

Mélanie Kaeser, Alexander F. Wyss, Shahid Bashir, Adjia Hamadjida, Yu Liu, Jocelyne Bloch, Jean-François Brunet, Abderaouf Belhaj-Saif and Eric M. Rouiller

J Neurophysiol 103:1630-1645, 2010. First published Jan 13, 2010; doi:10.1152/jn.00459.2009

You might find this additional information useful...

Supplemental material for this article can be found at:

<http://jn.physiology.org/cgi/content/full/00459.2009/DC1>

This article cites 95 articles, 43 of which you can access free at:

<http://jn.physiology.org/cgi/content/full/103/3/1630#BIBL>

Updated information and services including high-resolution figures, can be found at:

<http://jn.physiology.org/cgi/content/full/103/3/1630>

Additional material and information about *Journal of Neurophysiology* can be found at:

<http://www.the-aps.org/publications/jn>

This information is current as of March 17, 2010 .

Effects of Unilateral Motor Cortex Lesion on Ipsilesional Hand's Reach and Grasp Performance in Monkeys: Relationship With Recovery in the Contralesional Hand

Mélanie Kaeser,^{1,2} Alexander F. Wyss,¹ Shahid Bashir,¹ Adjia Hamadjida,¹ Yu Liu,¹ Jocelyne Bloch,² Jean-François Brunet,² Abderaouf Belhaj-Saif,¹ and Eric M. Rouiller¹

¹Unit of Physiology and Program in Neurosciences, Department of Medicine, Faculty of Sciences, University of Fribourg, Fribourg; and
²Department of Neurosurgery, Neurosurgery Clinic, University Hospital of Lausanne, Lausanne, Switzerland

Submitted 27 May 2009; accepted in final form 11 January 2010

Kaeser M, Wyss AF, Bashir S, Hamadjida A, Liu Y, Bloch J, Brunet J-F, Belhaj-Saif A, Rouiller EM. Effects of unilateral motor cortex lesion on ipsilesional hand's reach and grasp performance in monkeys: relationship with recovery in the contralesional hand. *J Neurophysiol* 103: 1630–1645, 2010. First published January 13, 2010; doi:10.1152/jn.00459.2009. Manual dexterity, a prerogative of primates, is under the control of the corticospinal (CS) tract. Because 90–95% of CS axons decussate, it is assumed that this control is exerted essentially on the contralateral hand. Consistently, unilateral lesion of the hand representation in the motor cortex is followed by a complete loss of dexterity of the contralesional hand. During the months following lesion, spontaneous recovery of manual dexterity takes place to a highly variable extent across subjects, although largely incomplete. In the present study, we tested the hypothesis that after a significant postlesion period, manual performance in the ipsilesional hand is correlated with the extent of functional recovery in the contralesional hand. To this aim, ten adult macaque monkeys were subjected to permanent unilateral motor cortex lesion. Monkeys' manual performance was assessed for each hand during several months postlesion, using our standard behavioral test (modified Brinkman board task) that provides a quantitative measure of reach and grasp ability. The ipsilesional hand's performance was found to be significantly enhanced over the long term (100–300 days postlesion) in six of ten monkeys, with the six exhibiting the best, though incomplete, recovery of the contralesional hand. There was a statistically significant correlation ($r = 0.932$; $P < 0.001$) between performance in the ipsilesional hand after significant postlesion period and the extent of recovery of the contralesional hand. This observation is interpreted in terms of different possible mechanisms of recovery, dependent on the recruitment of motor areas in the lesioned and/or intact hemispheres.

INTRODUCTION

Following hemiparesis, for instance after a unilateral stroke affecting motor control, there is wide variability in the extent of recovery of motor control that depends on several parameters (e.g., the precise location and extent of the lesion, type and rapidity of intervention after the cerebral vascular accident, type of rehabilitative therapy; see, e.g., Jørgensen et al. 1999a; Masiero and Carraro 2008; Nudo et al. 2001; Oujamaa et al. 2009; van der Lee et al. 1999; Ward and Cohen 2004; Zemke et al. 2003). A cursory evaluation may lead to the prediction that, when there is good functional recovery of the

contralesional hand, the patient uses the hand affected by the lesion in a sustained manner. In contrast, when there is poor recovery of the contralesional hand, the patient relies more, if not exclusively, on the ipsilesional hand (unaffected by the lesion) to accomplish most tasks, thus possibly acquiring, through experience, enhanced capabilities in the ipsilesional hand, compared with the prelesion situation or with normal subjects (e.g., Cauraugh and Summers 2005; Jørgensen et al. 1999b; Liepert et al. 2000a; Nakayama et al. 1994).

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

The mechanisms that may underlie a contribution of the intact hemisphere to the functional recovery after unilateral lesion of the motor cortex are not well understood (e.g., Misawa et al. 2008; Netz et al. 1997; Swayne et al. 2008), especially with respect to its anatomical substrate (corticospinal projection and/or other, indirect pathways). The role played by the intact hemisphere may depend on the degree of paralysis of the affected hand as a result of the lesion in the opposite hemisphere. When the paralysis is significant, the intact hemisphere is likely to be more engaged in the compensation than

Address for reprint requests and other correspondence: E. M. Rouiller, Unit of Physiology, Department of Medicine, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland (E-mail: Eric.Rouiller@unifr.ch).

that in the case of more residual manual performance (Calautti and Baron 2003; Carey et al. 2005; Johansen-Berg et al. 2002; Serrien et al. 2004). Since the intact hemisphere normally and primarily controls the unaffected hand (referred to in the following text as the *ipsilesional hand*), depending on the intact hemisphere's contribution to the recovery of the affected hand, the performance of the ipsilesional hand is likely to be influenced. In the case where the intact hemisphere is strongly engaged in the recovery of the affected hand (especially when the paralysis is great), then it may be less available for its "normal" task of controlling the ipsilesional hand, thus resulting in a decrease of motor skill and motor learning ability with the ipsilesional hand.

Following this reasoning, we hypothesize that a permanent unilateral lesion of the motor cortex hand area in monkeys generates, as expected, a loss of manual skills in the contralesional hand, which is then followed by spontaneous, but incomplete, functional recovery. We further hypothesize that, depending on the extent of functional recovery of the contralesional hand, the performance of the ipsilesional hand may also be influenced over the long term (i.e., over a period of several months), considering the slow process of recovery. The aim of the present study was to test the hypothesis that the better the functional recovery of the contralesional hand following unilateral lesion of the motor cortex, the more proficient the ipsilesional hand over the long term, as observed several months postlesion. In the present study we assessed the motor performance of the ipsilesional hand not only during the weeks immediately following the lesion but for ≤ 308 days following the lesion (Table 1).

METHODS

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

Treatments

As outlined in Table 1, the 10 monkeys subjected to permanent unilateral lesion of the motor cortex were included in two pilot studies aimed at assessing the possible effect of two treatments: 1) anti-Nogo-A antibody treatment; and 2) cell therapy with injection of autologous adult progenitor cells, collected from the same animal in the prefrontal cortex (see Brunet et al. 2005). The anti-Nogo-A antibody treatment was tested on monkeys with motor cortex lesions because it was found to significantly enhance functional recovery and sprouting of corticospinal axons after cervical cord injury in macaques (Freund et al. 2006, 2007, 2009). The anti-Nogo-A antibody paradigm was tested on a subgroup of three monkeys (Mk-VA, Mk-SL, Mk-MO) and compared with a subgroup of four monkeys also subjected to a unilateral lesion of the motor cortex but that did not receive any treatment (Mk-CE, Mk-JU, Mk-GE, and Mk-RO; see Table 1). Three additional monkeys (Table 1) were included in the pilot cell therapy

TABLE 1. Factors related to monkeys subjected to permanent primary motor cortex lesion and included in the present study with identification code

Treatment	Mk-CE	Mk-JU	Mk-GE	Mk-RO	Mk-VA	Mk-SL	Mk-MO	Mk-AV	Mk-JO	Mk-JA
Age at time of lesion (rounded to 0.5 yr)	None	None	None	None	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Sham-cell therapy	Cell therapy	Cell therapy
Weight at time of lesion, Kg	4.5	5.0	5.0	4.0	5.5	5.5	5.5	3.5	3.5	4.0
Time window for prelesion assessment of manual dexterity, days	3.8	3.6	2.8	3.2	4.9	4.6	5.6	4.3	3.4	4.3
Time window for assessment of long-term deficit of ipsilesional hand post-lesion, days	99	128	70	29	119	77	29	20	29	60
Volume of ibotenic acid injected, μL	150–287	154–264	78–115	48–77	153–225	99–128	70–93	100–166	140–164	260–308
Number of ICMS sites injected with ibotenic acid	40	40	13	18	15.5	18	20	15	15	38*
Total volume of lesion (in mm^3)	21	21	13	12	11	11	20	10	10	38
Gray matter (motor cortex + postcentral gyrus)	112.8	63.01	48.7	14	20	78.2	41.8	33.2	33.6	22.2
Volume of lesion in postcentral gyrus	10.1	0.0	7.6	0.0	5.8	1.8	0.0	0.0	3.8	2.5
Long-term performance of ipsilesional hand in the modified Brinkman board task "Score" (percentage of prelesion score)	93.9%	92%	100%	124%	119.2%	100%	109.1%	138.5%	105.7%	128.3%

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

project: two monkeys (Mk-JO and Mk-JA) received an implantation of autologous adult brain progenitors cells in the vicinity of the cortical lesion, whereas one monkey (Mk-AV) served as a sham control animal (infusion of vehicle only). The present study does not address the issue of the efficacy of the treatments; the therapeutic effects of the two treatments on the contralesional hand will be reported elsewhere.

Behavioral assessment of manual performance

A major consequence of lesion of the hand representation in the motor cortex is the loss of manual dexterity, thus requiring appropriate behavioral tests focused on fine finger movements to track the functional recovery (for recent contributions also see Darling et al. 2009; Murata et al. 2008; Pizzimenti et al. 2007). Monkeys were trained to perform our “modified Brinkman board” test (e.g., Freund et al. 2006, 2009; Liu and Rouiller 1999; Rouiller et al. 1998; Schmidlin et al. 2004), which requires a reach and grasp motor sequence to retrieve small food pellets from wells while using the precision grip (opposition of the thumb and index finger). Food pellets were made of dried banana powder or glucose powder, compressed into a round shape of about 4 mm in diameter. This test of hand motor capacity was performed on a perspex board (10 × 20 cm) containing 50 randomly distributed slots, each filled with a food pellet at the beginning of the test. The dimension of the slots was 15 × 8 × 6 mm (length × width × depth). Twenty-five slots were oriented vertically and 25 slots horizontally. As outlined in detail in a recent report (Freund et al. 2009), retrieval from horizontal slots was more challenging because it required a postural adaptation of the hand (specifically a forearm rotation) in addition to the precision grip, whereas for the vertical slots, the precision grip can be performed with the hand in its natural posture (in pronation of the forearm). The monkeys were not food deprived: the pellets, which served as positive reinforcement during the tests, were the animals’ first access to food in the morning. At the end of the tests, the monkeys received additional food (cereals, fruits). The body weight of the monkeys was checked before each behavioral session. The monkeys had free access to water in the animal room.

