° **At 30 dB** above the unimodal thresholds: the mean visual RT (357 msec) was statistically significantly shorter ($p < 0.05$) than the mean auditory RT (333 msec). The mean visuo-acoustic RT (309 msec) was significantly shorter than the unimodal mean auditory ($p < 0.0001$) and visual ($p < 0.01$) RTs, with a decrease of 24 msec as compared to the fastest unisensory stimulation (auditory).

**Second individual subject (Figure 33 B): S6**

1° **At 10 dB** above the unimodal thresholds: the results show that the mean visual RT (445 msec) was significantly longer (Mann-Whitney Test, $p < 0.05$) than the mean auditory RT (396 msec). The mean visuo-acoustic RT (374 msec) was significantly shorter ($p < 0.0001$) than the mean visual RT, and also shorter (by 22 msec) than the mean auditory RT, although this difference was not statistically significant ($p \geq 0.05$).

2° **At 20 dB** above the unimodal thresholds: the mean visual RT (345 msec) was significantly longer ($p < 0.0001$) than the mean auditory RT (287 msec). The mean visuo-acoustic RT (278 msec) was significantly shorter ($p < 0.0001$) than the mean visual RT, but no statistically significant difference ($p \geq 0.05$) was observed between the auditory and the visuo-acoustic mean RTs, the latter being 9 msec shorter than the mean auditory RT.

3° **At 30 dB** above the unimodal thresholds: the mean auditory RT (265 msec) was significantly shorter ($p < 0.0001$) than the mean visual RT (319 msec). The mean visuo-acoustic RT (260 msec) was significantly shorter ($p < 0.0001$) than the mean visual RT, and also shorter than the mean auditory RT (by 5 msec), but this last difference was not statistically significant.

**Third individual subject (Figure 33 C): S8**

From a statistical point of view, this individual human subject (S8) is very comparable to the second one (S6), in other words exhibiting statistically significant shorter mean auditory RTs than mean visual RTs, at all 3 intensities tested. Furthermore, the mean visuo-acoustic RTs were shorter than the mean visual RTs (statistically significant differences) and also shorter than the mean auditory RTs, but the latter difference was not statistically significant, at all 3 intensities tested. The detailed RTs can be seen in Figure 33.

For the purpose of yielding a more global view of the behavioural results in the whole group of human subjects, Figure 34 shows the distribution of the mean RTs in response to visual, auditory or visuo-acoustic stimulations, and for each condition, cumulating the 10 human subjects enrolled in this part of the study. For the analysis of the cumulated data, we have split the results according to the three intensity conditions.

1. **At 10 dB** above the unimodal thresholds: The results showed that mean visual RT (458 msec) was significantly longer (Mann-Whitney Test, $p < 0.0001$) than the mean auditory RT (422 msec). The mean visuo-acoustic RT (385 msec) was significantly shorter ($p < 0.0001$) than RTs for both unisensory stimulation. The difference between the auditory and the visual mean RTs was about 36 msec and the mean RT in response to the visuo-acoustic stimulation was shorter (37 msec) than the fastest mean RT obtained in response to unimodal stimulation (Auditory in the present case).
2. **At 20 dB** above the unimodal thresholds: The mean visual RT (422 msec) was significantly longer ($p < 0.001$) than the mean auditory RT (384 msec), representing a difference of ~38 msec. The mean visuo-acoustic RT (353 msec) was significantly shorter than the mean unimodal auditory ($p = 0.0002$) and visual RTs ($p < 0.0001$), representing a reduction of 31 msec with respect to the fastest unimodal stimulation (Auditory).

3. **At 30 dB** above the unimodal thresholds: The mean visual RT (392 msec) was not statistically different ($p \geq 0.05$) from the mean auditory RT (385 msec). However, interestingly the mean visuo-acoustic RT (341 msec) was significantly shorter ($p < 0.0001$) than the unimodal mean auditory and visual RTs, with a decrease of 44 msec as compared to the fastest unisensory stimulation (again Auditory in the present case).

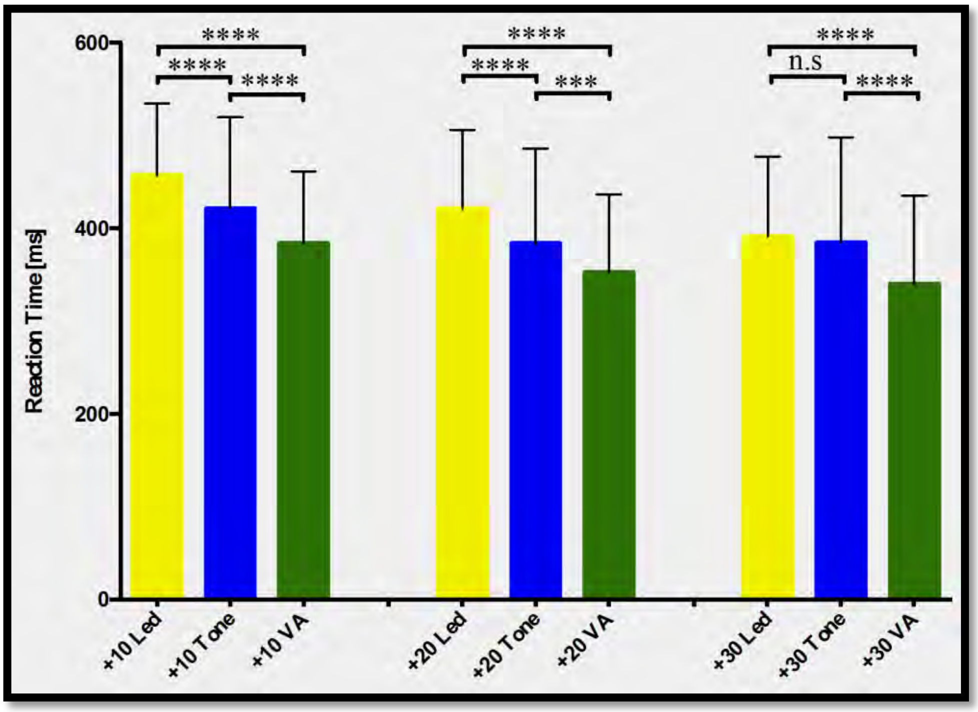


**Figure 34: Mean reaction times and their SDs obtained at 10, 20 and 30 dB above the unimodal thresholds. Data cumulated from 10 human subjects. n.s.: $p \geq 0.05$ ; ***: $p < 0.001$ ; ****: $p < 0.0001$ (Mann-Whitney Test).**

### III.2.3.2 Stimuli intensity effects on RTs

In Figure 35, for the same data pool, we have reported the variation of mean RTs as a function of stimuli intensities. The mean RT differences between all stimulation conditions have been tested statistically using a Mann-Whitney Test. The results showed that, as expected, an increase of the visual intensities led to a statistically significant decrease ($p < 0.0001$) of the RTs (yellow bars in Figure 35). The decrease in mean visual RTs was 36 msec between +10 and +20 dB above threshold, whereas it was 30 msec between +20 and +30 dB above threshold. For the auditory stimulation alone (blue bars in Figure 35), the effects of intensity were less systematic. Between +10 and +20 dB above threshold, the decrease of the mean RT (38 msec) was statistically significant ($p < 0.0001$). In contrast, the mean RT difference between +20 and +30 dB above threshold was not statistically significant (p≥0.05), limited to an increase of 1 msec. Concerning the visuo-acoustic stimulation condition (green bars in Figure 35), the effects of the intensity were comparable to those observed for the auditory stimulation alone. The results showed that increase of intensity from +10 to +20 dB above threshold led to a decrease of mean RT (32 msec), which is statistically significant (p<0.0001). Increasing the visuo-acoustic stimulation from +20 to +30 dB above threshold provoked a limited decrease of mean RT (12 msec), statistically significant (p<0.05). In all three modalities, the differences in mean RTs between stimulations at +10 dB versus +30 dB above threshold were statistically significant (Figure 35).

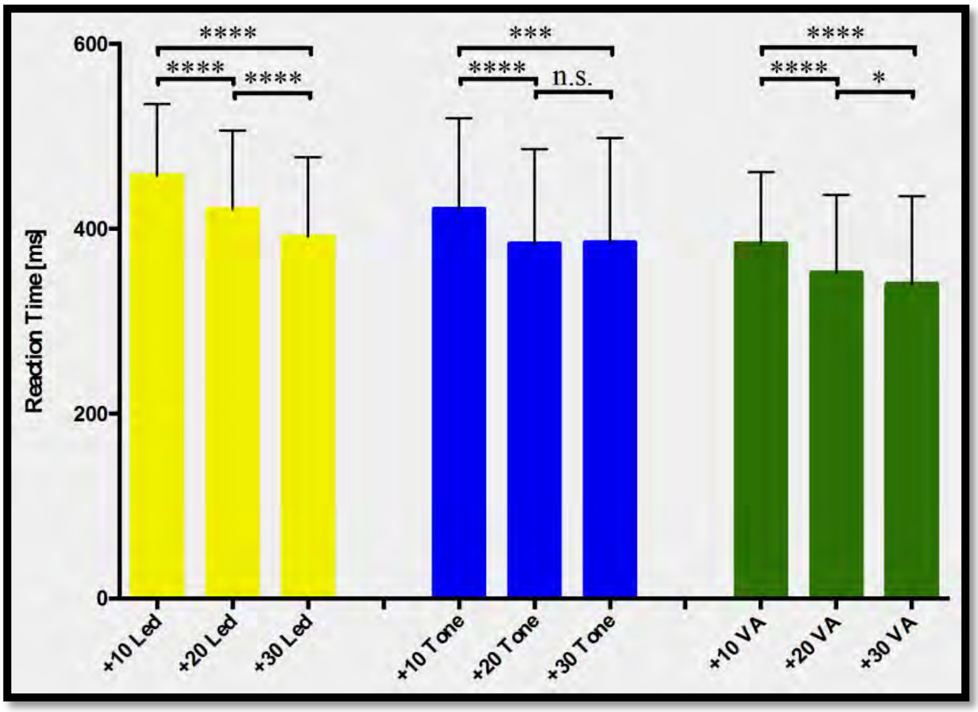


**Figure 35: Mean Reaction times and their SDs obtained for the three conditions of intensities within each modality (visual in yellow, auditory in blue and visuo-acoustic in green). Cumulated data obtained from 10 human subjects. n.s.: p≥0.05 ; *: p<0.05 ; ***: p<0.001 ; ****: p<0.0001 (Mann-Whitney Test).**

To follow the same logic as the analysis of data presented in the non-human primate article (Lanz et al., 2013b), we analyzed the cumulative distribution function and the Miller race model inequality (Miller, 1982) for the present human behavioural data. This analysis was conducted with the aim of determining if a redundant signal effect (RSE) is present or not. Consequently, we calculated for every condition the cumulative distribution functions, displayed in the left panels of Figure 36. The more the curve is shifted to the left, the shorter is the RT, meaning a higher probability of occurrence at a given RT value. For every intensity condition, if compared to the auditory and visual unimodal curves, one can observe a leftward shift for cross-modal stimulation (AV) (corresponding to green curves in Figure 36).

In parallel and in addition, an analysis of Miller's Inequality was tested for each intensity condition. As a reminder, this equation defines itself as follows: $P(\mathrm{RT}(\mathrm{VA})) < (P(\mathrm{RT}(\mathrm{A})) + 8\ P(\mathrm{RT}(\mathrm{V}))) - (P(\mathrm{RT}(\mathrm{A}))\ \mathrm{x}\ P(\mathrm{RT}(\mathrm{V})))$. When the real value is bigger than the value attended by the model, the model is violated (i.e. positive values for the probability difference). In case of positive value, it means that a neural response interaction is involved, which was the case in the 3 intensity conditions tested (10, 20 and 30 dB above threshold; right panels in Figure 36). However, in contrast to the results obtained with the non-human primates (in the § III.1), we did not obtain a decrease of the probability differences' values when the intensities increased (Figure 36, right panels).