Individual testing sessions typically lasted about 60 min, which included the time to transfer the monkeys to the primate chair and their transport to and from the animal room to the laboratory, as well as delivery of the additional food at the end of the session. An initial prelesion training phase was necessary to bring the monkeys to a stable level of performance that corresponded to a plateau in the reach and grasp score (represented by the red horizontal lines in Fig. 2A). The prelesion plateau, which was achieved within a time frame ranging from 20 to 128 days before the lesion, depending on the specific experimental protocol for each monkey (Table 1), was used to establish the median value of the prelesion score (Fig. 2A). Because the goal of the present study was to assess the long-term effect of the unilateral motor cortex lesion on the ipsilesional hand, the postlesion behavioral data were focused on a time window of several months (Table 1).

Monkeys performed the reach and grasp task first with one hand and then with the other hand, in two to five sessions per week during several months before and after the cortical lesion. For each daily session, the entire modified Brinkman board task was performed once with each hand, corresponding to 50 pellets retrieved by the left hand and 50 pellets retrieved by the right hand—in other words, 100 pellets in total when the monkey was successful for all slots (this was usually not the case for the contralesional hand following the lesion, due to a considerable deficit of manual performance). The temporal order in which each of the two hands was tested (left hand first or right hand first in a given session) was alternated on each consecutive behavioral session to avoid a possible bias toward one hand or the other. Testing one hand on the modified Brinkman board (retrieval of 50 pellets) took from 1 to 2 min. All tests were videotaped. In the present study, two parameters were assessed: 1) the retrieval score, defined as the

number of pellets successfully retrieved from the slots and brought to the mouth during the first 30 s of testing and established separately for the vertical and the horizontal slots; and 2) the contact time, defined as the time of contact (in seconds) between the fingers and the pellet. Specifically, the contact time corresponds to the time interval between the insertion of the first finger (usually the index) into the slot to contact the pellet and the retrieval of the pellet from the slot (grasped in between the index finger and the thumb), as previously reported (Freund et al. 2009). The contact time represents the amount of time it takes to retrieve a pellet from a slot and thus specifically reflects the manual dexterity. In the present study, the contact time was calculated for the first five vertical slots and the first five horizontal slots targeted by the monkey in an individual session. The manual reach and grasp task as performed on the modified Brinkman board can be seen on the following web page: <http://www.unifr.ch/neuro/rouiller/motorcontcadre.htm>.

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

Surgery

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

Electrophysiology: intracortical microstimulation

To guide the lesion procedure, electrophysiological intracortical microstimulation (ICMS) sessions were first performed to map the primary motor cortex (M1): a tungsten microelectrode (0.1–1 MΩ impedance; FHC, Bowdoinham, ME) was used to microstimulate M1, along penetrations performed at a distance of 1 mm from each other (see, e.g., Schmidlin et al. 2004, 2005). Along each electrode track, ICMS was applied below the surface of the dura mater at intervals of 1 mm. When the electrode penetration was located

slightly rostral to the central sulcus, ICMS effects were obtained along a distance of ≤ 10 –12 mm, along the rostral bank of the central sulcus, forming a band of gray matter perpendicular to the cortical surface (see red dashed line in Fig. 1*B*). When the electrode penetration was located more rostral, the cortical layers were oriented parallel to the cortical surface and the distance along which ICMS effects were present was shorter (≤ 4 –5 mm; see green dashed line in Fig. 1*B*). Depths of the ICMS sites were determined with the zero corresponding to the surface of the dura mater, which became progressively thicker with time (it was regularly scratched to facilitate the electrode penetrations every 3–4 wk). The effect of ICMS was assessed by visual inspection and/or palpation of the body part (articulation) where a movement was

elicited. The minimal current (ICMS threshold) producing this movement was determined at each stimulation site. The repeated ICMS electrode penetrations were performed for several weeks prelesion. The ICMS map as seen from the surface was finally represented in the form of an unfolded map of M1 (Supplemental Fig. S1),¹ as previously reported (Park et al. 2001, 2004) and served as the basis to guide injections of ibotenic acid to produce a permanent lesion of the motor cortex, targeting the hand area of M1 (see following text).

¹ The online version of this article contains supplemental data.

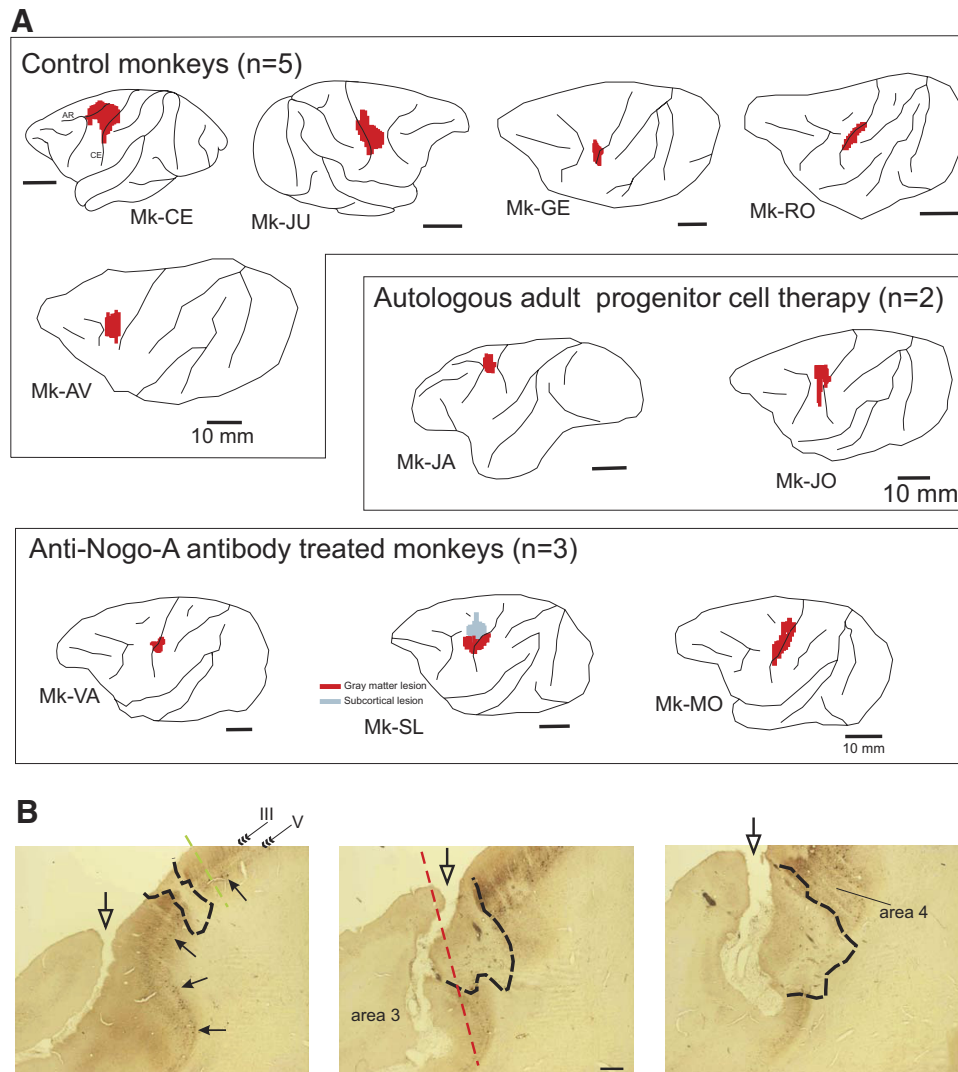


FIG. 1. *A*: location and extent of the permanent unilateral lesion of the hand representation in the motor cortex, as seen on corresponding lateral views of the brain for the 10 monkeys included in the present study (see Table 1). The lesion area, represented in red, was determined from the lesioned zone of cerebral cortex visible on consecutive frontal histological sections. The red area corresponds to a lesion affecting the gray matter. Spread of the lesion to the subcortical white matter below the gray matter is not represented, except in monkey Mk-SL in which a region of subcortical white matter was lesioned (gray spot), in a zone located medial to the red territory. The motor cortex lesion was performed in the left hemisphere for all monkeys, except in Mk-JU in which the lesion was in the right hemisphere. Of the 10 monkeys, 5 (*top*) were control animals for 2 pilot treatment studies, 3 were treated with anti-Nogo-A antibody (*bottom*), and 2 were subjected to an autologous adult progenitor cell therapy (see METHODS and Table 1). *B*: SMI-32 stained frontal sections of the left hemisphere of Mk-VA (the most rostral section is on the *left*). The interval between the left and the middle sections is 1 mm and 0.5 mm between the middle and the right sections. The open arrows point to the central sulcus, separating area 3 on the *left* from area 4 on the *right*. In area 4, SMI-32 stains the pyramidal cells in layers III and V (triple-headed arrows on the *left*). The 4 black arrows point to typical large pyramidal cells in layer V. The area of the lesion in the gray matter is delineated on each section by the dashed line. The red dashed line schematically represents the trajectory of a fictive intracortical microstimulation (ICMS) electrode penetration, showing that some sites in layer V with low ICMS thresholds are indeed located deep, near the fundus of the rostral bank of the central sulcus. The green dashed line schematically represents the trajectory of a fictive electrode penetration in the primary motor cortex (M1), located more rostral to the central sulcus. Scale bar = 1 mm.

Permanent lesion of M1 hand representation with ibotenic acid

On the day of ibotenic acid injections, selected electrode penetrations were repeated to verify the ICMS effects and the precise depths at which ibotenic acid would be injected. As reported earlier (Schmidlin et al. 2005), there was good reproducibility of the ICMS data derived from electrode penetrations performed several weeks apart. Each site selected for ibotenic acid injection corresponded to a locus where ICMS produced a movement of the digits at low threshold and thus included the hand area of M1. Typically, along a penetration such as that represented by the red dashed line in Fig. 1B, three sites were selected for ibotenic acid injection (at depths of 3, 6, and 9 mm), whereas along a more rostral penetration (green dashed line in Fig. 1B), a single site was selected at the depth of layer V (which exhibited the lowest ICMS threshold).

Ibotenic acid (10 $\mu\text{g}/\mu\text{l}$ in phosphate buffer) was infused using a Hamilton microsyringe at selected ICMS sites of the hand area in M1 unilaterally, as previously reported in detail (Liu and Rouiller 1999). The number of ICMS sites injected and the total volume of ibotenic acid infused in M1 are indicated for each monkey in Table 1. The unilateral lesion was performed in the left hemisphere, except in Mk-JU (Fig. 1A). After a delay of several minutes, the ibotenic acid infusion produced a significant paralysis in the contralesional hand.

Data analysis

Within the pre- and postlesion time frame of behavioral analysis defined for each monkey (see Table 1), the pellet retrieval score was plotted as a function of time in days (e.g., Fig. 2A). The prelesion period was used to establish the reach and grasp performance of reference, indicated by the median value (red horizontal lines in Fig. 2A). Postlesion, behavioral sessions were conducted for several months (e.g., Fig. 2A). To assess the long-term effects of unilateral lesion of the motor cortex on the hand's reach and grasp capacity, the long-term score was also represented by its median value (green horizontal lines in Fig. 2A). Finally, the comparison of the two median values (prelesion vs. long-term postlesion) allowed a quantitative assessment of the effect of the lesion several months thereafter. The behavioral data were analyzed statistically using an unpaired nonparametric Mann-Whitney test. A similar analysis was conducted on the second behavioral parameter, the contact time. The prelesion data used to address the issue of hand dominance (see *Hand dominance for the modified Brinkman board?* in RESULTS) were analyzed using a paired comparison of daily scores obtained for the left hand and the right hand (paired *t*-test or Wilcoxon test). The statistical analysis and related graphs were obtained using the software SigmaStat 3.5 and SigmaPlot 10.0.

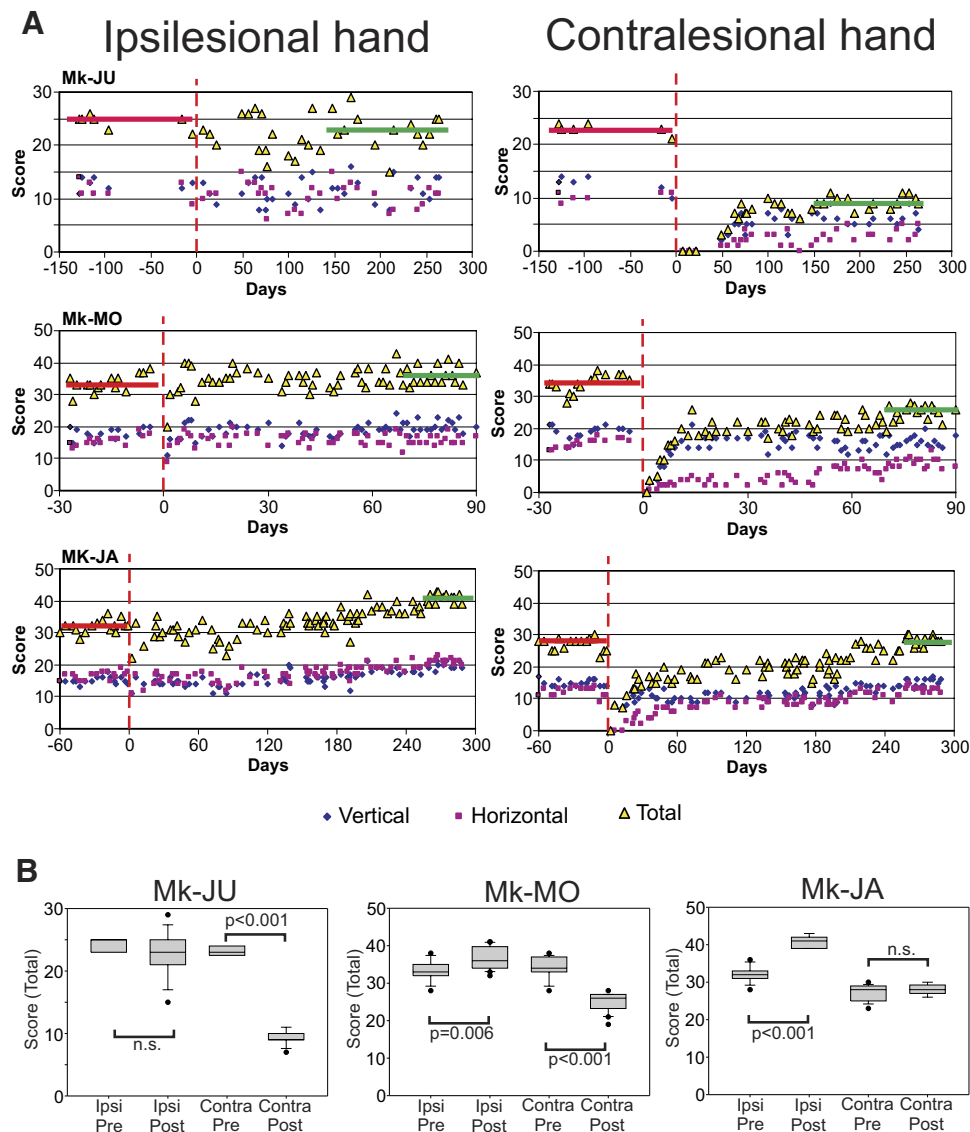


FIG. 2. *A*: number of pellets retrieved in 30 s as a function of time (days) in the modified Brinkman board task for 3 monkeys (Mk-JU, top; Mk-MO, middle; and Mk-JA, bottom). The numbers of retrieved pellets (scores) are indicated separately for the vertical wells (blue diamonds) and the horizontal wells (red squares); the yellow triangles represent the total score determined by summing the vertical and horizontal scores. The vertical red dashed line at time 0 is the day of the unilateral motor cortex lesion (prelesion days are negative and postlesion days are positive). Prelesion, the median total score is given by the red horizontal line (plateau); postlesion, the median long-term total score is given by the green horizontal line (plateau). Prelesion, sessions took place earlier than the first day indicated on the abscissa, but the animals' performance had not yet reach a stable plateau. Because Mk-JU was generally slower than the other 2 monkeys (fewer pellets collected in 30 s), to preserve resolution of the individual data points. *B*: for the same 3 monkeys as in *A*, the total score is represented in the form of box and whisker plots for the ipsilesional hand (2 left boxes) and the contralesional hand (2 right boxes), allowing comparison of the pre- and postlesion performances for each hand. In the box and whisker plot, the boundary of the box closest to zero corresponds to the 25th percentile, the line within the box is for the median value, and the boundary of the box farthest from zero is for the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Black dots are for outlying points. The result of the statistical comparison pre- vs. postlesion (Mann-Whitney test) is indicated with the corresponding *P* value (n.s., not statistically significant at $P > 0.05$).

At the end of the experiments, the animals were sacrificed with an intraperitoneal overdose of pentobarbital sodium (90 mg/kg body weight). Transcardiac perfusion with 0.9% saline (500 ml) was followed by fixative (4,000 ml of 4% phosphate-buffered paraformaldehyde). The brains were placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection for 3–5 days. Frontal sections (50 μ m thick) of the brain were prepared and collected in five series. One series of sections was Nissl stained with cresyl violet, whereas a second series was processed to visualize the marker SMI-32, as previously described (Beaud et al. 2008; Liu et al. 2002; Wannier et al. 2005). The epitope recognized by the SMI-32 antibody lies on nonphosphorylated regions of neurofilament protein and is expressed only by specific categories of neurons (Campbell and Morrison 1989; Tsang et al. 2006). The two series of sections were then used to reconstruct on consecutive sections the position and extent of the permanent lesion in the cerebral cortex, especially using the SMI-32 stained sections (Fig. 1B), on which the pyramidal neurons in layers III and V are clearly visible. Finally, the lesion was transposed onto a lateral view of the cortical surface of the lesioned hemisphere (Fig. 1A). Using an ad hoc function of the NeuroLucida software (based on the Cavalieri method; see, e.g., Pizzimenti et al. 2007), the volume of the cortical lesion (in mm^3) affecting the cortical gray matter was extrapolated from the reconstructions of the lesion on consecutive histological sections of the brain (see Table 1).

RESULTS

Unilateral lesion of the motor cortex

The unilateral lesion of the motor cortex was produced by infusion of ibotenic acid at multiple sites defined by intracortical microstimulation (ICMS; see Supplemental Fig. S1). Most ICMS sites selected for infusion of ibotenic acid were located in the rostral bank of the central sulcus, where most of the hand is represented in the primary motor cortex (Supplemental Fig. S1; Fig. 1B, *middle* and *right*). Because ibotenic acid was also injected at a few sites more rostrally, the lesion also extended onto the part of the motor cortex at the brain surface (Fig. 1B, *left*). The infusion of ibotenic acid at multiple sites did not produce a uniform lesion, but rather several distinct zones, the areas of which were added to compute the total volume of the lesion in the gray matter (Table 1). The total volume of the lesion is used to correlate with the behavioral data to assess the effect of the lesion size.

The aim of our motor cortex lesions was to permanently inactivate the M1 hand area. The lesion extent was variable from one monkey to another (red areas in Fig. 1A), corresponding in most animals to an extent of 4–5 mm on surface views of the brain (Fig. 1A). The lesion area is consistent with the known size of the hand area in macaque monkeys. However, in a few monkeys, along one dimension or another, the lesion extended further, with the largest lesions extending ≤ 10 mm. In one monkey (Mk-SL), the lesion spread medially to the subcortical white matter (Fig. 1A). There was also limited, but lesser, subcortical damage of the white matter in some of the other monkeys (Table 1) and the damage remained below the gray matter injury (and is therefore not apparent on the brain surface views in Fig. 1A). In some monkeys, the lesion spread to adjacent areas, including the premotor cortex and/or the somatosensory cortex (S1), as indicated in Table 1 for the latter area. The impact of the lesion spread in premotor cortex and/or S1 is considered in the DISCUSSION.

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

The number of pellets retrieved by the monkey in 30 s from the modified Brinkman board is shown in detail for three representative monkeys (Fig. 2A: Mk-JU, Mk-MO, and Mk-JA), separately for the vertical and the horizontal slots, as well as a total score representing the sum of both slot orientations.

First, focusing on the total number of pellets retrieved, Mk-JU achieved a stable (plateau) prelesion retrieval score about 130 days before the lesion. This monkey's median prelesion score for the ipsilesional hand was 25 pellets and for the contralesional hand 23 pellets. This monkey's behavioral assessment continued for 264 days after the lesion. A long-term (154 to 264 days after the lesion) postlesion median score of 23 pellets was obtained for the ipsilesional hand, which represents a manual performance of 92% of the prelesion score. This pre- and postlesion difference for the ipsilesional hand in Mk-JU was not statistically significant (Fig. 2B). Note, however, that the contralesional hand of Mk-JU recovered only incompletely, given that the postlesion retrieval score (median value = 9 pellets) represented only 39% of the prelesion score, a pre- versus postlesion difference that was highly significant ($P < 0.001$; Fig. 2B).