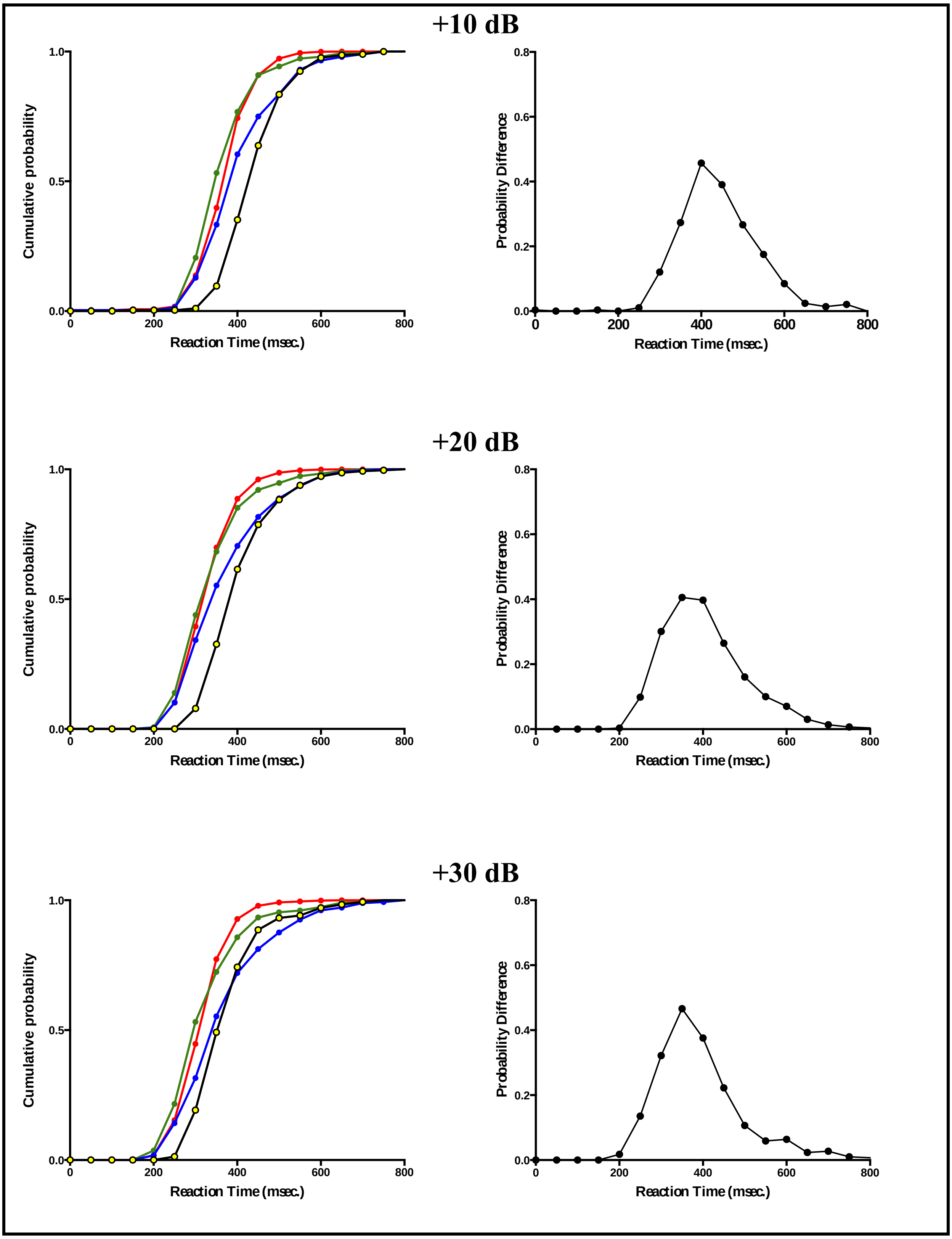


**Figure 36: Cumulative distribution function for three intensity conditions and cumulated for the 10 human subjects. Left panels are for the cumulative distribution function and the right panels for the Miller's Inequality. In the cumulative distribution functions, the yellow/black curves represent the visual condition, the blue curve the auditory condition and the green curve the visuo-acoustic condition. The red curve is the Miller's race model inequality prediction (Miller, 1982).**

### III.2.3.3 Bimodal gain

As reported in the article on the non-human primates (Lanz et al., 2013b), as well as in the literature (Stein and Meredith, 1993; Cappe et al., 2010a), the bimodal or multisensory gains were plotted in Figure 37, representing the decrease in percent of the mean RTs in response to the visuo-acoustic stimulation as compared to the fastest unimodal stimulation. We can observe that in the intensity conditions 1 and 2, which are +10 and +20 dB above threshold respectively, the bimodal gains were similar (between 8.9 and 8.1 %). However in the intensity condition 3 (+30 dB above threshold), surprisingly the bimodal gain even increased up to 11.6 %, thus representing an unexpected increase of bimodal gain with intensity. In other words, this observation does not coincide with the principle of inverse effectiveness, at least in the range of intensities tested here in the present sample of 10 subjects.

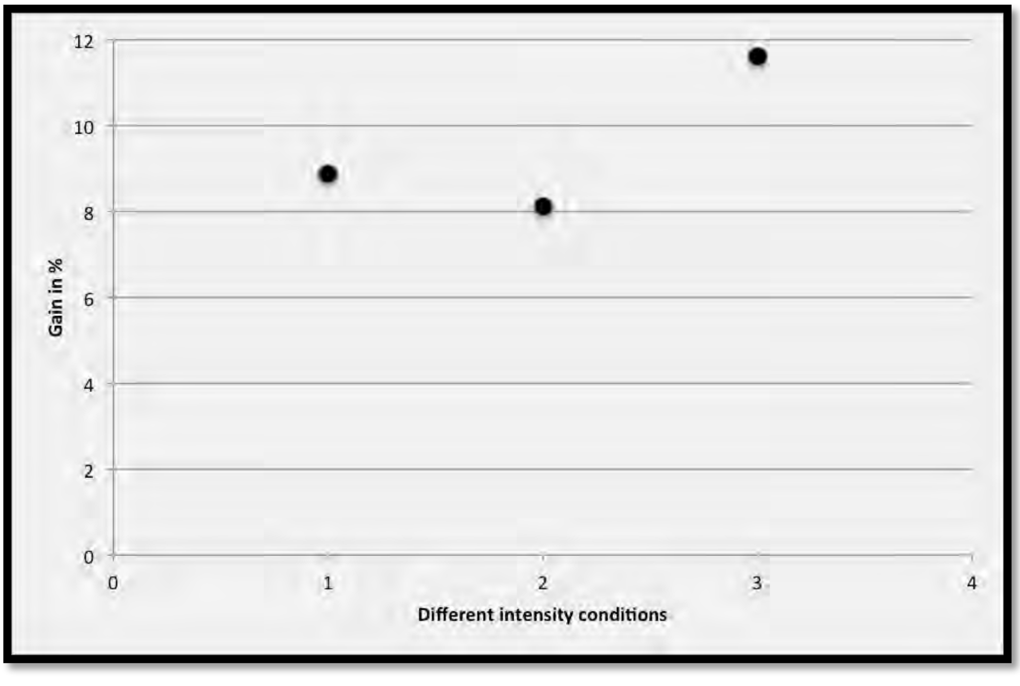


**Figure 37: Representation of the bimodal gain. These bimodal gains are expressed in percent, corresponding to the decrease of mean visuo-acoustic RT in % with respect to the mean RT of the fastest unisensory modality. Intensity condition 1 = 10 dB above the sensory thresholds. Intensity condition 2 = 20 dB above the thresholds and Intensity condition 3 = 30 dB above the thresholds.**

### III.2.3.4 Electrophysiological results

Before the description of our pilot (feasibility) study per se, we start first with preliminary results derived from the very first subject enrolled in this detection task associated with EEGs recordings. This initial subject did not appear in the feasibility study, because this session was used only to test the equipment and the protocol. However, and in spite of higher intensities used than the sensory thresholds (+ 30 dB), the results are interesting and worth to be to introduce into this thesis.

The first results obtained were the analysis of global field power (GFP). To do it, we had analyzed the GFP for every modality (Figure 38). The GFP was obtained by averaging 400 trials during a single session. The visual examination of the three GFPs revealed differences between the latency and intensity of the evoked potentials (EPs), for each modality. The EEG follow the trend observed for the behavioural results. The EPs latency in response to the visuo-acoustic stimulation was shorter than the EP latencies in response to unimodal stimulations (between 15 and 30 msec). Furthermore, the auditory EP latency was shorter than the visual EP latency. This difference was only a few milliseconds (around 15 msec). The amplitude of the EPs also varied according to the modalities. The amplitude of EPs in response to bimodal stimulation was larger than the unimodal EPs amplitude. The amplitude of the auditory EP was smaller than that of the visual EP.

In addition, we performed an analysis of the overlapped waveforms and the GFPs between the three modalities and the sum of the individual visual GFP and auditory GFP (Figure 39). The latency observed for the multisensory stimulation was shorter than the latency for the sum GFP (around 30 msec).

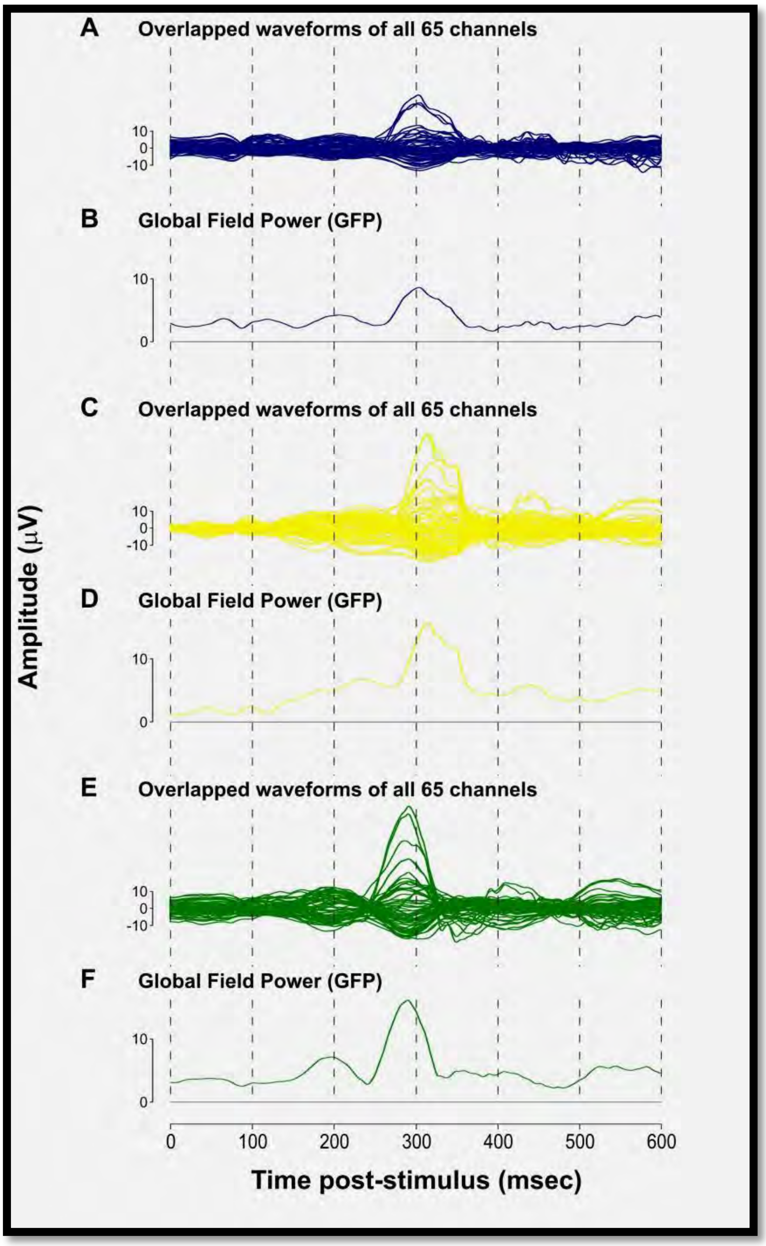


**Figure 38: Representation of the overlapped waveforms of all electrodes (A,C,E) and the Global Field Power (B,D,F) for each modality (Auditory in blue; Visual in yellow; Audio-visual in green).**

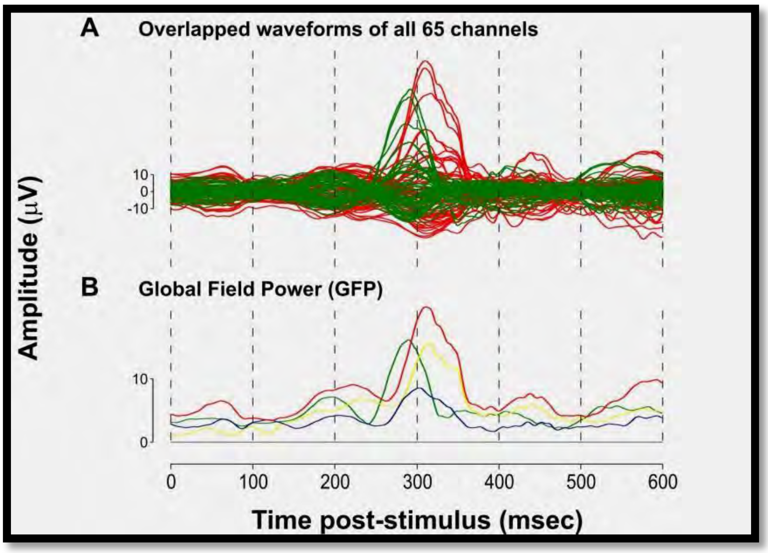


**Figure 39: Representation of the overlapped waveforms of all electrode (A) and the Global Field Power (B) for each modality (Auditory in blue; Visual in yellow; Audio-visual in green) and for the sum of unisensory evoked potentials (Sum in red).**

Following the report of this initial, preliminary subject, we introduce below the results relative to the "final" protocol, in which 4 subjects were recorded in the context of the feasibility study. For reminder (Chapter "Material and Methods), the protocol has been slightly changed compared with the results obtained for the behavior. Indeed, the auditory stimulus was not any more a tone burst, but it was a white noise. This modification was introduced in order to match more closely the detection task used in the non-human primates. The analysis was focused on the difference between the summation (auditory plus visual) of unimodal ERPs and the ERPs obtained in combined stimulation (visuo-acoustic). These differences were observed for all recorded electrodes (n=64) and are represented, according to the used helmet, in Figure 40 (top panel). This representation is not easy to read and to interpret and therefore an additional representation was generated in the form of a global activity GFP) of the brain (Figure 40 bottom panel).