Second, in Mk-MO, the median prelesion retrieval score was 33 pellets for the ipsilesional hand and 34 pellets for the contralesional hand. Behavioral sessions ended 95 days after the lesion. The long-term postlesion retrieval score (last 23 days on the plots in Fig. 2A) showed an enhanced score for the ipsilesional hand (median value of 36 pellets) compared with the prelesion value (thus representing 109% of the prelesion score). This pre- versus postlesion difference for the ipsilesional hand in Mk-MO was statistically significant ($P = 0.006$; Fig. 2B). For the contralesional hand of Mk-MO, recovery was again incomplete with a median postlesion retrieval score of 26 pellets, representing 76% of the prelesion score (the pre- vs. postlesion difference was statistically significant for the contralesional hand as well, but in the other direction: $P < 0.001$; Fig. 2B).

Third, Mk-JA reached a plateau in performance 60 days prelesion, with a median retrieval value score of 32 pellets for the ipsilesional hand and 28 pellets for the contralesional hand (Fig. 2A). Behavioral data were acquired for 290 days postlesion. For Mk-JA, the long-term ipsilesional hand performance was dramatically enhanced, 128% that of prelesion performance, reaching a median value of 41 pellets ($P < 0.001$; Fig. 2B). For this monkey (Mk-JA), the contralesional hand recovered completely from the lesion, achieving a postlesion retrieval score of 28 pellets, the same score as prelesion (thus representing 100% of recovery; Fig. 2B).

The data shown in Fig. 2 for three representative monkeys suggest that, when the contralesional hand recovered well from the lesion (e.g., Mk-JA), the long-term postlesion performance in the ipsilesional hand was enhanced postlesion over the long term. In contrast (Mk-JU), in the case of poor recovery of the contralesional hand, the manual performance in the ipsilesional hand is not affected, maintaining a level of performance that is close to or slightly worse than the prelesion performance. In between these two extreme cases (Mk-JU and Mk-JA), in the monkey with an intermediate recovery of the contralesional hand (Mk-MO), the ipsilesional hand also exhibited enhanced

long-term postlesion performance, but to a somewhat lesser degree than that in Mk-JA, although the pre- versus postlesion difference was nevertheless statistically significant. As shown in Fig. 2A, the preceding observations for the total retrieval scores also hold true when considering either the vertical slots or the horizontal slots separately.

The general trend for the three monkeys shown in Fig. 2 was found to be true when considering the other seven monkeys included in the present study (Fig. 3). Four of the seven monkeys in Fig. 3 also showed a long-term enhancement of manual performance (total score) in the ipsilesional hand (Mk-VA, Mk-RO, Mk-JO, Mk-AV), as evidenced by a better postlesion than prelesion retrieval score. In the other three monkeys (Mk-SL, Mk-GE, Mk-CE), the long-term performance in the ipsilesional hand remained at the same level of performance as that of prelesion (Fig. 3). Note that the latter three monkeys exhibited relatively incomplete contralesional recovery of their manual dexterity postlesion, compared with the relatively better recovery of the contralesional hand in the other four monkeys (Fig. 3).

Overall (Figs. 2 and 3), long-term postlesion enhancement of reach and grasp performance in the ipsilesional hand was found in six of ten monkeys, as assessed by the total retrieval score in the modified Brinkman board task. In these six monkeys, this enhancement was associated with relatively good recovery of the contralesional hand. To better analyze the dependence between the two hands, the long-term postlesion manual performance in the ipsilesional hand (expressed in % of prelesion score) was plotted as a function of the percentage of recovery of the contralesional hand (Fig. 4). There is a strong correlation between these two parameters, with a coefficient of correlation

$r = 0.932$ ($P < 0.001$), consistent with the notion that, after unilateral lesion of the motor cortex, a good recovery with the contralesional hand is associated over the long term with an enhancement of manual performance in the ipsilesional hand.

The above-cited data are based on an analysis of manual performance as assessed by the total retrieval score (sum of vertical and horizontal slots) in the modified Brinkman board task. Because the synergy of movements is somewhat different for the vertical and horizontal slots (Freund et al. 2009), it is of interest to analyze the same data considering the vertical and horizontal slots separately (Figs. 5 and 6; Supplemental Figs. S2 and S3). For the three representative monkeys (Mk-JU, Mk-MO, and Mk-JA depicted in Fig. 5), the separate data for vertical and horizontal slots are consistent with the total retrieval score data for two monkeys (Mk-JU and Mk-JA; Fig. 2B). For Mk-MO, the vertical slot data led to the same conclusion as that of the total score data. Interestingly, contralesional hand performance for Mk-MO was poor when retrieving pellets from the horizontal slots, which was associated with insignificant long-term enhancement of manual performance in the ipsilesional hand (Fig. 5). In other words, in Mk-MO considering the vertical and horizontal slots separately, the data are consistent with the notion of enhancement of ipsilesional performance only if recovery of the contralesional hand is complete or at least substantial.

As for the total retrieval score, analysis of vertical and horizontal slots separately revealed a correlation between long-term reach and grasp performance in the ipsilesional hand and the percentage of recovery of the contralesional hand, although the correlation was less pronounced than that for the total score (Fig. 6). Nevertheless, the correlation was statistically signifi-

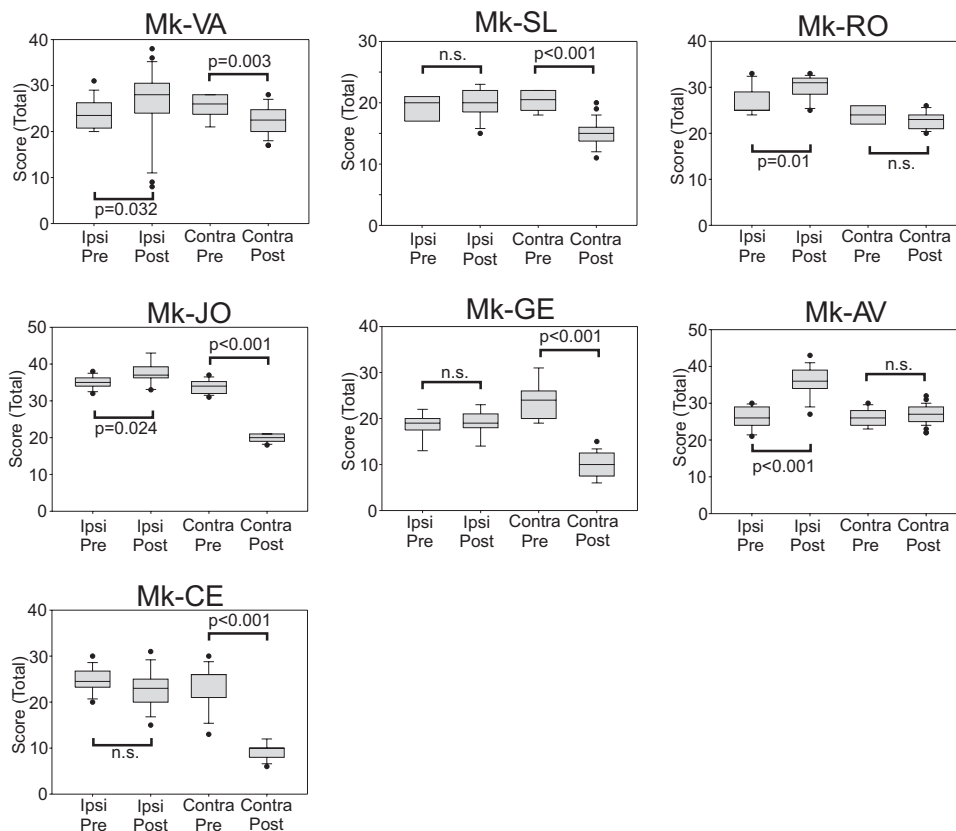


FIG. 3. Box and whisker plots (as in Fig. 2B) for the other 7 monkeys included in the study, showing the total score in the ipsilesional hand (2 left boxes) and in the contralesional hand (2 right boxes) for comparison of the pre- and postlesion scores for each hand. As in Fig. 2, note that the maximal value of the ordinate is not the same for all monkeys, to provide maximal resolution for the comparison of the median pre- and postlesion values for each hand. The result of the statistical comparison (Mann-Whitney test) is indicated with the corresponding P value (n.s., not statistically significant with $P > 0.05$).

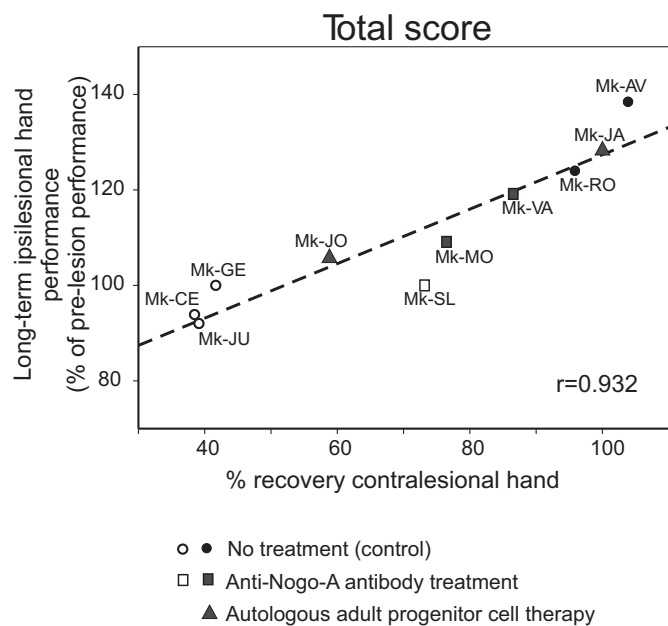


FIG. 4. Long-term ipsilesional hand performance (expressed in % of the prelesion total score in the modified Brinkman board task) was plotted as a function of the percentage of recovery in the contralesional hand (compared with the prelesion total retrieval performance). The dashed line is the regression line representing a significant correlation between these 2 parameters ($r = 0.932$). The different symbols distinguish the 3 subgroups of monkeys. Filled symbols are for the 6 monkeys exhibiting significant long-term enhancement of ipsilesional hand performance (see Figs. 2B and 3), whereas there was no enhancement in the 4 monkeys represented by open symbols.

cant for both the vertical slots ($P < 0.01$) and the horizontal slots ($P < 0.05$).

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

The cited data indicate that the long-term enhancement of manual performance in the ipsilesional hand is strongly correlated with the degree of recovery for the contralesional hand (Figs. 4 and 6). One may wonder whether the same parameter is correlated with the extent of the motor cortex lesion. Manual performance in the ipsilesional hand assessed over the long

term (expressed as percentage of the prelesion score) was plotted as a function of the volume of the lesion, expressed in cubic millimeters, encompassing primarily the gray matter in M1 and, to a lesser extent, gray matter in S1 in some monkeys (see also Table 1). As shown in Fig. 7 (top), there is a significant inverse correlation ($r = -0.735$; $P < 0.01$) between the long-term performance in the ipsilesional hand and the volume of the lesion (in the motor cortex and S1). Clearly, the six monkeys with a significant long-term enhancement of manual performance of the ipsilesional hand (filled symbols in Fig. 7, top) had a smaller lesion than that of the other four monkeys. For a more comprehensive description of the relationship between the three relevant parameters, the bottom panel of Fig. 7 shows a three-dimensional plot of the long-term performance in the ipsilesional hand versus the percentage of recovery of the contralesional hand and the volume of the cortical lesion.

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

The pellet retrieval score data take into account the entire sequence of movements to collect the pellets (including reaching, withdrawing). In contrast, the contact time parameter is restricted to the time of contact between the fingers and the pellet while it is in the slot (the retrieval time). The contact time specifically reflects the grasping capability during execution of the precision grip and thus may be a more precise measure of manual dexterity. The contact time was measured during each session for the first five vertical slots and the first five horizontal slots. The data were then cumulated for the prelesion plateau period and for the long-term postlesion period during the same time windows as for the pellet retrieval score data (Table 1). The contact time data are presented similar to the pellet retrieval score data (see Fig. 5 and Supplemental Figs. S1 and S2), in the form of box and whisker plots and analyzed statistically using the Mann–Whitney test (Supplemental Figs. S4 and S5).

The median contact time for the contralesional hand was largely in line with the retrieval score data. The majority of monkeys for which the long-term retrieval score for the contralesional hand remained significantly lower postlesion, com-

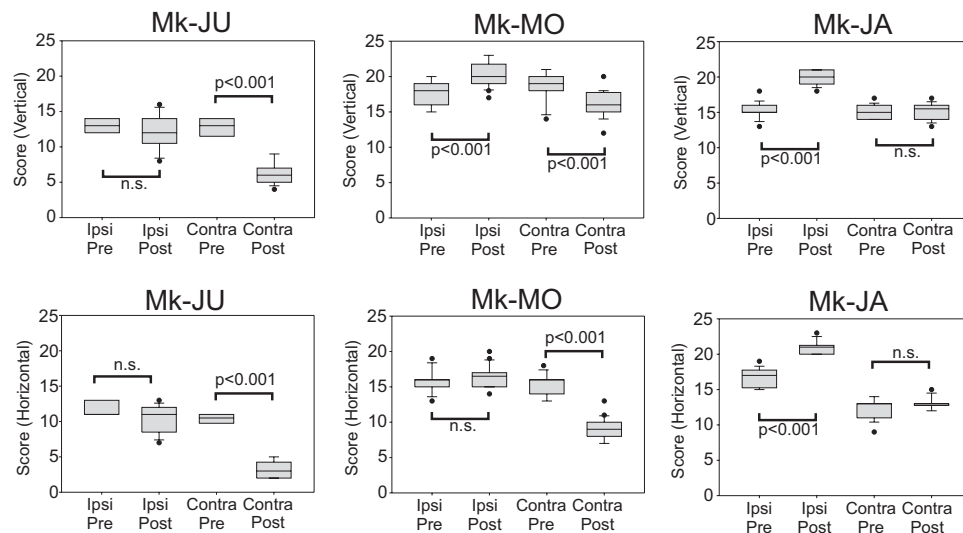


FIG. 5. Same data as in Fig. 2B (Mk-JU, Mk-MO, and Mk-JA), but for the 2 slot orientations (vertical and horizontal) analyzed separately.

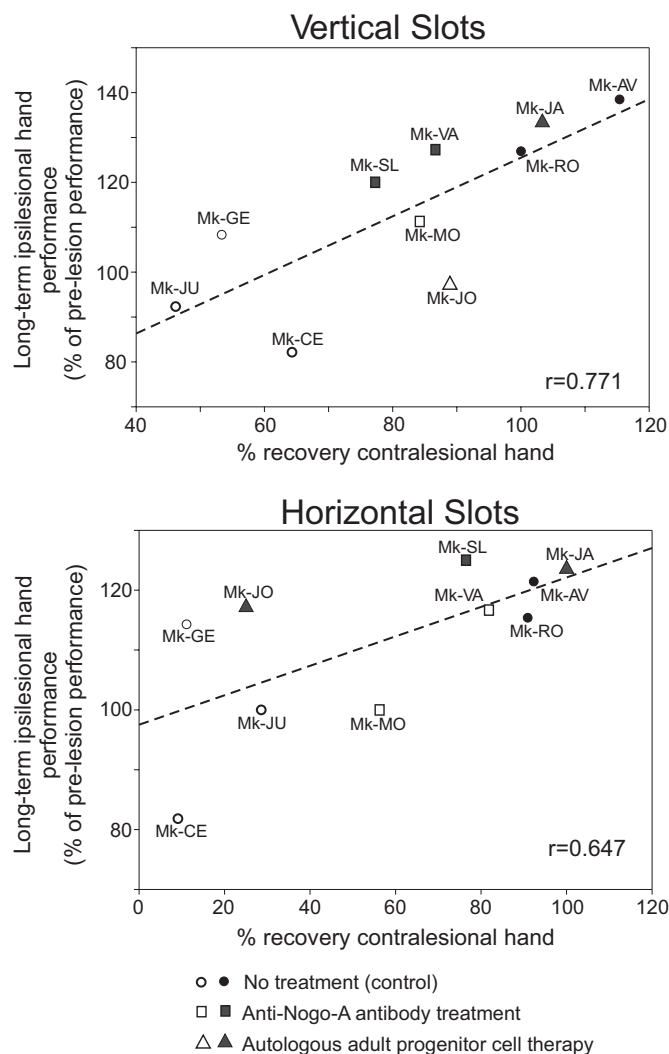


FIG. 6. Same data as in Fig. 4 (correlation between long-term postsilesional ipsilesional hand performance and percentage recovery of the contralesional hand) for the 2 slot orientations (vertical and horizontal) analyzed separately. Filled symbols are for the monkeys exhibiting significant long-term enhancement of ipsilesional hand performance (see Fig. 5 and Supplemental Figs. S1 and S2), whereas there was no enhancement for the ipsilesional hand in the monkeys represented by open symbols.

pared with prelesion, exhibited a consistent long-lasting increase in contact time (i.e., more time was needed to grasp the pellet). This was true for the vertical slots for Mk-MO, Mk-JU, Mk-SL, Mk-JO, Mk-CE, and Mk-GE (Supplemental Fig. S4), whereas for the horizontal slots, this was true for Mk-JU, Mk-SL, Mk-JO, Mk-GE, and Mk-CE (Supplemental Fig. S5). One monkey (Mk-VA) showed contact times postlesion that did not increase or even decreased, compared with prelesion, inconsistent with a poor postlesion pellet retrieval score over the long term. The three monkeys (Mk-JA, Mk-RO, and Mk-AV) with a complete recovery of retrieval score (>95%) for the contralesional hand exhibited a contact time that was not statistically different pre- versus postlesion or was even shorter postlesion (Supplemental Figs. S4 and S5).

With respect to the ipsilesional hand, the contact time data showed less difference between the prelesion and the long-term postlesion periods than did the retrieval score data. An en-

hancement of postlesion ipsilesional hand manual dexterity over the long term, evidenced by a decrease in contact time, was observed in four monkeys for the vertical slots (Mk-JA, Mk-RO, Mk-VA, and Mk-CE; Supplemental Fig. S4) and in two monkeys for the horizontal slots (Mk-VA and Mk-JO; Supplemental Fig. S5). As observed for the retrieval score data (Fig. 6), there was also a correlation between the contact time observed over the long term for the ipsilesional hand and the extent of recovery of contact time for the contralesional hand (Supplemental Fig. S6), for both the vertical slots ($r = 0.579$) and the horizontal slots ($r = 0.349$). However, these correlations for the contact time were only a trend because they were not statistically significant ($P > 0.05$).

Differences with clinical studies

In the present study, each monkey was able to serve as its own control by comparing the prelesion manual score with the postlesion score, a very sensitive approach that allows the detection of moderate differences between pre- and postlesion performances, as presented here for the ipsilesional hand (Figs. 2B and 3). Is such long-term enhancement of the ipsilesional hand performance detectable in a clinical study, devoid of available prelesion data for the patients (e.g., for instance after a cortical lesion)? Clinical studies rely on group comparisons, intact subjects versus lesioned patients. To address this issue (Fig. 8), the postlesion ipsilesional total retrieval score over the long term in the group of 10 monkeys included in the present study was compared with a different group of 12 intact monkeys (before they were subjected to spinal cord injury [SCI]; see Freund et al. 2006, 2007). As shown in Fig. 8 (left part of the plot), the variability of manual performance as assessed by the total retrieval score in the modified Brinkman board across 12 intact monkeys was substantial. Plotting on the same graph the long-term ipsilesional total retrieval score observed postlesion for the 10 monkeys included in the present study (Fig. 8, right) yields complete overlap between the two groups, preventing statistical detection of the long-term enhancement of motor performance in the ipsilesional hand in the group of 10 monkeys subjected to the motor cortex lesion (Mann-Whitney test, n.s., $P = 0.241$).

Hand dominance for the modified Brinkman board?

The data presented in Fig. 8 are also pertinent to address the issue of whether intact monkeys have a dominant hand when performing the modified Brinkman board task, as assessed by the total retrieval score. In other words, is prelesion performance different for the left hand versus that for the right hand? Comparing the total number of pellets retrieved for the 12 intact monkeys shown in the left panel of Fig. 8 reveals that there was no significant difference in left hand versus right hand performances for 9 of the 12 monkeys (paired t -test or Wilcoxon test: $P > 0.05$; range: 0.088–0.885). In the other three intact monkeys, the prelesion total retrieval score was significantly higher for one hand compared with that for the other hand ($P < 0.05$), with a better score for the right hand in two monkeys and for the left hand in one monkey. Comparing the total number of pellets retrieved prelesion by the left or the right hand in the group of 10 monkeys included in the present study was