### III.2.3.4.1 Global Field Potential (GFP)

A visual analysis of the GFPs (Figure 40 bottom panel) shows a waveform difference between the pair (bimodal) ERP and the sum of the individual ERPs post-stimulation (0 to

450 msec post-stimulus). This difference began at ~60 msec after the onset of the stimulation. This latency is coherent with previously reported data (Giard and Peronnet 1999; Foxe et al., 2008; Cappe et al., 2010b). For the continuation of our study, we focused on a period of interest (POI) ranging from 60 to 90 msec.

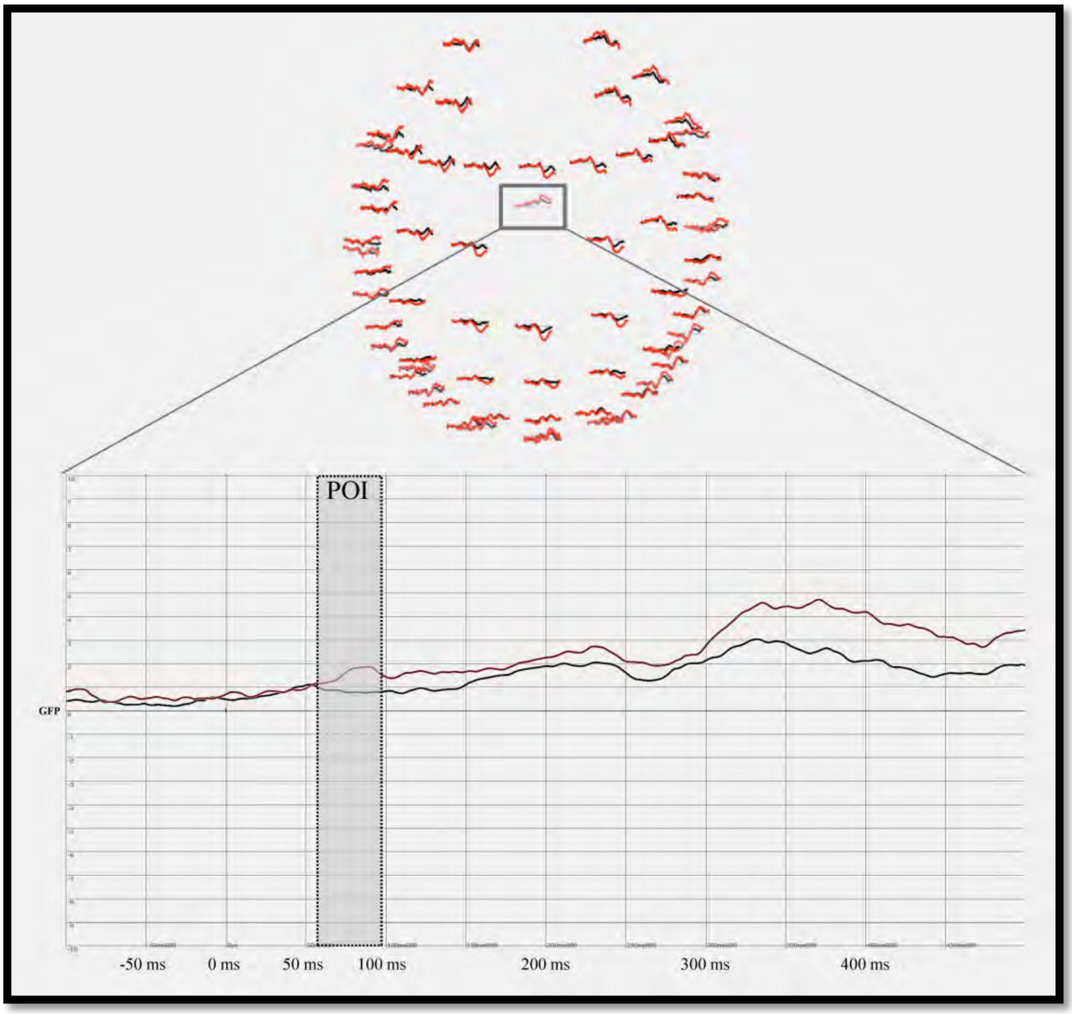


**Figure 40: Representation of the waveforms differences between the pair and sum ERPs. Top panel: Representation of ERPs for all electrodes (n=64), displayed for the "pair" (bimodal) in black and the "sum" in red. Bottom panel: Waveform from GFP (global field potential) for the pair in black and the sum in red. A difference between these curves is observed during a period of interest (POI); between 60 and 90 msec post-stimulation.**

### III.2.3.4.2 ERP topography

For this analysis we estimated the topographic differences between the pair ERPs and the sum ERPs. The period of analysis corresponded to the previously quoted period that is between 60 and 90 msec. The topography resulting from this analysis showed a maximal difference over the right temporal scalp (Figure 41). This maximal difference, pointed in the Figure by a cross, as the results obtained by analysis of the Miller's race model Inequality, indicates that a neuronal interaction took place during the combined (AV) stimulation.

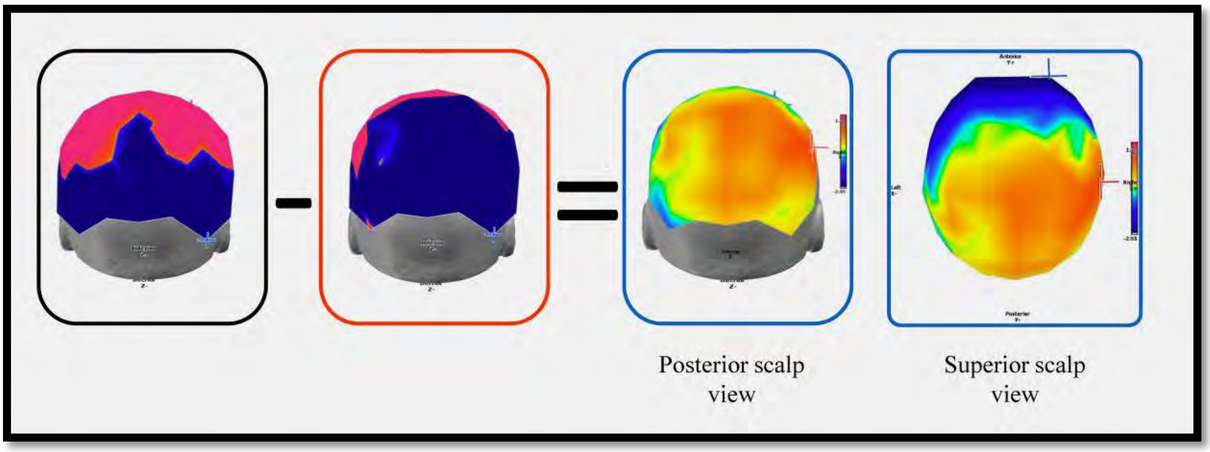


**Figure 41: Representations of the ERPs topography between 60 and 90 msec after stimulus onset. The two voltage maps on the right in blue represent the difference between the AV condition minus the sum of the unimodal conditions (A plus V stimuli). The positivity obtained on the posterior and temporal regions in both views indicates a subbaditive interaction.**

### III.2.3.4.3 Source estimation

With this analysis and during our POI, we wanted to localize electric sources in the brain. For this we have used a distributed source estimation procedure; the "LAURA" based on the MNI template with 5007 points of inverse solution. Recent studies on auditory and visual integration support the application of the LAURA approach (Murray et al., 2004; Murray et al., 2006).

According to the obtained results (Figure 42), we could notice that neuronal interactions during the period going from 60 to 90 msec post-stimulation took place to the right at the level of the visual cortex, but also at the level of the auditory cortex. Similar results were obtained in a previous study by Cappe and collaborators (Cappe et al., 2010b). However it seems that the part of the visual cortex activated in our case is more caudal, that is in the posterior parietal cortex. This would correspond to the activation of areas 18/19, which are secondary and tertiary visual areas respectively, but also the area 7 (associative parietal cortex).

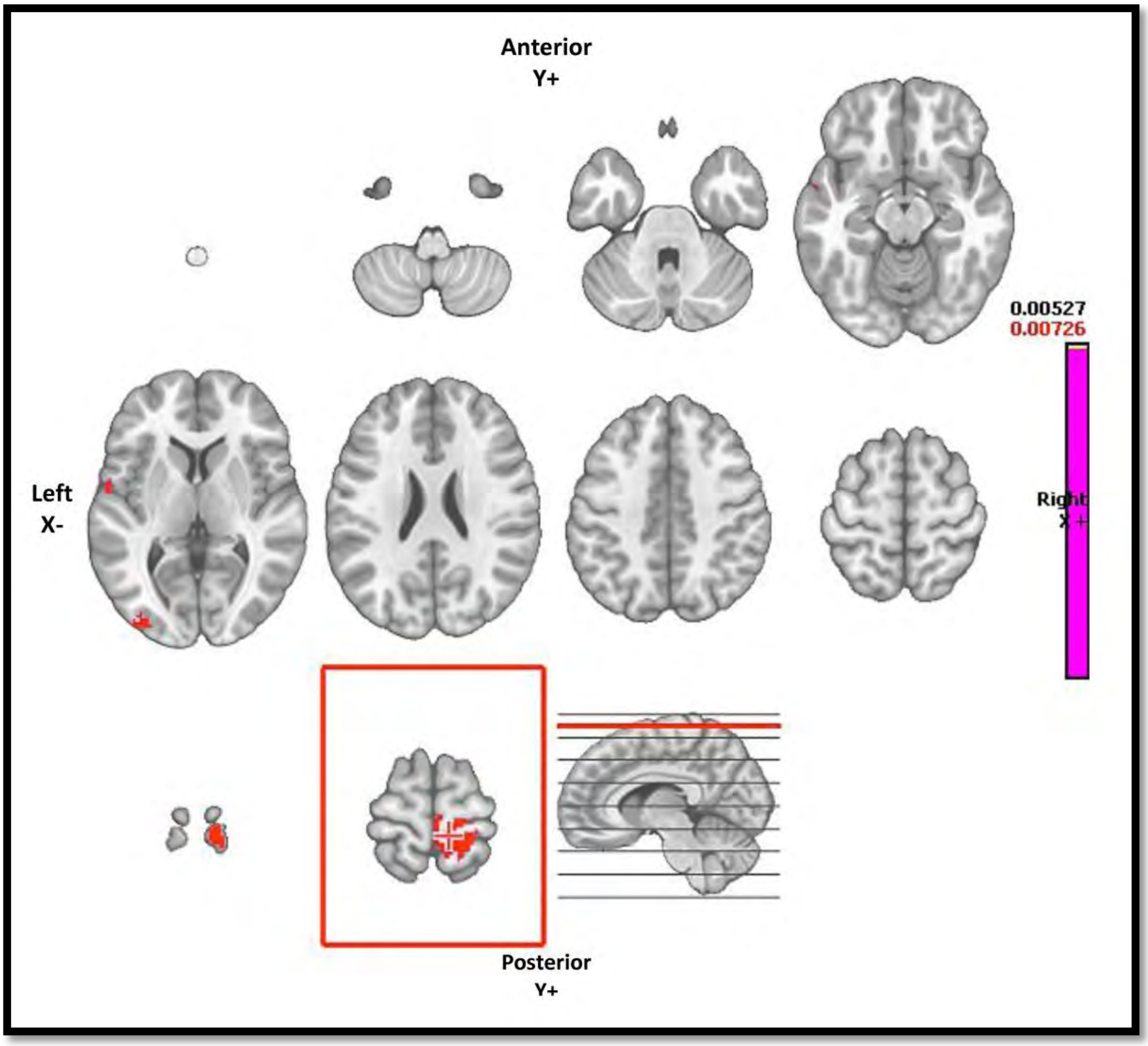


**Figure 42: Representation of the inverse solution (POI: 60 – 90 msec).**

### III.2.4 Discussion

Our present data collected on human subjects (feasibility study) showed a facilitatory effect when the unimodal stimuli were combined, as previously reported (Giard and Peronnet, 1999; Forster et al., 2002; Molholm et al., 2002; Lovelace et al., 2003; Martuzzi et al., 2007; Senkowski et al., 2011). This effect was reflected by reaction times shorter during the visuo-acoustic stimuli presentation than during unimodal stimuli. This effect occurred for all tested intensities conditions (10, 20 and 30 dB above the thresholds). We also notice that the mean auditory reaction times (RTs) (in unimodal condition) were shorter than the mean visual reaction times. This difference was also observed by Giard and Peronnet (1999), reported as auditory dominance. It means that an auditory dominant subject will have shorter reaction times for the auditory stimuli and conversely in case of visual dominance. This notion of sensory dominance was important during the reading of the event-related potentials (ERPs) topography. Indeed, differences at the level of the activated areas during the early neuronal interactions occur according to the sensory dominance of the subject. This notion is detailed in the general discussion (in the § IV.2.2). Like Senkowski et al. (2011), we performed an assessment of the stimuli intensity effects on RTs, this with the aim of confirming or contradicting the inverse effectiveness (IE) principle, principle according to which the gain during a bimodal stimulation is more important when the intensities of stimulation are close to absolute sensory thresholds. In contrast to what we obtained in the non-human primates (Lanz et al., 2013b) and Senkowski and al. (2011) obtained in humans, we were not able to demonstrate an IE here on our human subjects. Our result demonstrated even a more important gain when we increased the intensities of stimulations, though in the 10 to 30 dB range tested.