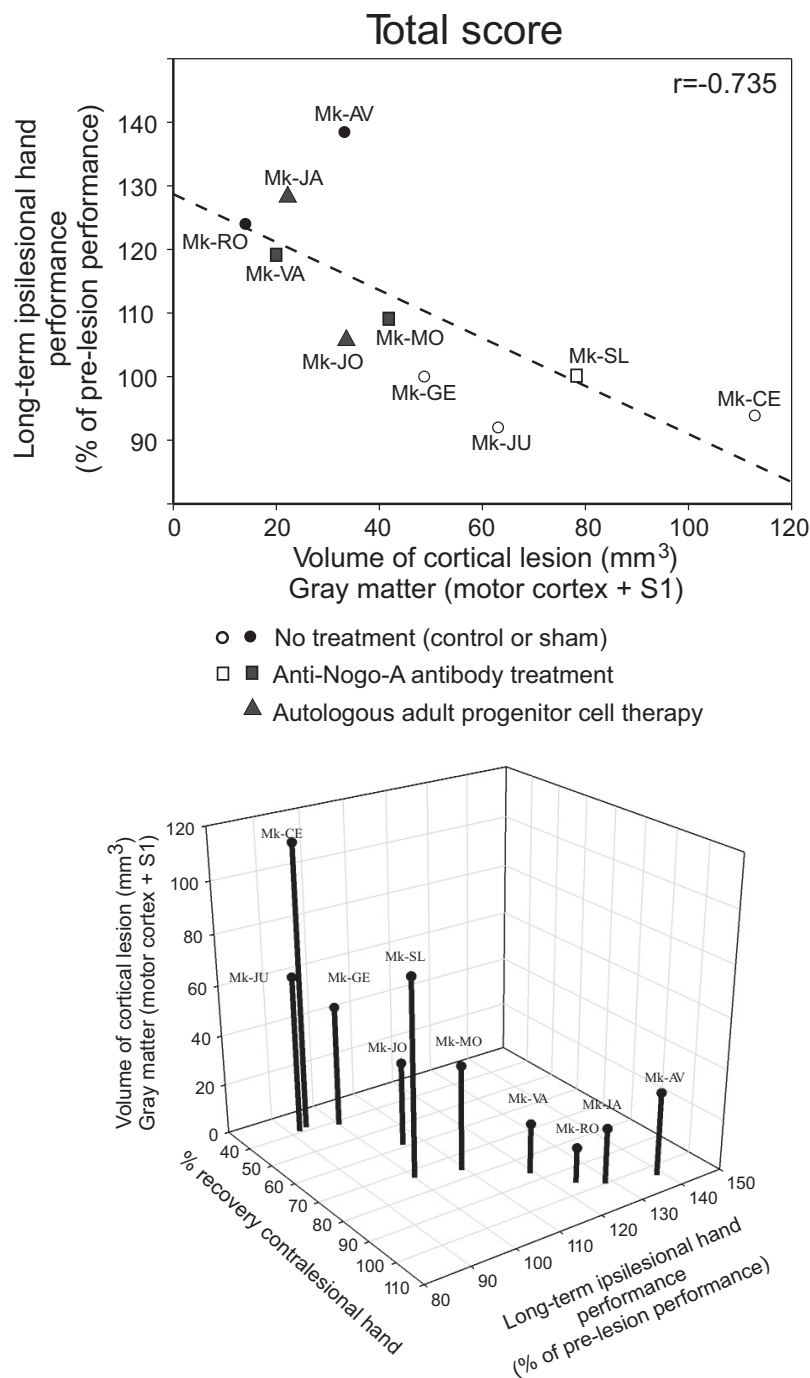


FIG. 7. *Top*: the postlesion long-term ipsilesional hand performance (compared with prelesion) is plotted as a function of the volume of the motor cortex lesion (corresponding to the volume of gray matter affected by the lesion in the motor cortex and in the primary somatosensory cortex; see Table 1 for more details). The dashed line indicates a statistically significant inverse correlation between these 2 parameters ($P < 0.01$). *Center inset box* gives the symbol code referring to the 3 groups of monkeys (see text). Filled symbols are for the monkeys exhibiting a significant long-term enhancement of ipsilesional hand performance (see Figs. 2 and 3). Open symbols are for the monkeys without such enhancement of ipsilesional hand performance. *Bottom*: 3-dimensional summary representation of the data of the 3 parameters analyzed in the 10 monkeys, the long-term retrieval performance in the ipsilesional hand, the percentage of recovery of the contralesional hand, and the volume of the lesion. The coefficient of correlation between the “long-term ipsilesional hand performance” and the “% recovery contralesional hand” is indicated on Fig. 4. The coefficient of correlation between the “long-term ipsilesional hand performance” and the “volume of motor cortex lesion” is indicated on the *top panel*. The coefficient of correlation between “% recovery contralesional hand” and the “volume of motor cortex lesion” is $r = -0.698$.

consistent with this general trend: three monkeys (Mk-JU, Mk-JA, and Mk-GE; see Figs. 2 and 3) exhibited a statistically significant difference between the left and the right hands ($P < 0.05$), whereas in the other 7 monkeys there was no significant difference (the P value was >0.05 , ranging from 0.108 to 0.898). In the 3 monkeys exhibiting hand dominance prelesion, 2 monkeys had a better score for the left hand and one for the right hand. In summary, in a total population of 22 monkeys, only 6 animals exhibited hand dominance (3 for the left hand and 3 for the right hand). It can thus be concluded that, for the modified Brinkman board task, there was no clear and systematic hand dominance, at least as revealed by the total retrieval score.

DISCUSSION

Based on the retrieval score data and the contact time data, but to a lesser extent for the latter (see following text), the results of the present study are consistent with our hypothesis that, after unilateral motor cortex lesion, long-term manual performance in the ipsilesional hand covaries with the extent of postlesion recovery of the contralesional hand. To the best of our knowledge, this is an original observation because most previous studies on unilateral motor cortex lesions focused on the recovery of the contralesional hand and the behavioral assessment was limited to the period immediately following the lesion until a performance plateau was reached. The ipsile-

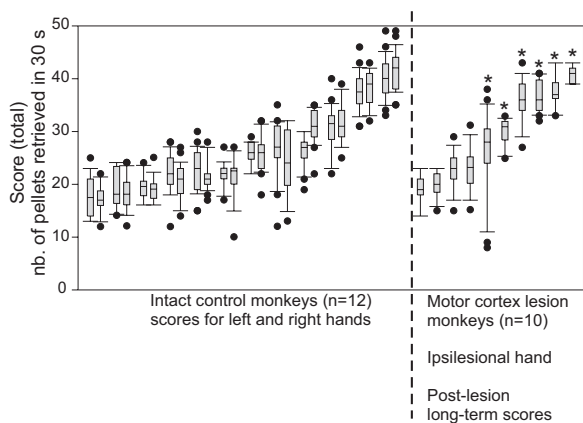


FIG. 8. Box and whisker plots showing the total number of pellets retrieved (score) in the modified Brinkman board task for 2 groups of monkeys. On the left, the performance is shown for a group of intact control monkeys (in fact prelesion score of monkeys subjected later on to spinal cord injury [SCI]; see text). In the control group, performance is shown for each hand for each monkey (box plots are grouped by 2 for each monkey), allowing between-hand comparison. The group of monkeys on the right consists of the 10 monkeys included in the present study with their postlesion total score over the long term in the ipsilesional hand only. The stars point to the monkeys that were characterized by a statistically significant enhancement of manual performance for the ipsilesional hand over the long term (see Figs. 2B and 3).

sional effect observed here appeared, in some cases, only several months postlesion, although there is no systematic relationship between the extent of enhancement of manual performance and the time frame in which it occurs (Table 1).

As expected, the extent of recovery in the contralesional hand is inversely correlated with the lesion volume (Fig. 7, bottom). Because the manual performances of the contralesional and ipsilesional hands are positively correlated (Fig. 4), it follows that the enhancement of manual performance in the ipsilesional hand is negatively correlated with the lesion size (Fig. 7, top). This result contrasts with the observation in rats of a postlesion facilitation of motor skill learning in the nonaffected hand, an augmentation that parallels increasing lesion size, within a certain range, and as observed 20 days postlesion (Allred and Jones 2004). This discrepancy may be related to the different time points (i.e., several months postlesion in our monkeys) and the very different organization of the corticospinal system between rodents and primates.

The long-term enhancement of manual performance in the ipsilesional hand was found in six of ten monkeys, specifically those exhibiting the best recovery in the contralesional hand. In the other four monkeys, there was no such enhancement or even a decrease in manual performance in the ipsilesional hand, over the long term. For example, a decrease in ipsilesional manual performance was observed in the two monkeys with the largest lesions of the motor cortex (Mk-CE and Mk-JU; see Fig. 4). Data from these two monkeys are thus consistent with data in humans, in which a unilateral lesion of the motor cortex leads to a deficit of manual performance in the ipsilesional hand, although different motor parameters were affected depending on which hemisphere was lesioned (Hermsdörfer and Goldenberg 2002; Hermsdörfer et al. 1999a,b).

Limitations of interpretation

The interpretation of our results concerning a covariation between the long-term extent of recovery in the contralesional

hand and manual performance in the ipsilesional hand after a unilateral lesion of the motor cortex may be limited by confounding factors. The study comprises multiple variables, raising some uncertainties about the interpretation of this main finding. In particular, the protocol was disparate to some extent between monkeys—for example, the time windows of behavioral assessment and long-term follow-up period, the lesion size, the precise position of the lesion, as well as the type of treatment. The limited number of monkeys in each group prompted a pooling of all animals, to allow a correlation on a sufficiently large number of data points ($n = 10$). Indeed, the serious ethical concerns for the use of nonhuman primates in research limit the design of studies based on large groups of animals. One obvious limitation of interpretation of the present study is that the five untreated animals represent extreme values (Fig. 4). Ideally, a study conducted on a larger pool of untreated monkeys only may have produced more reliable data, although a constraint with the control monkeys is that, above a certain volume of lesion (40 mm^3 ; see Fig. 7), the extent of recovery was largely incomplete ($\sim 40\%$). In the present study, as a result of the two treatments (anti-Nogo-A antibody; autologous progenitor cell therapy), some monkeys with a fairly large lesion exhibited a substantial recovery, clearly $>40\%$ (Mk-SL, Mk-MO, Mk-JO). A possible direct effect of the treatments on the enhancement of manual performance in the ipsilesional hand over the long term after a unilateral lesion of the motor cortex is difficult to evaluate. Among the treated monkeys ($n = 5$), two animals showed a marked enhancement of manual performance with their ipsilesional hand, whereas the other three treated monkeys did not (this depends on the slot orientation; Figs. 4 and 6). Thus there is apparently no systematic relationship between long-term manual performance in the ipsilesional hand and the presence or absence of treatment. Both the extent of functional recovery in the contralesional hand and the manual performance in the ipsilesional hand over the long term appear to be more dependent on the lesion size than on the treatments applied to some of the monkeys. The bottom panel of Fig. 7 emphasizes the interdependence between the three parameters (extent of recovery in the contralesional hand; manual performance in the ipsilesional hand over the long term; volume of cortical lesion), as well as the limitations of interpretation due to the presence of multiple variables in the present study.

Spread of the lesion to cortical areas adjacent to M1

Although our lesions targeted M1 (see Supplemental Fig. S1), they sometimes spread into adjacent cortical areas, such as premotor cortex (PM: Mk-CE, Mk-JU, Mk-AV, Mk-JA, Mk-SL) or postcentral in the somatosensory cortex (Mk-CE, Mk-GE, Mk-VA, Mk-SL, Mk-JO, Mk-JA). As quantified for the postcentral gyrus (Table 1), the spread of the lesion into the somatosensory cortex was generally limited. However, what is the impact of the lesion's spread in PM or in the postcentral gyrus on the present data? In an intact monkey, reversible inactivation of PM had no effect on reach and grasp manual tasks (Kermadi et al. 1997; Liu and Rouiller 1999). However, it may be different in a monkey subjected to a lesion affecting mainly M1, given that PM and the somatosensory cortex contribute to functional recovery (e.g., Dancause et al. 2005). The spread of the lesion postcentrally did not influence the present

data because there was no correlation between the enhancement of the ipsilesional manual performance and the spread of the lesion into primary somatosensory cortex (Table 1). For instance, in two monkeys with comparably reduced postlesion performance in the ipsilesional hand (Mk-CE and Mk-JU), one had a part of the somatosensory cortex lesioned (10 mm³), whereas the other monkey did not. At the other extreme, one monkey with enhancement of ipsilesional hand's performance (119%) had a lesion encroaching on the somatosensory cortex, whereas in another monkey (124% performance), the postcentral gyrus was not affected by the ibotenic acid infusion. There was also no systematic relationship between the extent of the recovery of the contralesional hand and the presence/absence or size of lesion affecting the somatosensory cortex. The reasons for this are likely twofold. First, a lesion of the somatosensory cortex does not necessarily affect the hand representation and, second, the monkeys were overtrained on this task, suggesting that the contribution of the somatosensory cortex may be less crucial than during training or during early phases of regular practice or immediately after the lesion. With respect to the spread of the lesion in PM, there is also no correlation with the long-term enhancement of ipsilesional manual performance. In the group of monkeys with spread in PM, some exhibited behavioral enhancement (Mk-JA, Mk-AV), whereas others did not (Mk-CE, Mk-JU, Mk-SL); however, note that the extent of the lesion in PM and in the somatosensory cortex was included in the total volume of the lesion in gray matter considered in the analysis of correlation with the behavioral parameters (Fig. 7).

Note that the monkey exhibiting the best recovery of the contralesional hand together with the most extensive enhancement of the performance in the ipsilesional hand (Mk-AV; see Fig. 4) is characterized by a lesion affecting only the rostral part of the primary motor cortex, with spread into PM (Fig. 1). As expected, for such a lesion position, recovery was better compared with that of a lesion including the caudal part of the primary motor cortex.

Comparison of score and contact time data

The contact time data specifically reflect the grasping function by measuring the time of manipulation of the pellet with the fingers before successful retrieval. The retrieval score data also reflect this manipulation but, in addition, comprise other facets of the task, such as arm reaching, arm withdrawal, and transport of the pellet to the mouth. The observation of long-term enhancement of manual performance in the ipsilesional hand after unilateral motor cortex lesion in six monkeys comes largely from score data (Figs. 4 and 6), whereas the contact time data showed only a trend in that direction (Supplemental Fig. S6). How can it be explained that contact time data are not fully corroborating with the retrieval score data? To address this question, the strategy used by the monkey to perform the modified Brinkman board was investigated. To assess one facet of the strategy, the cumulative distance between consecutive slots was determined, both prelesion and postlesion. If monkeys visit the slots in a systematic manner (e.g., starting at a given extremity of the board and then moving progressively toward the other extremity of the board), then the cumulative distance is smaller than that in the case of random spatial choice of the slots. For each monkey, the difference of cumulative distance between consecutive slots (postlesion minus

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

Comparison with functional recovery in human subjects

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

Potential mechanisms: cortical contribution

Two potential mechanisms will be presented for the observed correlation between the extent of contralesional recovery and the ipsilesional manual performance over the long term, starting here at the level of the cerebral cortex (see next section for potential subcortical mechanisms). In human subjects, transient unilateral disruption of the motor cortex with repetitive transcranial magnetic stimulation (TMS) increased excitability of the unaffected motor cortex, resulting in improved motor learning with the hand ipsilateral to the motor cortex disrupted with TMS (Kobayashi et al. 2009). These observations were interpreted in terms of interhemispheric competition. Suppression of motor control in M1 on one side may transcallosally disinhibit the contralateral motor cortex,

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

Potential mechanisms: subcortical contribution

One cannot exclude the possibility of a facilitation of the intact hemisphere on the ipsilesional hand mediated indirectly via the brain stem, involving for instance rubrospinal or reticulospinal neurons. For the red nucleus magnocellularis (RNm), output fibers decussate just after exiting the RNm, thus providing an indirect crossed pathway from the intact motor cortex to the spinal motoneurons of the ipsilesional hand, via the contralesional red nucleus. It has been shown that the rubrospinal projection can reorganize after lesion of the corticospinal tract, presumably to restore function to flexor muscles (Belhaj-Saif and Cheney 2001). The reticulospinal system projects bilaterally to the spinal cord. Stimulus-triggered averaging studies in awake monkeys (Davidson and Buford 2004, 2006) confirmed bilateral stimulus effects on mostly proximal muscles, with a common pattern of facilitation in flexor muscles and inhibition in extensor muscles ipsilaterally and the opposite effect on the contralateral side. Similar data were

obtained when using the spike-triggered averaging technique (Davidson et al. 2007). However, the magnitude of the effects was weak and rare (5%). In a more recent study, Riddle et al. (2009) used intracellular recording in anesthetized monkeys to study synaptic connections between the reticulospinal tract and identified cervical motoneurons. The main finding was that the electrical stimulation of the reticulospinal tract activates motoneurons projecting to proximal and distal (wrist and hand) forelimb muscles: out of 140 motoneurons tested, the activation was exerted via direct monosynaptic (13% of the motoneurons) and disynaptic reticulospinal pathways (46% of the motoneurons), indicating that the reticulospinal system may contribute to an enhancement of the motor performance of the ipsilesional hand after unilateral motor cortex lesion.

Hand dominance and pertinence of the nonhuman primate model

The data presented in Fig. 8 support the notion that macaque monkeys do not show a systematic manual dominance for the present task (modified Brinkman board), at least in 16 of 22 monkeys. It can thus be concluded that the choice of the lesioned hemisphere in the present study did not influence the results. This may be different in human subjects due to the known disparity in motor performance between the dominant and nondominant hands, but this has not yet been investigated by using a task similar to the modified Brinkman board. A more important conclusion of the data presented in Fig. 8 is the significance of the present experimental model of cortical lesion in monkeys. First, sophisticated manual motor skills are a prerogative of primates (for review, see Lemon 2008; Lemon and Griffiths 2005). Second, the observed enhancement of manual performance in the ipsilesional hand after unilateral motor cortex lesion, although statistically significant, can be observed only in an animal model, where the prelesion data are compared with the postlesion data within the same subject. This is likely the reason why such enhancement of manual dexterity in the ipsilesional hand correlated with the degree of functional recovery of the contralesional hand was not observed in previous clinical studies investigating the possible effect of unilateral stroke on the ipsilesional hand (Nowak et al. 2005; Sunderland 2000). Therefore the present study emphasizes the crucial need to maintain animal models of major brain dysfunctions or pathologies (such as the consequences of stroke, for instance), especially monkey models as discussed earlier for several neuropathologies (e.g., Capitanio and Emborg 2008; Courtine et al. 2007). The monkey model is pertinent to decipher subtle mechanisms involved in functional recovery after a lesion. Such knowledge together with the assessment of possible secondary effects of a treatment represent a solid basis for translating and refining therapeutic strategies to human patients, as recently demonstrated for anti-Nogo-A antibody treatment after spinal cord injury in macaque monkeys (Freund et al. 2006, 2009).

ACKNOWLEDGMENTS

We thank V. Moret, C. Roulin, F. Tinguely, C. Marti, L. Grollimund, and C. Pythoud (technical assistance with histology and behavioral evaluations); J. Savidan and A. Meszaros (lesion reconstruction); J. Corpataux, L. Bossy, B. Bapst, and B. Morandi (animal housekeeping); and A. Gaillard (mechanics), B. Aebischer (electronics), and L. Monney (informatics). The data derived from the spinal cord injured monkeys (see Fig. 8) were collected with the

contributions of Drs. P. Freund, E. Schmidlin, T. Wannier, A. Mir, and M. Schwab. We also thank Dr. E. Schmidlin and J. Aebischer for contributions to some experiments and M. Rouiller-Schaer and Dr. Pamela Banta-Lavenex for editing the English language.

GRANTS

This work was supported by Swiss National Science Foundation Grants 31-61857.00 and 310000-110005 to E. M. Rouiller and 3100A0-103924 to J. Bloch; Novartis Foundation; The National Centre of Competence in Research on "Neural Plasticity and Repair"; and the Christopher Reeve Foundation (Springfield, NJ). The antibodies were provided by Novartis Pharma.