The Miller's Inequality analyses demonstrated us that this facilitatory effect, commonly named redundant effect signal (RSE) was greater than the prediction by the Race model (Raab, 1962). Consequently, the audiovisual integration was not due to an independent processing from sensory inputs (=Race model), but these interactions were due to early neuronal processing. Several candidates, such as subcortical regions could influence this facilitatory effect. As such, we could cite the thalamus. Indeed, anatomical studies (Morel et al., 2005; Cappe et al., 2009b) highlighted the thalamic projections to the premotor cortex, originating from multisensory thalamic nuclei. Consequently, the interactions in the thalamus may contribute to faster motor responses triggered by the visuo-acoustic stimulation.

In the second part of this study, we recorded the global field power (GFP) during the same multisensory-motor task. As compared with the literature (Giard and Peronnet, 1999; Molholm et al., 2002; Cappe et al., 2010; Senkowski et al., 2011), the ERPs topography revealed an early interaction during the bimodal stimulation. The latency observed in the literature demonstrated early interaction between 45 – 50 msec post-stimulation; in our case the interaction occurred after 60 msec. With regard to the literature we can mention that our intensities were weaker, what could explain this difference of latency. Indeed, as proven in the results on behavior, a stimuli intensity increase caused a decrease of reaction times. This notion is not to be confused with the multimodal gain decrease when we increase the intensities (IE principle). From a topographical point of view we observed a maximal difference over the right temporal scalp. That is in line with the literature.

In conclusion, we saw that the results obtained from a behavioural and electrophysiological point of view are generally consistent with the results of various studies available in the literature. The low number of human subjects (n=4) does not allow us to apply relevant statistics. However, we can say that this protocol, used previously in the non-human primates, is stable and repeatable. Thereby we can satisfy our second hypothesis according to which the multisensory properties observed in the monkeys can be reproduced in the human subjects. This conclusion supports the aim of transferring in the future the knowledge acquired in the non-human primates towards the humans (translational research) (in the § IV and V).

# IV. General Discussion

This general discussion will articulate around the three axes delineated by three main lines of research conducted in the course of the present Ph.D. thesis: (i) the initial methodological developments; (ii) the behavioural investigations; (iii) the electrophysiological studies. It is proposed at that step to treat altogether the behavioural and electrophysiological data obtained in both the non-human primates and in the human subjects. The present work is a logical continuation of a previous work conducted in the laboratory of Professor Eric Rouiller, concerning the multisensory integration at the level of the auditory cortex (Céline Cappe's Ph.D. thesis, 2007). More specifically, in her thesis work Céline Cappe has established in non-human primates the presence of various multisensory cortico-cortical (heteromodal connections) interactions, as well as at thalamo-cortical level; furthermore, she demonstrated the presence of neurons responsive to visual and visuo-acoustic stimuli in the auditory cortex. The present thesis represented a crucial step towards a necessary refinement of the experimental protocol and an extension of the investigations to another cortical area, the premotor cortex in non-human primates. In addition, the present work successfully established the translation of the experimental protocol from the non-human primates to human subjects. The main facets of the present thesis work are: 1) adapt and improve the previous multisensory detection protocol in both non-human primates and humans (Lanz et al. 2013 a et b; feasibility study), in order to allow the recording of neurons in other cortical areas (e.g. premotor cortex, thalamus) in monkeys and EEG in humans; 2) compare our behavioural data for this detection paradigm with previous studies available in the literature (in the § Behavioural results); 3) analyze and interpret the electrophysiological data, single unit recordings in the premotor cortex of two monkeys (in the § Electrophysiological Results in Lanz et al., 2013b) and EEG in four human subjects (feasibility study).

## IV.1 Methodology

For the general discussion related to the initial methodological developments, we will linger mainly on further issues dealing with the various chronic implants used in the non-human primate model. However, let’s start with a few considerations related to the modifications introduced to the different parameters defining the detection protocol finally applied to both non-human primates and human subjects. As compared to the previous protocol (Cappe's thesis), a major change affected the visual parameters, such the properties of the stimulus itself and the target for the gaze fixation. These modifications are the result of preliminary discussions with Dr Pascal Würth, specialized in the acquisition of data on the visual system (University hospital of Bern). The modifications are as follows:

- **The target** for the gaze fixation is no more a cross or a square, but a round shaped target placed in the center of a black background screen. Indeed, by using a round target, we naturally "facilitate" the gaze fixation by the subject. The previous choice of a cross as target presented the inconvenience that the subject was prompted make saccadic eye movements with the aim of scanning the four extremities of the cross. Indeed it is crucial to avoid eyes' movement artifacts during the electrophysiological recordings.
- **The shape of the flash** stimulus was also modified. We used a round green LED (1.9 mm circle). This LED was placed in the center of the target. The advantage of this central position is again to focus the gaze during the presentation of the visual or visuo-acoustic stimuli. Furthermore, the use of a LED allowed increasing the precision in the control of the light intensity. Indeed, the intensity can be finely modulated by subtle modifications of its frequency of excitation (more details in § Stimuli Lanz et al., 2013b). Therefore, and in contrast to the previous protocol, we could here precisely measure the detection visual threshold. This notion fits into the logic applied for the search of the auditory thresholds thanks to a detection task.

As far as the auditory stimulus is concerned, no modification was introduced here, as compared to Céline Cappe's thesis. We may question the possibility (in the future) to modify the loudspeakers' position. Indeed, according to the literature (Hughes et al., 1994; Frens et al., 1995), the multisensory integration is facilitated when stimuli are located in the same spatial alignment. Consequently, we may consider placing our loudspeakers in front of the subject and not laterally. Nevertheless, in the present experimental conditions, the expected multisensory facilitatory effect (e.g. decrease of the reaction time) was clearly present, thus fulfilling the behavioural prerequisite requested to conduct the electrophysiological investigations.

The second, main methodological development conducted in the course of the present work concerns the various devices and chronic implants, as well as the 3D replica of the monkeys' skull with the aim to construct personalized implants (see detail in Lanz et al., 2013a). As reminder, it is a headpost fixation implant in titanium and chronic recording chambers in tekapeek. The use and the design of these devices were developed with the aim of firstly increasing the animal's comfort (3Rs Initiative) and, secondly, to guarantee the stability of the implants on a long period of time, in order to prolong the time window of electrophysiological investigations. The refinement of the method can be summarized by the following characteristics:

- Non-use of dental or orthopedic cement to anchor the chronic implants to the skull.
- Natural osseo-integration by using hydroxyapathite coating.
- Decrease of the post-operative risk of infections by an antibacterial agent applied to the hydroxyapathite coating (Medicoat AG).
- Longevity of the devices and no accidental loss of the implants.
- Custom-fitted implant according the 3D replica of the skull.

For the experimenter, these newly developed implants have also advantages:

- Decrease of daily care (e.g. cleaning).
- Fewer risks of infections or loss of the implants.
- Possibility to easily remove and replace the implants.
- Skull and bone thickness visualization with the 3D replica of the skull.

Moreover, there are further advantages linked to the non-use of dental or orthopedic cement, as already argued by Adam et al. (2007, 2011), as well as by McAndrew et al. (2012). The use of screws in titanium only, instead of cement covering screws for the implants anchor, results in a decrease of the implant's weight on the animal's head, for a better daily comfort. The use of screw only without cement necessitates however some precautions during the surgery. It was indeed important to continuously flow saline solution during the insertion of screws into the bone, in order to avoid an increase of the temperature, which may affect the health of the bone. This precaution thus decreases the subsequent risks of loss and infections at the level of the skull. Finally, the use of screws only without cement reduced dramatically duration of surgery, as one can avoid the tedious and highly time consuming application of cement layers by layers around the implant. In summary this first part of the thesis, extending the techniques of Adam et al. (2007 and 2011) and of McAndrew et al. (2012) defines new ethical guidelines in the spirit of the 3 Rs initiative, with the goal to improve the conditions of the laboratory animals.

According to the overall chronology of the experimental protocol in non-human primates (see Figure 32 in the § II.6), the first device implanted is the headpost fixation. This implant in titanium is placed on the skull at its foreseen position, most rostrally as possible in order to leave open all options to access brain areas, from the prefrontal cortex to the occipital cortex. The legs of this device, forming a K shape of a thickness of 1.5 mm, allowed instantly adjustments during the surgery to optimally fit the morphology of the animal's skull. The preoperative pre-shaping based on the use of the 3D Replica of the skull is however largely sufficient to ensure a good fit with the skull contour, thus making the final adjustment a minor task for the surgeon. The 3D replica of the skull was developed on the basis of a CT scan, and then in the form of a collaboration with the school of architects and engineers of Fribourg. The 3D replica of the skull is a major technical advancement as provides a precise morphological representation of the skull (thickness of the bone, the roughness of the bone), leading to personalize the various implants according to their anticipated location on the skull's surface. The length of the legs of the K-shape headpost, determined by the number of screws per leg, is defined according to the weight of the animal. However the length of the axis, the visible part of the device, is of 2 cm to avoid any problem with the environment of the animal (e.g. height of the wire mesh of transfer cages). However, and according to strengths and resistance calculations (S+D Scherly, La Roche, Switzerland), it may well be that this length can be reduced.

A further improvement concerning this headpost device maybe the choice of the material used to fabricate it. Indeed, we opted for a titanium rank 2, as used by Adams et al. (2007). However, after more recent discussion with the S+D Scherly's engineers, the use of a titanium rank 5 may be a more adequate choice, especially if the experimental protocol requires MRI acquisitions afterward. The significant difference between the rank 2 and the rank 5 lies in the "amount" of artifacts engendered by the rank 2 titanium. The rank 5 titanium is expected to generate none or very few artifacts during MRI acquisitions.

The second newly developed implant device is the chronic recording chamber. The design of this chamber was also based towards the idea to not use cement for its anchoring. For that, we again used titanium screws and the principle of osseo-integration. This approach offered the same advantages for the animal as those obtained for the headpost fixation. Thanks to the use of a home-made adapter, this chamber allows the use of a variety of multi-electrodes or a single electrode drivers.

The advantage of using tekapeek to fabricate the chronic recording chambers lies in the reduced weight (as compared to metal), as well as the possibility to conduct MRI session acquisitions with little artifacts. For the future, and based on its validation by resistance calculations, we may also produce the headpost fixation from tekapeek, to further reduce artifacts during MRI or CT scan acquisitions. Nevertheless, in this context, there is still the issue of using titanium screws, creating small artifacts. We can circumvent this problem in the future with the insertion of porcelain screws or trying to stick the device with some glue composed of hydroxyapatite (e.g. HydroSet Injectable HA Bone Substitute, Stryker®), with a glue hardening in a few minutes and composed of synthetic bone as well.

In the future, and as mentioned above for the headpost fixation device, the size of chronic recording chamber may also be reduced (based on a discussion with Prof. Stuart Baker, Newcastle University). In our case, such size reduction would be an advantage, because it would limit the length of the electrodes guides and thus decrease the risk of error as for the angle of penetration with the recording electrodes. To reduce this kind of errors as much as possible, we may have to manufacture for every non-human primate personalized guides. For the next generation of chronic chambers, that will be used for the thalamic recordings for instance (in the § V), a reduced length chamber will be considered, in addition to a protrusion laterally in order to cover the skull opening caused by the previous chamber above the premotor cortex (in the § Recording chamber, Lanz et al., 2013a).

In conclusion for this methodological part, and according to the results described in the two recently published articles (Lanz et al., 2013 a and b), we can say that our general experimental protocol in non-human primates is stable, reproducible and thus can be considered as validated for future experiments.

## IV.2 Behavior

For the general discussion related to the behavioural data obtained in non-human primates and in human subjects, we will dwell on further issues dealing with the facilitatory effect induced by a reaction time decrease in response to a visuo-acoustic stimulation, as compared to unimodal stimulation. This part of the discussion will be split into two to sub-chapter. The first one dedicated to the results obtained in the non-human primates and the second for the results obtained in the human subjects.

### IV.2.1 Behavior in non-human primates

In this sub-chapter, we will treat the various aspects bound to the behavioural results obtained during the various phases of this thesis. We can mention that this sub-chapter was already widely discussed in the second article (Lanz et al., 2013b). As described in the part "Material and Methods", the behavioural evaluation was divided into two parts: the absolute sensory thresholds research (auditory and visual) and the measure of various behavioural parameters (RTs, percentage of success, types of errors, effect of the intensity during unisensory and combined stimuli) bound to the multisensory task. This division follows the temporal aspect of the project. In other words, the determination of the sensory thresholds was a crucial step in the animal training for the purpose of achieving the final version of the mutisensory-motor integration task, during which the various parameters (enumerated above) were measured.

Consequently the first obtained behavioural data were the sensory absolute thresholds (Appendix 1). It is easy to compare the auditory thresholds obtained with those of the literature. Indeed, several studies conducted on the non-human primates are available. Briefly (§ "Results, Absolute sensory thresholds", Lanz et al., 2013b), the obtained auditory thresholds (6.6 dB SPL for Mk-JZ and 7.9 dB SPL in Mk-LI) were very similar to auditory thresholds (1-8 dB SPL) reported in the literature (Stebbins et al., 1966; Stebbins, 1973; 1975; Pfingst et al., 1978; Smith and Olszyk, 1997) in various monkey species and using other psychophysical methods.

Concerning the other modality, that was the visual thresholds (~24 dB), a comparison with the literature (e.g. Cappe et al., 2010a) is difficult, if not impossible. For example: by taking Céline Cappe's thesis (protocol the most comparable to ours), too many modifications at the level of the visual stimulus as well as in the measure of the threshold were brought, making a direct comparison impossible. Consequently the comparison with previous studies the literature, in which the protocols were even more different than in Cappe's work, is not possible either. However, there is apparently not strong reason to question the pertinence of the visual thresholds measurements we have performed, as this psychophysical method has already been conducted in the field of auditory threshold assessment (e.g. Smith and Olszyk, 1997).

Following the sensory thresholds evaluations, we continued the training of the animal, to obtain behavioural data acquired when performing the multisensory-motor task per se. Among the many parameters considered in the analysis and discussion parts of the corresponding article (Lanz et al., 2013b), we shall focus here on the essential attribute, namely the reaction times (RT). In a general manner, the RTs obtained in response to cross-modal stimuli in the different intensity conditions tested are consistent with a facilitatory effect, reflected by a decrease of RTs, as compared to individual auditory or visual stimulation. This RT decrease was statistically significant in most cases in comparison to the RTs obtained in unisensory conditions. These observations are in agreement with those obtained in the study of Cappe et al. (2010a) in non-human primates, as well as other studies conducted in human subjects (Giard and Peronnet, 1999; Molholm et al., 2002; Sperdin et al., 2009; Fiebelkorn et al., 2011; Senkowski et al., 2011). This behavioural facilitation usually known as redundant signals effect (RSE) might be best simulated with one or the other of two different models: a) the race model (Raab, 1962; in the § I.4.2 and in Lanz et al., 2013b) and b) the co-activation model (Miller 1982; in the § I.4.2 and in Lanz et al., 2013b). In our case, the cross-modal RTs exceed the predictions established during the summation of probability, what demonstrates a "redundant effect signal" (RSE). To determine if this RSE is the consequence of one of the two models quoted above, we applied Miller's inequality for every condition. The results (Figures 6 Lanz et al., 2013b) showed a violation of these inequalities; meaning the involvement of a neuronal interaction occurring early before the subject generates a movement (Lanz et al., 2013b). Several anatomical (e.g. Morel et al., 2005; Cappe et al., 2009b) and electrophysiological (Giard and Peronnet, 1999; Molholm et al. 2002; Calvert et al., 2001) studies suggest possible brain locations and mechanisms possibly involved in such early multisensory interactions. At cortical level, a network of inter-connected polymodal areas could play a role as a putative origin of these RSE. For example, we could quote the VIP area which receives inputs from somatosensory, auditory, visual and vestibular systems (Duhamel et al., 1998; Schroeder and Foxe, 2002), or the prefrontal cortex, receiving inputs from visual and auditory cortical areas (e.g. Romanski et al., 1999). These projections or inputs confer to some neurons in these brain areas the capacity to respond to several modalities and maybe integrate them into a unified perception. At the beginning of the 2000s, it was unexpectedly demonstrated that cortical areas considered so far as purely unimodal had direct connections, namely heteromodal connections, with other unimodal cortical areas (Schroeder et al., 2001; Falchier et al., 2002; Rockland and Ojima, 2003, Cappe et al., 2007). Older studies also demonstrated the multimodal neurons existence within an unimodal area (e.g. in the auditory cortex of the monkey: Watanabe and Iwai, 1991). Consequently, the results derived from these studies diverge from the usual concept that multisensory integration is involved only and late in the hierarchy in higher-order associative areas; in contrast, these new data revealed that cross-modal integration may take place early already in unisensory cortices, at rather low levels of cortical processing (Giard and Peronnet, 1999, Molholm et al., 2002, Schröder and Foxe, 2005; Ghazanfar and Schröder, 2006; Céline Cappe's Thesis, 2007). In our experimental protocol, the very fast generation of movements triggered by a multisensory stimulus is likely to involve low level polysensory areas of convergence, possibly sub-cortically, providing rapid access to cortical responsible for the initiation of the movements (e.g. premotor cortex). According to previous anatomical studies (Morel et al., 2005; Cappe et al., 2009b), inputs from polysensory nuclei of the thalamus to the premotor cortex have been demonstrated and indeed may represent a rapid pathway for the visuo-acoustic-motor integration. A potential candidate would be the pulvinar nucleus of the thalamus. Indeed, the study of Cappe (Cappe et al., 2009b) demonstrated that this nucleus receives projections from different sensory cortices. Furthermore, the thalamus is in position to relay transthalamically quick and secure information between two remote cortical areas (e.g. Rouiller and Welker 2000; Sherman and Guillery, 2006; Cappe et al., 2009b), including the premotor cortex. Based on these anatomical and connectional data, the pulvinar nucleus is an ideal candidate as anatomical support for the fast initiation of movements in response to the detection of multisensory stimuli. This "thalamic" concept is elaborated in more detail in the discussion of the article (Lanz et al., 2013b).

We have just seen that the RTs analysis based on the application of the Miller's inequality demonstrated that the multi-modal RTs decrease is due to a neuronal interaction. However, and in the views of the various used conditions (modulations of intensities), other parameters relating to RTs can be analyzed, such as the principle of inverse effectiveness. As mentioned in the Introduction, the facilitatory effect, expressed in RT decrease (in msec.) or in bimodal gain (in percent; see Lanz et al, 2013b), shows a relationship between its efficacy and the intensities of the respective stimuli. This relationship, commonly named inverse effectiveness (Stein and Meredith, 1993; Holmes, 2009), specifies that when the intensity of the stimuli increases, the bimodal facilitatory effect decreases. In our case, this would mean the more the visual and auditory intensities increase the more the difference between the unimodal and the multi-modal RTs decreases. In line with the results obtained by Cappe et al. (2010a), as well as in her thesis, and in spite of an intensity range limited between 10 and 40 dB over the threshold, we have also observed the inverse effectiveness effect, for both monkeys enrolled in the non-human primate study.

In recent publications (Cappe et al., 2010a; Sendowski et al. 2011), a relation between the inverse effectiveness and the Miller's inequality can be applied: the more the intensity is close to the threshold, bigger is the amplitude of the inequality. However, after analyses of the Miller's inequality, it turns out that we did not notice this relation between the intensity and the inequality amplitude. As a reminder, this observation of the inverse effectiveness was demonstrated first of all during electrophysiological acquisitions (Meredith and Stein, 1986), in the superior colliculus (SC). The fact of not being able to observe this effect on the inequality may be explained by two factors:

1 ° According to Klemen and Chambers (2011), multisensory interactions can occur without the criteria established in the models of the SC. This argument follows the Angelaki and al. (2010) hypothesis, suggesting that the super-additive responses are not necessarily a criterion for all multisensory interactions.

2° The intensities tested were close to the sensory thresholds, in order to emphasize the robustness of the facilitatory effect due to the multisensory integration. The application of higher intensities may have better demonstrated this inverse effectiveness effect on inequality possibly more predominant at higher ranges of intensity.

As additional information to the analyses of RTs (important part of the behavioural data), we analyzed the errors (percentages, types of errors). The importance of these data must not be minimized, but their impact is less important. Nevertheless, like mentioned in the literature (e.g. Meredith and Stein, 1986; Giard and Peronnet, 1999; Calvert and Thesen, 2004; Wallace and Stein, 2007; Stein and Stanford, 2008), better performance occurred during a multi-modal integration, as compared to unimodal conditions. For us, the same conclusion was met, as shown by the statistically significant decrease of the number of errors in the bimodal condition (Figure 4 C and D, Lanz et al., 2013b).

In conclusion, these behavioural data obtained in non-human primates enables us to validate the entire experimental protocol (in the § I.5). Indeed, we observed the expected bimodal behavioural facilitation with a significantly decrease of the RTs in response to combined stimuli and the principle of inverse effectiveness was largely followed in the non-human primate model (in the § III.1). Consequently, these behavioural results prompted the new development of methodological skills, with the aim to test our initial hypothesis, suggesting that the premotor cortex may be a site for early convergence of visuo-acoustic integration.

### IV.2.2 Behavior in human subjects

As mentioned throughout this thesis, a feasibility study was conducted on human subjects. The basic idea being to be able to compare the human results with those obtained on the non-human primates with an identical protocol. Consequently, and according to this aim, the behavioural analyses were identical in the two groups of subjects (monkeys and humans). The structure of the discussion relative to the human subjects will be also the same as for monkeys for the sake of consistency.

We will begin here with some initial comments concerning the sensory thresholds. Indeed, the average auditory and visual thresholds were not calculated in the course of the behavioural assessment per se. Indeed, the sensory thresholds (in the § II.2.3) were determined once before the behavioural session per se and for every subject. Furthermore, every subject carried out only once the behavioural session. Consequently, the variability inter-subject is too large for, from a scientific point of view, to cumulate these data. However, the absolute auditory thresholds obtained in response to 1000 Hz pure tones were consistent with the values reported in the literature, i.e. between 5 and 10 dB SPL (Botte et al., 1989). The comparison of the visual thresholds with those of the literature is again more difficult, because, to our knowledge and as commented in the non-human primate behavioural discussion, no similar protocol was used previously. We are aware that this aspect of the protocol differs from that of the monkeys, in which the thresholds were averaged from data collected over several months. However, there are several arguments which can explain this difference:

- The human subjects (n=10) involved in this behavioural protocol did not perform electrophysiological recordings. Consequently the duration necessary for the task execution is not long enough to observe an attention decrease.
- In contrast to monkeys, the human subjects do not need a learning period, requiring consolidation periods to carry out this sensory-motor task.

- In contrast to the humans, the two monkeys were involved both in behavioural and electrophysiological assessments. Consequently, it would be tedious to evaluate, for example, the sensory thresholds before each electrophysiological data acquisition. Indeed, between the time required for the sensory measures and the time required for the electrodes positioning, the animal would be demotivated or would not be concentrated enough for the continuation of the experiment.
- Furthermore, the multisensory task learning required several steps including consolidation/stabilization phases. For example, during the period of auditory threshold measurement (several months), the data of the first weeks were not considered, because the animal exhibited too much variability.

The choices as for the various parameters of stimuli (type of acoustic cue, shape of the flash, the color of the flash, the intensity) do not allow easy comparisons with the literature concerning the audio-visual integration in human subjects. Nonetheless, several studies (e.g. Giard and Peronnet, 1999; Molholm et al., 2002; Senkowski et al., 2011) were conducted on human subject in the context an audiovisual integration, though with significant differences in their protocol.

As discussed for the non-human primates, we are going to detail the RTs aspect. Indeed, the study of Giard and Peronnet (1999) showed shorter mean auditory RTs than mean visual RTs during audiovisual integration (RTa < RTv). In contrast, the study of Sun and al. (2009) demonstrated shorter RTs for the visual stimuli than for the auditory stimuli (RTv < RTa). In our case, the results are comparable to those obtained by Giard and Perronet (RTa < RTv). Consequently, if we believe in the rules of the sensory dominance, as proposed by Giard and Peronnet (1999), our subjects would present an auditory dominance (auditory RT < visual RT). We shall discuss impacts of this sensory dominance on the results in the next sub-chapter (in the § IV.3.2).

The observation of RTs during the various conditions (+10, +20 and +30 dB) indicates a facilitatory effect when the presented stimuli were combined. This effect is statistically significant by comparison with mean RTs obtained in response to unisensory stimuli. This observation is consistent with previous data available in the literature (Giard and Peronnet, 1999, Molholm et al., 2002; Ross et al., 2007; Senkowski et al., 2011). These observations in the human subjects are also in line with those observed in non-human primates (Cappe et al., 2010a; Lanz et al., 2013b). The expression in percent of this facilitatory effect, the so-called bimodal gain, also revealed a substantial gain in term of RTs decrease. Therefore, we applied the same analyses as in the non-human primates, in order to demonstrate the existence of the redundant signal effect (RSE), as well as the notion that the cross-modal RTs exceed the predictions established during the summation of probability. Additionally, we applied Miller's inequality, which showed a violation of the model. Consequently, in line with the monkeys data, these results cannot be simply explained by the shorter modality processing, indicative then of neuronal integration occurring early.

However, and in contrary to what was expected, we did not see the inverse effectiveness (IE) effect. It is possible that our choice of intensities tested was not the most pertinent and that higher intensities would have required to address this inverse effectiveness effect issue. The latter interpretation is supported by data derived from the study of Senkowski and al. (2011). These authors were able to determine during an audio-visual task in human subjects the principle of IE. It is important to note this effect is low (but present) and that the chosen intensities were clearly higher (40, 65 and 90 dB) than ours.

The relationship (Senkowski et al., 2011) between the intensity increase and the amplitude increase of the Miller's Inequality could not be demonstrated in our case. Indeed, in our study, the amplitude of the facilitatory effect had no relation with the intensity of stimulation.

In conclusion, the behavioural results allowed to validate the multisensory-motor task newly developed in human subjects as well. The human behavioural results demonstrated the facilitatory effect obtained with a combined stimulus. What suggests that the induced RSE takes place early in neuronal integrating processing at cortical level. Furthermore, the similarity of the results between the non-human primates and the human subjects with the same task allows validating this psychophysical protocol with the goal, in the future, to offer an extrapolation from the animal model to the human model. Besides the protocol validation itself, we confirmed (at least for the behavioural part) our working hypothesis that the multisensory facilitation properties observed in the non-human primates with this specific experimental protocol can be successfully reproduced in humans.

## IV.3 Electrophysiology

For the general discussion related to the electrophysiology data obtained in non-human primates and in human subjects, we will dwell on further issues dealing with the single-unit recordings in non-human primates (n=2) (detailed in Lanz and al. (2013b)) and with electroencephalography data (EEG) in the human subjects (n=4). This part of the discussion will be split into two to sub-chapter according the subjects and the type of recordings.

The electrophysiological part aimed at responding to our main hypothesis, according to which the premotor cortex (PM) may be a site for early convergence of audiovisual inputs in the context of the present bimodal detection task, as well as based on previous data in other contexts (Graziano 1999 and 2001). As an initial introduction to this chapter, a brief reminder is needed concerning two articles of reference dealing with audiovisual integration at the electrophysiological level (Giard and Perronet 1999; Molholm et al. 2002). These two articles make collectively reference to the precocity of the neuronal interactions (=early convergence). These interactions were observed as early as from 40 msec (45 msec for Molholm et al. 2002) post-stimulation. In complement to these observations, which can be compared with our results, it is interesting to notice the effect of visual or auditory dominance mentioned by Giard and Perronet (1999), a property observed for the first time.

In parallel to these human studies, electrophysiological recordings conducted in the non-human primates in the 90s (e.g. Wise et al., 1997) demonstrated the role of PM in the preparation of the voluntary movement. The very fast motor response and neuronal responses (with certain pattern) to a multisensory stimulation can be the consequence of a premature convergence of sensory information, for example at the subcortical level. This hypothesis is supported by various studies at subcortical levels, linked with the motor cortex, such as the superior colliculus (Meredith and Stein, 1986; Rezvani and Corneil, 2008; Cuppini, 2010).

### IV.3.1 Neuronal recordings in non-human primates

The discussion about the non-human primates' electrophysiological recordings was already treated (in detail) in the article specifically concerning the results (Lanz and al., 2013b). However, we propose here to summarize the content and to bring some additional arguments.

Data based on the activity of a total of 132 neurons (pooled between the two monkeys) during a visuo-acoustic detection task allowed us to determine/define several neuronal patterns. We were able to classify the neurons into 7 patterns of activity distributed into two main classes (Table 6): 1) the "responses" patterns, defined by neuronal activity modifications during the presentation of the stimulus and 2) "modulation" patterns, neuronal spiking changes taking place after the sensory stimulation. In the first class, four patterns were identified:

a) **ONSET**: strong and sharp excitation at the stimulus **onset.**
b) **Sustained**: excitatory neuronal activity present during the entire stimulation period.
c) **Late**: strong and sharp excitation in the second part of the stimulation period (~100 msec latency and latter).
**d)** **Inhibition**: the neuronal activity decreased during the stimulus below 1 SD subtracted from the average activity of reference.

In the second class the modulations of activity are composed by 3 patterns:

a) **OFFSET**: strong and sharp excitation after the stimulation period.
b) **Post-sustained**: enhanced neuronal activity along the post-stimulation period
c) **Inhibition**: decrease of neuronal activity after the stimulations period.

**Table 6: Distribution of the single neurons' activity type into two main classes, with different patterns obtained during electrophysiological recordings in non-human primates.**

| Response Patterns | Modulation Patterns |
|---|---|
| ONSET | OFFSET |
| Sustained | Post-sustained |
| Late | Inhibition |
| Inhibition | |

This classification was introduced according to criteria relative to the neuronal activity (enhancement or decrease) in comparison to a 200 msec-reference period preceding the stimulation, as well as to the latency of these activities (during the stimulation or post-stimulation). The terms, "Response and Modulation" (< 250 msec), chosen to define these neuronal classes are very important. In the case of response patterns neurons, we could conclude that the stimulation affected rather directly the spiking activity of a PM neuron. In this case, especially with ONSET neurons, we are in presence of multisensory neurons or of unisensory neurons able to respond either to an auditory stimulus or a visual stimulus. Although we are in PM, such neurons behave more like "sensory" cells than neurons linked to the motor attributes of the behavioural task. In the case of modulation patterns, although the neuronal activity is also linked to the stimulation, such activity is likely to be more related to the preparation of the motor response itself, possibly modulated by the modality of the sensory stimulus. The two classes of neurons in PM (Responses and Modulation Patterns) may thus reflect two successive epochs along the entire sensory-motor integration leading to the generation of the motor act triggered by the detection of an expected sensory stimulus. Due to the massive training, if not overtraining, of the two monkeys for this task, it is very likely that the motor acts are highly automatized and may thus be delegated to other (lower) structures than the motor cortical areas (e.g. subcortically in the brain stem or in the basal ganglia). As a result, the "motor" activity in PM may be reduced for such a detection task after intensive training, as compared to an earlier training phase. This may explain the relatively low proportion of neurons in PM truly influenced during the present study, as activity was recorded when the monkeys were overtrained. It would certainly be interesting to record activity in PM earlier during the training phase, although this is clearly more challenging as the monkeys exhibit more chaotic behaviors, not necessarily compatible with stable and long enough periods of activity to collect enough trials. As far as the comparison with the existing literature is concerned, besides what was said in the related article (Lanz et al., 2013b), especially studies in non-human primates, there are few electrophysiological studies, such as EEGs, or imaging studies, such as fMRI, conducted in human subjects (e.g. Jones and Callan, 2003; Lee and Noppeney, 2011; Okada et al., 2013) in the context of multisensory integration in the premotor cortex (see below human data). However, and to the best of our knowledge, no specific study based on single unit recordings was made to date in PM in the context of a sensory detection task per se, as most studies were based on the principle of a conditional task with delay. More precisely, no similar study requiring a detection behavioural paradigm, and comparing unisensory stimulations and bimodal stimulations, was made to date in non-human primates.

In conclusion, these PM single-unit electrophysiological data in non-human primates demonstrated the presence of two main classes of neurons, the Response and the Modulation patterns, which were both influenced in the course of the detection behavioural task performed by the monkeys. According to the neuronal activity patterns and the latencies of these activities observed, these PM neurons remain candidates as players in the rapid translation from sensory detection into motor acts, with the possibility that differences in responses between modalities may be the substrate for differences in RT depending on whether the stimulation was unimodal or bimodal. Further studies with recordings in the thalamus, combined with reversible inactivation investigations also in the thalamus (e.g.

pulvinar nucleus), are needed to further elucidate the precise neuronal circuit underlying early multisensory facilitation, as observed in such bimodal detection paradigm. Separate reversible inactivations of primary, secondary visual and auditory cortical areas, as well as associative cortical areas, may also indicate to what extents each of them are involved or not in such detection task. Such an approach may also help to decipher the precise role played by subcortical associative nuclei (SC, thalamus) in early multisensory integration.

### IV.3.2 Electrophysiological recordings (EEG) in humans

As mentioned along this Ph.D thesis, a feasibility study conducted on human subjects was developed and analyzed according to comparable criteria as those applied in the non-human primate study. The feasibility study aimed at establishing the link between the single-unit recordings obtained in the non-human primates and the EEGs results obtained in the human beings. This concept of link will be further developed in the paragraph "Perspective" (in the § V). It is true that it is not straightforward to compare electrophysiological data when recording techniques and the systems of acquisition are not the same between animals and humans. In the direction of unifying the experimental approaches, at the moment, the EEGs recordings in the monkeys require several adjustments, especially in awake and behaving monkeys (see Gindrat et al., 2013 in preparation; EEG data in anesthetized monkeys). Indeed, no study so far reported EEG recordings during a sensory-motor task in the non-human primates. The EEG studies conducted on awake non-human primates (Gardner et al., 1984; McCarthy et al., 1991; Peterson et al., 1995; El Kharroussi et al., 1997) are very rare and they do not involve a protocol requiring a voluntary action of the animal. At the other extreme, intra-cortical recordings in human beings is not possible at the moment, due to technical and ethical limitations. Consequently, and as described in the next chapter (in the § V), the EEG recordings in non-human primates remains an option, although the technical difficulties are serious for an application to behaving monkeys.

Back to our EEG data in humans, we can mention that the data were derived from three human subjects (n=3), the fourth one was eliminated from the analysis due to substantial artifacts during the acquisition which may have biased the results. Consequently, an extension of this study to additional subjects is necessary to obtain more satisfactory results on statistical point of view.

Therefore, this limited number of subjects at that step restricts the direct comparison with the literature, with studies based on larger numbers of human subjects (Giard and Peronnet, 1999; Molholm et al., 2002; Fort et al., 2002; Talsma et al., 2007; Cappe et al., 2010b; Senkowski et al., 2011). Nevertheless, we observed in our EEG data early interactions at ~60 msec. latency, consistent with those obtained by the authors mentioned above. These data are indicative of an early interaction at neuronal level in relation to the multisensory process. Consequently, we focused our analysis on a latency period ranging from 60 to 90 msec post-stimulation. This latency range is comparable to one considered by Cappe et al. (2010b), as well as Giard and Peronnet (1999): 40 to 90 msec post-stimulation. This temporal analysis (early neuronal interactions) is complementary to the behavioural results described in the previous chapters and, altogether, they suggest that the facilitatory bimodal effect reflected by the RTs may result from these early neural interactions (Lanz et al., 2013b). The analysis of the EEG in the humans offers a more comprehensive spatial representation of activities than the single-unit recordings, limited to the restricted brain area investigated. The spatial analysis (ERPs topography analysis) is likely to provide useful indications regarding the very first brain regions involved in the audiovisual integration required in our detection paradigm. To determine the brain areas involved in relevant information processing during periods going from 60 to 90 msec post stimulation onset, we have compared the ERPs obtained during bimodal stimuli (= Pair ERPs) and the ERPs representing the sum of the visual and auditory ERPs (= Sum ERPs). In other words we applied the following formula:

$$x = AV - (A + V)$$

where x represents the robust activity difference regions; AV the pair ERPs' topography and A+V the sum ERPs' togography. Several studies used the same approach (Giard and Peronnet, 1999, Cappe et al., 2010b; Senkowski et al., 2011). In our case, we observed an activation of the posterior et right temporal region. These results, in spite of a limited number of subjects, are consistent with those reported by Giard and Peronnet (1999), Cappe et al. (2010), as well as Senkowski et al. (2011). In contrast however to Senkowski et al. (2011), but in agreement with Cappe and al. (2010b), we did not find multisensory interaction at the level of the anterior electrodes. According to Talsma and al. (2007, 2010), interactions at such frontal level indicates an implication of attentional mechanisms. What do we understand by attention? By this question, we are going to introduce here some additional information about the role played by attention in multimodal integration. Indeed, this theme was not developed in the introduction of this Ph.D thesis. Attention is a cognitive process defined as, according to William James (1890):

> *"Everyone knows what attention is. It is the taking possession by the mind, in clear and vivid form, of one out of what seem several simultaneously possible objects or trains of thought. Focalization, concentration, of consciousness are of its essence. It implies withdrawal from some things in order to deal effectively with others, and is a condition which has a real opposite in the confused, dazed, scatterbrained state which in French is called distraction, and Zerstreutheit in German"*

Attention can be divided into two principle components: the selective attention and the divided attention. Briefly, in the selective attention, we can speak about visual and auditory attention. The selective attention brings in a concept of facilitation and inhibition. Indeed, a modality is facilitated whereas the other one is inhibited. In our case, the divided attention is pertinent because it represents the ability to pay attention to more than one task at same time. This definition is very similar then to the definition which we may formulate to characterize multisensory integration. And just like multisensory integration, the divided attention improves with training (Sternberg and Sternberg, 2009). Nevertheless what are the neurological principles necessary for the attention? How do we pay our attention? Researches allowed to advance two processing (also similar from a terminological point of view to those used in multisensory): it is the « bottom-up » and the « top-down » processing:

- The bottom-up is also called exogenous attention or stimulus-driven attention (Fort's thesis, 2002; Cappe's thesis, 2007; Talsma et al., 2010). This form of attention can be oriented by the physical properties of stimuli, for example. This orientation is automatic and may consequently be not voluntary. According to Posner and Petersen (1990), this process of attention involves the parietal and temporal cortices, as well as the brainstem. In case of exogenous attention, the display of a stimulus of different modality, but spatially matched, the processing of this second one will be facilitated.
- The top-down is also known as endogenous attention or goal-driven attention (Talsma et al., 2010). The endogenous attention allows selecting an attribute over another. This process is voluntary. Consequently, the person pays its attention towards a specific sensory information. This process takes place in the frontal cortex, as well as in the basal ganglia (Posner and Petersen, 1990; Posner and Rothbart, 1998; Pessoa et al., 2003; Pessoa et al., 2009). Endogenous attention also improves the performances during conditions of low sensitivity (facilitatory effect).

In conclusion, we can see that the attention has an influence on the cross-modal interactions. According to Fort (Fort's Ph.D Thesis, 2002), the attention has effectively an influence on the bimodal interactions, but this effect depends on the sensory dominance of the subjects (in the § IV.2.2) (e.g. auditory or visual) and on the cortical area under investigation (e.g. auditory cortex / visual cortex). Fort's Ph.D thesis, very interestingly, demonstrated a non-parallelism, according to the terms of her author, as for the phenomena observed in the various cortices. Indeed, the interactions in the auditory cortex were dependent on the attention. It means that, in subjects with auditory dominance (what is the case in our study with human) and in the auditory cortex, an inhibition of the processing of combined stimuli is observed when the subject pays its attention on the visual stimuli. However, the recordings in the visual cortex demonstrate activities during combined stimuli, irrespective of the attention carried out by the subject.

To return to the analysis of the EEGs, it appears that defining the process of attention solely on the activation of the anterior electrodes is inadequate. Indeed, the various processes of attention (bottom-up and top-down), as well as the study of Talsma and Woldorff (2005a), suggest neural activations in broader brain areas in relation to attentional processing. For example, the study of Talsma (2005a), dealing with selective attention and multisensory integration, allowed the identification of brain regions involved, based on the latency period. It turned out that the process of attention began at the level of the frontal cortex after 100 msec., followed by an activity in the centro-medial region between 160 and 190 msec. In our case, we did not notice significant early interactions at the level of the frontal cortex. For this, two explanations are plausible: 1) Our analysis focused on the latency period going from 60 to 90 msec, precedes the relevant period reported by Talsma and Woldorff (2005a). 2) EEGs have a high temporal resolution, but less so on the spatial aspect and therefore the present spatial analysis may have missed some focus of activity.

The analysis of sources estimations, an approach developed by Murray et al., (2004, 2006), confirmed that neuronal interactions occuring during this early period (60-90 msec), based on the topographic analyses of the ERPs, yield comparable results as the analysis LAURA. Nevertheless, we were able to notice that some activated areas are not as posterior as that seen in the topography analysis. It is the case for example for the areas 18/19 (visual secondary areas), as well as the area 7 (somatosensory area).

In conclusion, we were able to observe early evoked brain activity during EEGs recordings. These results are consistent with those obtained in the literature (Giard and Peronnet, 1999; Molholm et al., 2002; Fort et al., 2002; Talsma et al., 2007; Cappe et al., 2010; Senkowski et al., 2011). The localization, as well as the early activity period, is compatible with the information concerning subjects with auditory dominance (Giard and Peronnet, 1999). Indeed, for this type of subjects, interactions take place between 40 and 150 msec, in occipital regions. Our feasibility study represents a first step towards the goal to collect reproducible electrophysiological data in human subjects, when executing a protocol comparable to that conducted on the non-human primates.

## IV.4 Necessity to evaluate the behavior and the electrophysiology

In the discussion developed above, we separated voluntarily the behavioural and electrophyisological results. However it is necessary to keep in head that generally these results cannot be separated. Indeed they form a whole. For it we are going to refer to the studies of Gu et al., (2008) as well as Angelaki et al., 2009 which demonstrated the importance to integrate a psychophysical behavioural task during the study of the neuronal mechanisms.

It was demonstrated that the multisensory integration follows predictions where the Bayesian statistical inferences (in the § I.4.5.1) can be applied. Indeed, psychophysical studies (between 2000 and 2005) (e.g. Alais and Burr, 2004; Kersten et al., 2004), against the studies led to the anesthetized animals (e.g. Meredith and Stein, 1986, Wallace et al., 1993) demonstrated that the integration of various cues followed a combined linear integration. From a probabilistic point of view, when a subject is in the presence of two perceptual cues, the prediction wants that this subject shows a greater perceptual sensitivity than during the presence of a single signal (Gu et al., 2008). In other words the unified perception is due to the estimation of individual cue and this according to a linear model (For review (Angelaki and al., 2009). Consequently to understand better the neural processing of sensory integration, it is important to conduct studies on multisensory tasks assessment, that is psychophysics. E.g. Studies similar to those led to the humans (e.g. Alais and Burr, 2004).

## IV.5 Perspectives

In the present work data collected in both human and non-human primates represent an important step in better understanding how the brain integrates two sensory modalities to bring a unified perception of the environment. However, along the collection of these data, we could figure out that the behavioral data collected from our non-human primate model could be further used by trying to put them directly in relation with the various neuronal distributions, classes and neuronal discharge patterns in order to label each behavioral during the sensorimotor task. More precisely, an analysis establishing the connection between RT and the electrophysiological recordings could be done for example for the purpose of understanding the timing between the neuronal discharge/inhibition and the phase of movement initiations. A mapping of the various neurons according to their classification could be also made. This would allow determining if there are groups of neurons active according to common criteria (of the same category) or instead more spreading.

Another important point would be to find a way to “bridge” the studies between non-human and human primates. To achieve this the monkeys’ recording chambers should be removed some EEGs’ should be recorded in awaken monkeys engaged in the same protocol. . Therefore, we would have electrophysiological recordings like EEGs, LFPs and unitary recordings at the level of the auditory cortex (previous work, Céline Cappe’s Thesis, 2007) and in the premotor cortex and we would be able to compare these data with the EEGs recorded from humans. It would result that in case of similarities, some multisensory integration principles could be described in humans at neuronal level. To go one step forward, a new recording chamber could be implanted in the non-human primate model to collect neuronal responses from the thalamus in order to demonstrate from an electrophysiologcial point of view the presence of cortico-thalamic projections that we indicated in the discussion section of the present work.

Finally this approach would have a clinical impact by contributing to a better adjustment of different biomedical devices for example by compensating a modality during a cross-modal perception. One can also imagine that the discovery of new principles regulating the exchange between high and low hierarchical levels could bring new insights in the treatment of affections like autism or Asperger’s disease.

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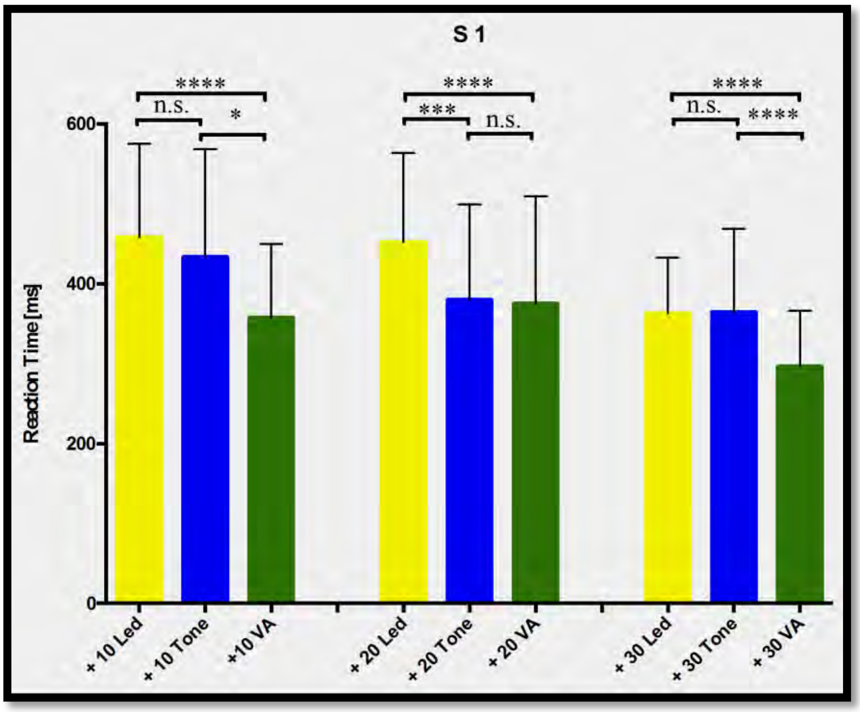
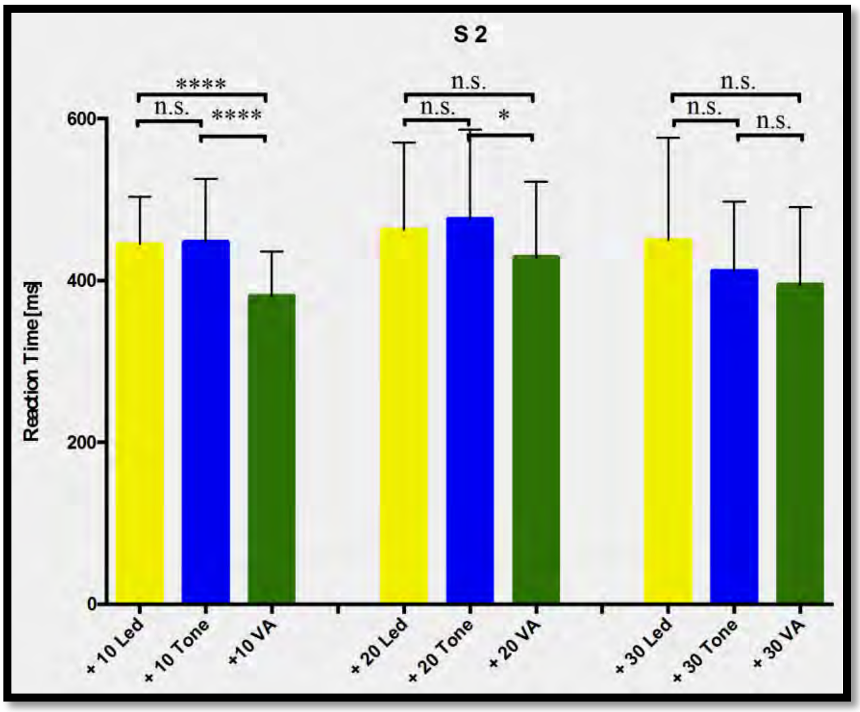
# VI. Appendix

**<u>Appendix 1</u> : Mean reaction times and their SDs obtained from 7 individual subjects**

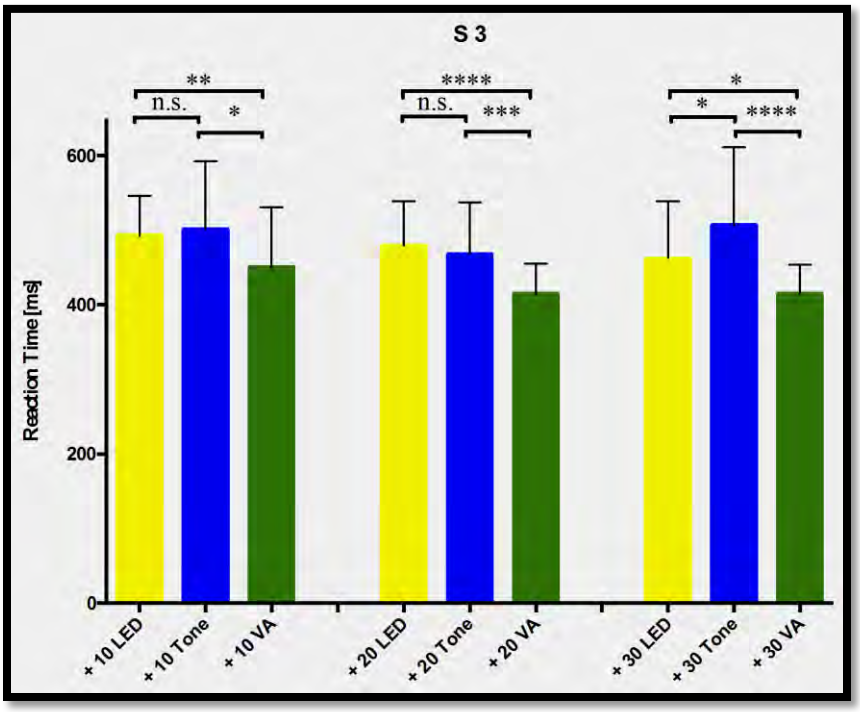
**A**



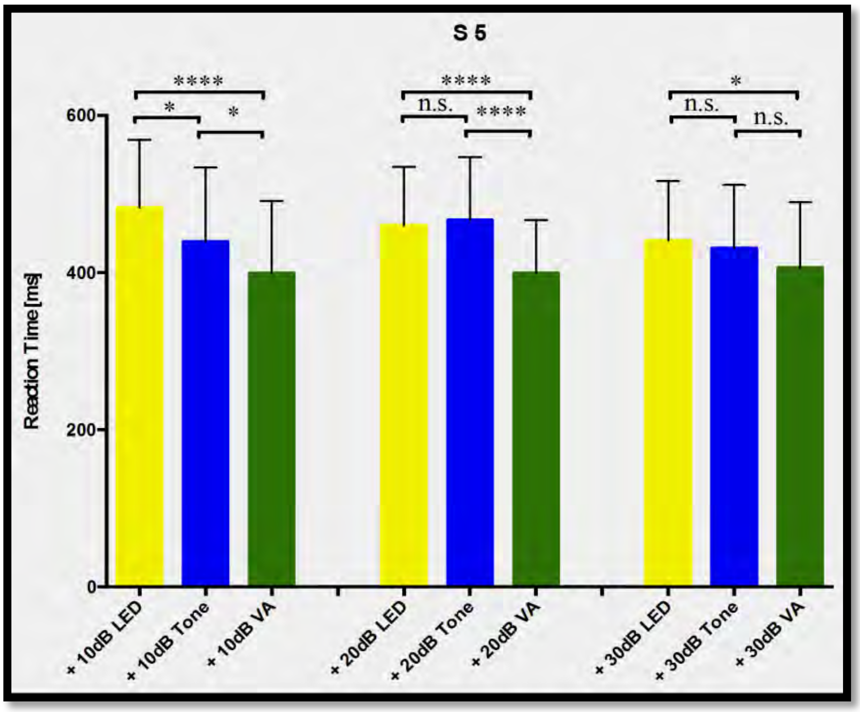
**B**



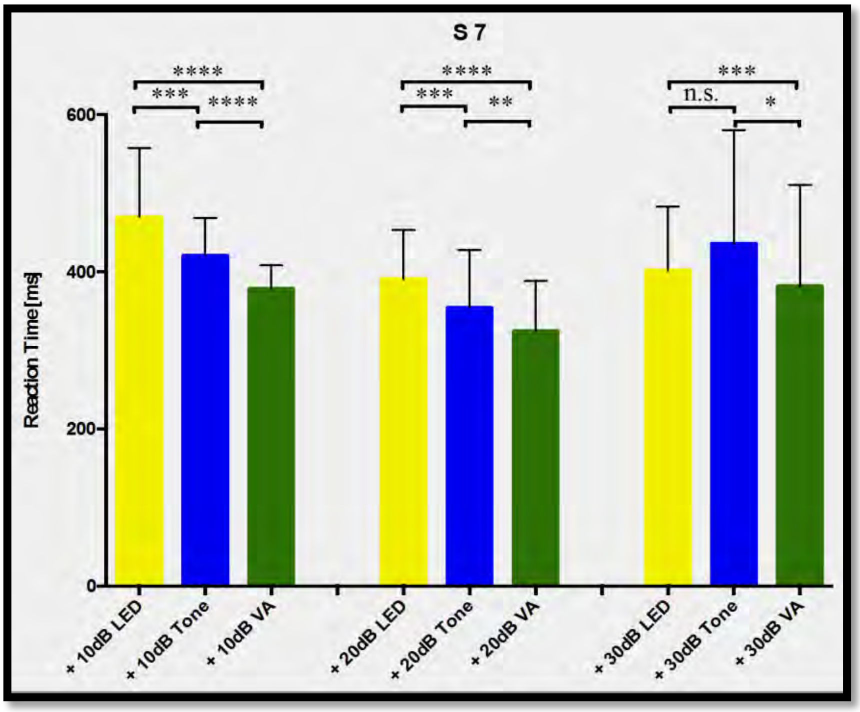
**C**


S 3
Reaction Time [ms]
0
200
400
600
**
n.s.
*
****
n.s.
***
*
*
****
+ 10 LED
+ 10 Tone
+ 10 VA
+ 20 LED
+ 20 Tone
+ 20 VA
+ 30 LED
+ 30 Tone
+ 30 VA

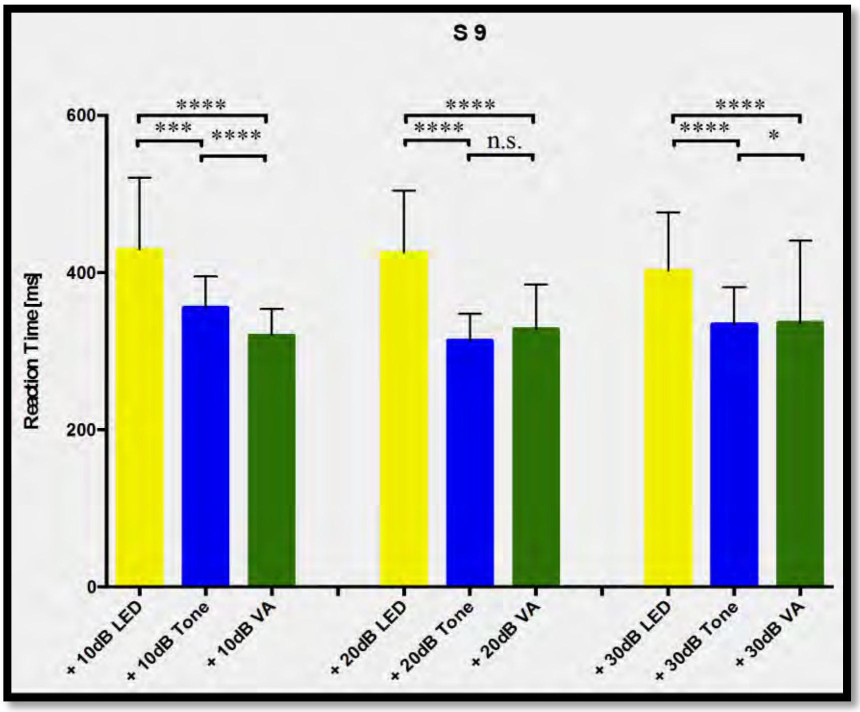
**D**


S 5
Reaction Time [ms]
0
200
400
600
****
*
*
****
n.s.
****
*
n.s.
n.s.
+ 10dB LED
+ 10dB Tone
+ 10dB VA
+ 20dB LED
+ 20dB Tone
+ 20dB VA
+ 30dB LED
+ 30dB Tone
+ 30dB VA

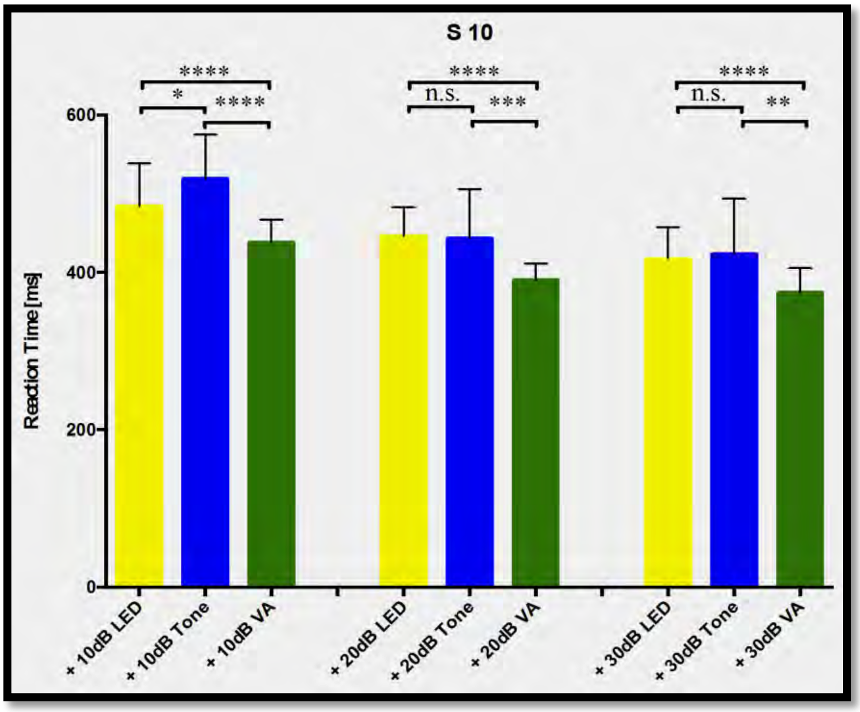
**E**


S 7
Reaction Time [ms]
0
200
400
600
****
*** ****
****
*** **
***
n.s. *
+ 10dB LED
+ 10dB Tone
+ 10dB VA
+ 20dB LED
+ 20dB Tone
+ 20dB VA
+ 30dB LED
+ 30dB Tone
+ 30dB VA

**F**

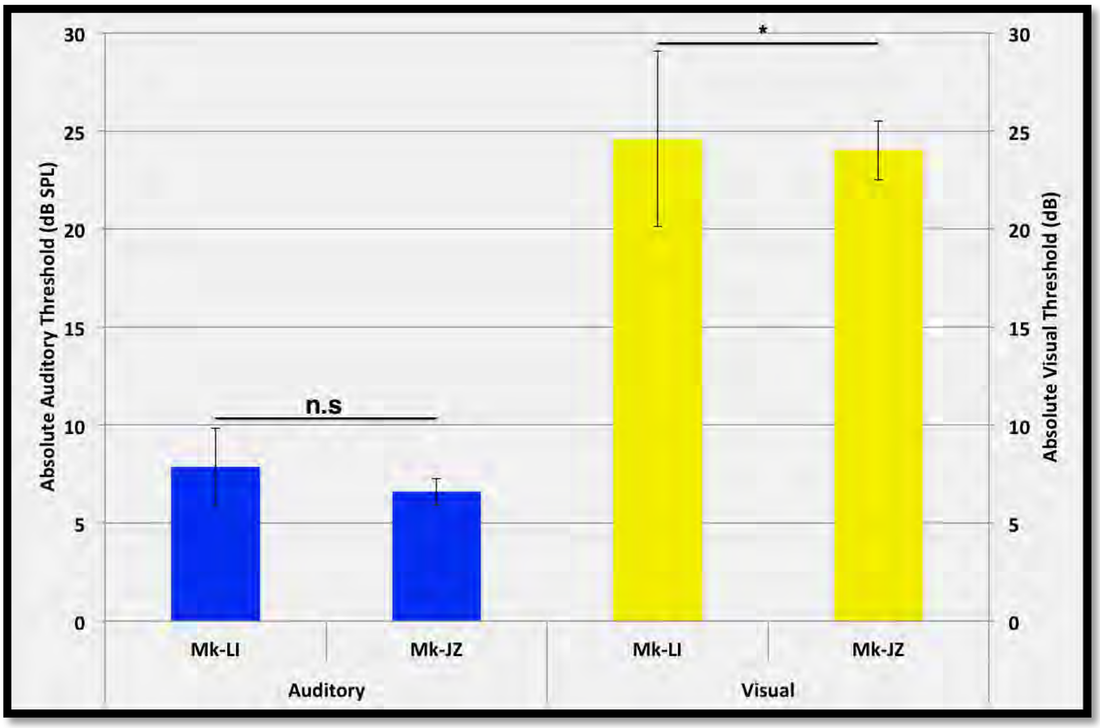

S 9
Reaction Time [ms]
0
200
400
600
****
*** ****
****
**** n.s.
****
**** *
+ 10dB LED
+ 10dB Tone
+ 10dB VA
+ 20dB LED
+ 20dB Tone
+ 20dB VA
+ 30dB LED
+ 30dB Tone
+ 30dB VA

**G**



**Appendix 1 Mean reaction times and their SDs obtained at 10, 20 and 30 dB above the unimodal thresholds. Data from 7 individual human subjects (S1, S2, S3, S5, S7, S9 and S10). n.s. : p≥0.05 ; * :p<0.05 ; ** :p<0.01 ; *** :p<0.001 ; **** :p<0.0001 (Mann-Whitney Test).**

## **Appendix 2** : Sensory thresholds in non-human primates



**Appendix 2: Auditory (in blue) and visual (in yellow) mean absolute thresholds with their SDs obtained in the two monkeys (Mk-LI and Mk-JZ). The auditory threshold assessment was conducted with daily tests corresponding overall to 5 months of training in Mk-LI and to 2 months of training in Mk-JZ. Then, after training, the auditory threshold was determined based on 8 daily tests. The visual thresholds were then determined from daily training tests conducted during 4 months in Mk-LI and during 3 months in Mk-JZ. After training, the visual threshold was determined based on 8 daily tests. n.s. $p \geq 0.05$ ; *: $p < 0.05$.**

# VII. Curriculum Vitae

**Florian Lanz**
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Single
31 year's old
Swiss

## Formation

**MBA, Venture Challenge, mini-MBA** 2013- Present
High School of Management of Fribourg

**Ph.D Student**, 2007- Present
University of Fribourg, Switzerland

- **Neurophysiology:** Topic: Multisensory Integration in human and non-human primates.

**M.Sc. in Biology**, Option Developmental and Cell Biology 2005 - 2007
University of Fribourg, Switzerland

- Language: English
- Master thesis in neurophysiology (Group Prof. E.M. Rouiller):

*"Development of a battery of behavioural tasks relevant for measurement of hearing and for cochlear implant research in the Macaca fascicularis"*

**Bachelor of Science in Biology**, 2002 - 2005
University of Fribourg, Switzerland

- Languages: French and German
- Bachelor thesis in neurophysiology (Group Prof. E.M. Rouiller):

*"Enregistrement des seuils auditifs comportementaux chez le macaque (Macaca fascicularis)"*

**General Certificate of Education**, Option Biology / Chemistry 1998 - 2002
Collège du Sud, Bulle, Switzerland

## Research

Auditory behavior and physiological acoustics

Electrophysiological recording in monkey cochlear nucleus and pulvinar nucleus

Multisensory integration in human and monkey

## Teaching

| | |
|---|---|
| Practical works in neurophysiology (medical and biomedical students) | 2008 - present |
| Bachelor and Master Science students supervision | 2008 - present |

## Certificate

- Certificate of animal experimenter (Module 1)

## Professional Experience

| | |
|---|---|
| Voluntary fireman | 2002 – present |
| Guard of swimming pool, Charmey, Switzerland | 2002 - 2010 |
| Caretaker of old people's home | 1998 - 2005 |
| Ski and snowboards instructor | 1998 - 2002 |

## Collaborations

Medicoat, A.G., 5506 Mägenwil, Switzerland

Ecole d'architectes et d'ingénieurs, 1700 Fribourg, Switzerland

S + D Scherly, 1634 La Roche, Switzerland

Hôpital fribourgeois (HFR), Hôpital cantonal, radiology, 1700 Fribourg, Switzerland

## Memberships

| | |
|---|---|
| Swiss Society for Neurosciences (SSN) | 2005 - present |
| Acoustical Society of America | 2006 - 2008 |
| Society of Neurosciences (SfN) | 2013 - present |

## Languages

French : Native language

English : Proficient speaking and writing level

German : B1

## Informatics

Windows 7, MS-Office 2010, SigmaStat, TDT, Matlab (Basis), Neurolucida

Mac OSX, MS-Office 2011, Osirix, Papers, R (statistics and graphics) (Basis), Graphpad Prism

## Centre of interest

Music: practice one instrument

Endurance sport: racing cycle, rock-climbing

## References

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