REFERENCES

- Allred RP, Jones TA. Unilateral ischemic sensorimotor cortical damage in female rats: forelimb behavioral effects and dendritic structural plasticity in the contralateral homotopic cortex. *Exp Neurol* 190: 433–445, 2004.
- Altschuler EL, Wisdom SB, Stone L, Foster C, Galasko D, Llewellyn DME, Ramachandran VS. Rehabilitation of hemiparesis after stroke with a mirror. *Lancet* 353: 2035–2036, 1999.
- Beaud ML, Schmidlin E, Wannier T, Freund P, Bloch J, Mir A, Schwab ME, Rouiller EM. Anti-Nogo-A antibody treatment does not prevent cell body shrinkage in the motor cortex in adult monkeys subjected to unilateral cervical cord lesion (Abstract). *BMC Neurosci* 9: 5, 2008.
- Belhaj-Saïf A, Cheney PD. Plasticity in the distribution of the red nucleus output to forearm muscles after unilateral lesions of the pyramidal tract. *J Neurophysiol* 83: 3147–3153, 2000.
- Brunet JF, Rouiller E, Wannier T, Villemure JG, Bloch J. Primate adult brain cell autotransplantation, a new tool for brain repair? *Exp Neurol* 196: 195–198, 2005.
- Calautti C, Baron JC. Functional neuroimaging studies of motor recovery after stroke in adults: a review. *Stroke* 34: 1553–1566, 2003.
- Campbell MJ, Morrison JH. Monoclonal antibody to neurofilament protein (SMI-32) labels a subpopulation of pyramidal neurons in the human and monkey neocortex. *J Comp Neurol* 282: 191–205, 1989.
- Capitano JP, Emborg ME. Contributions of non-human primates to neuroscience research. *Lancet* 371: 1126–1135, 2008.
- Caramia MD, Palmieri MG, Giacomini P, Iani C, Dally L, Silvestrini M. Ipsilateral activation of the unaffected motor cortex in patients with hemiparetic stroke. *Clin Neurophysiol* 111: 1990–1996, 2000.
- Carey JR, Kimberley TJ, Lewis SM, Auerbach EJ, Dorsey L, Rundquist P, Ugurbil K. Analysis of fMRI and finger tracking training in subjects with chronic stroke. *Brain* 125: 773–788, 2002.
- Carey LM, Abbott DF, Egan GF, Bernhardt J, Donnan GA. Motor impairment and recovery in the upper limb after stroke: behavioral and neuroanatomical correlates. *Stroke* 36: 625–629, 2005.
- Cauraug JH, Summers JJ. Neural plasticity and bilateral movements: a rehabilitation approach for chronic stroke. *Prog Neurobiol* 75: 309–320, 2005.
- Chollet F, DiPiero V, Wise RJS, Brooks DJ, Dolan RJ, Frackowiak RSJ. The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. *Ann Neurol* 29: 63–71, 1991.
- Courtine G, Bunge MB, Fawcett JW, Grossman RG, Kaas JH, Lemon R, Maier I, Martin J, Nudo RJ, Ramon-Cueto A, Rouiller EM, Schnell L, Wannier T, Schwab ME, Edgerton VR. Can experiments in nonhuman primates expedite the translation of treatments for spinal cord injury in humans? *Nat Med* 13: 561–566, 2007.
- Cramer SC, Nelles G, Benson RR, Kaplan JD, Parker RA, Kwong KK, Kennedy DN, Finklestein SP, Rosen BR. A functional MRI study of subjects recovered from hemiparetic stroke. *Stroke* 28: 2518–2527, 1997.
- Dancause N, Barbay S, Frost SB, Plautz EJ, Chen DF, Zoubina EV, Stowe AM, Nudo RJ. Extensive cortical rewiring after brain injury. *J Neurosci* 25: 10167–10179, 2005.
- Dancause N, Barbay S, Frost SB, Zoubina EV, Plautz EJ, Mahnken JD, Nudo RJ. Effects of small ischemic lesions in the primary motor cortex on neurophysiological organization in ventral premotor cortex. *J Neurophysiol* 96: 3506–3511, 2006.
- Darling WG, Pizzimenti MA, Rotella DL, Peterson CR, Hynes SM, Ge J, Solon K, McNeal DW, Stilwell-Morecraft KS, Morecraft RJ. Volumetric effects reaching kinematics and prehensile dexterity in non-human primates. *Exp Neurol* 220: 90–108, 2009.
- Davidson AG, Buford JA. Motor outputs from the primate reticular formation to shoulder muscles as revealed by stimulus-triggered averaging. *J Neurophysiol* 92: 83–95, 2004.
- Davidson AG, Buford JA. Bilateral actions of the reticulospinal tract on arm and shoulder muscles in the monkey: stimulus triggered averaging. *Exp Brain Res* 173: 25–39, 2006.
- Davidson AG, Schieber MH, Buford JA. Bilateral spike-triggered average effects in arm and shoulder muscles from the monkey pontomedullary reticular formation. *J Neurosci* 27: 8053–8058, 2007.
- Duque J, Hummel F, Celnik P, Murase N, Mazzocchio R, Cohen LG. Transcallosal inhibition in chronic subcortical stroke. *NeuroImage* 28: 940–946, 2005.
- Eisner-Janowicz I, Barbay S, Hoover E, Stowe AM, Frost SB, Plautz EJ, Nudo RJ. Early and late changes in the distal forelimb representation of the supplementary motor area after injury to frontal motor areas in the squirrel monkey. *J Neurophysiol* 100: 1498–1512, 2008.
- Feydy A, Carlier R, Roby-Brami A, Bussel B, Cazalis F, Pierot L, Burnod Y, Maier MA. Longitudinal study of motor recovery after stroke: recruitment and focusing of brain activation. *Stroke* 33: 1610–1617, 2002.
- Freund P, Schmidlin E, Wannier T, Bloch J, Mir A, Schwab ME, Rouiller EM. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. *Nat Med* 12: 790–792, 2006.
- Freund P, Schmidlin E, Wannier T, Bloch J, Mir A, Schwab ME, Rouiller EM. Anti-Nogo-A antibody treatment promotes recovery of manual dexterity after unilateral cervical lesion in adult primates: re-examination and extension of behavioral data. *Eur J Neurosci* 29: 983–996, 2009.
- Freund P, Wannier T, Schmidlin E, Bloch J, Mir A, Schwab ME, Rouiller EM. Anti-Nogo-A antibody treatment enhances sprouting of corticospinal axons rostral to a unilateral cervical spinal cord lesion in adult macaque monkey. *J Comp Neurol* 502: 644–659, 2007.
- Fridman EA, Hanakawa T, Chung M, Hummel F, Leiguarda RC, Cohen LG. Reorganization of the human ipsilesional premotor cortex after stroke. *Brain* 127: 747–758, 2004.
- Frost SB, Barbay S, Friel KM, Plautz EJ, Nudo RJ. Reorganization of remote cortical regions after ischemic brain injury: a potential substrate for stroke recovery. *J Neurophysiol* 89: 3205–3214, 2003.
- Glees P, Cole J. Recovery of skilled motor functions after small repeated lesions of motor cortex in macaque. *J Neurophysiol* 13: 137–148, 1950.
- Hermsdörfer J, Goldenberg G. Ipsilesional deficits during fast diadochokinetic hand movements following unilateral brain damage. *Neuropsychologia* 40: 2100–2115, 2002.
- Hermsdörfer J, Laimgruber K, Kerkhoff G, Mai N, Goldenberg G. Effects of unilateral brain damage on grip selection, coordination, and kinematics of ipsilesional prehension. *Exp Brain Res* 128: 41–51, 1999b.
- Hermsdörfer J, Ulrich S, Marquardt C, Goldenberg G, Mai N. Prehension with the ipsilesional hand after unilateral brain damage. *Cortex* 35: 139–161, 1999a.
- Heuninckx S, Wenderoth N, Swinnen SP. Systems neuroplasticity in the aging brain: recruiting additional neural resources for successful motor performance in elderly persons. *J Neurosci* 28: 91–99, 2008.
- Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol* 5: 708–712, 2006.
- Jenny AB. Commissural projections of the cortical hand motor area in monkeys. *J Comp Neurol* 188: 137–146, 1979.
- Johansen-Berg H, Rushworth MFS, Bogdanovic MD, Kischka U, Wimalaratna S, Matthews PM. The role of ipsilateral premotor cortex in hand movement after stroke. *Proc Natl Acad Sci USA* 99: 14518–14523, 2002.
- Jørgensen HS, Nakayama H, Raaschou HO, Olsen TS. Stroke. Neurologic and functional recovery: the Copenhagen Stroke Study. *Phys Med Rehabil Clin N Am* 10: 887–906, 1999b.
- Jørgensen HS, Reith J, Nakayama H, Kammersgaard LP, Raaschou HO, Olsen TS. What determines good recovery in patients with the most severe strokes? The Copenhagen Stroke Study. *Stroke* 30: 2008–2012, 1999a.
- Kermadi I, Liu Y, Tempini A, Rouiller EM. Effects of reversible inactivation of the supplementary motor area (SMA) on unimanual grasp and bimanual pull and grasp performance in monkeys. *Somatosens Mot Res* 14: 268–280, 1997.
- Kobayashi M, Theoret H, Pascual-Leone A. Suppression of ipsilateral motor cortex facilitates motor skill learning. *Eur J Neurosci* 29: 833–836, 2009.
- Kozlowski DA, James DC, Schallert T. Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. *J Neurosci* 16: 4776–4786, 1996.
- Leasure JL, Schallert T. Consequences of forced disuse of the impaired forelimb after unilateral cortical injury. *Behav Brain Res* 150: 83–91, 2004.
- Lemon RN. Descending pathways in motor control. *Annu Rev Neurosci* 31: 195–218, 2008.

- Lemon RN, Griffiths J.** Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? *Muscle Nerve* 32: 261–279, 2005.
- Liepert J, Bauder H, Miltner WHR, Taub E, Weiller C.** Treatment-induced cortical reorganization after stroke in humans. *Stroke* 31: 1210–1216, 2000a.
- Liepert J, Hamzei F, Weiller C.** Motor cortex disinhibition of the unaffected hemisphere after acute stroke. *Muscle Nerve* 23: 1761–1763, 2000b.
- Liu J, Morel A, Wannier T, Rouiller EM.** Origins of callosal projections to the supplementary motor area (SMA): a direct comparison between pre-SMA and SMA-proper in macaque monkeys. *J Comp Neurol* 443: 71–85, 2002.
- Liu Y, Rouiller EM.** Mechanisms of recovery of dexterity following unilateral lesion of the sensorimotor cortex in adult monkeys. *Exp Brain Res* 128: 149–159, 1999.
- Luft AR, McCombe-Waller S, Whittall J, Forrester LW, Macko R, Sorkin JD, Schulz JB, Goldberg AP, Hanley DF.** Repetitive bilateral arm training and motor cortex activation in chronic stroke: a randomized controlled trial. *J Am Med Assoc* 292: 1853–1861, 2004b.
- Luft AR, Waller S, Forrester L, Smith GV, Whittall J, Macko RF, Schulz JB, Hanley DF.** Lesion location alters brain activation in chronically impaired stroke survivors. *NeuroImage* 21: 924–935, 2004a.
- Masiero S, Carraro E.** Upper limb movements and cerebral plasticity in post-stroke rehabilitation. *Aging Clin Exp Res* 20: 103–108, 2008.
- Miltner WHR, Bauder H, Sommer M, Dettmers C, Taub E.** Effects of constraint-induced movement therapy on patients with chronic motor deficits after stroke: a replication. *Stroke* 30: 586–592, 1999.
- Mima T, Toma K, Koshy B, Hallett M.** Coherence between cortical and muscular activities after subcortical stroke. *Stroke* 32: 2597–2601, 2001.
- Misawa S, Kuwabara S, Matsuda S, Honma K, Ono J, Hattori T.** The ipsilateral cortico-spinal tract is activated after hemiparetic stroke. *Eur J Neurol* 15: 706–711, 2008.
- Murase N, Duque J, Mazzocchio R, Cohen LG.** Influence of interhemispheric interactions on motor function in chronic stroke. *Ann Neurol* 55: 400–409, 2004.
- Murata Y, Higo N, Oishi T, Yamashita A, Matsuda K, Hayashi M, Yamane S.** Effects of motor training on the recovery of manual dexterity after primary motor cortex lesion in macaque monkeys. *J Neurophysiol* 99: 773–786, 2008.
- Nakayama H, Jørgensen HS, Raaschou HO, Olsen TS.** Compensation in recovery of upper extremity function after stroke: the Copenhagen Stroke Study. *Arch Phys Med Rehabil* 75: 852–857, 1994.
- Nelles G, Spiekermann G, Jueptner M, Leonhardt G, Müller S, Gerhard H, Diener HC.** Reorganization of sensory and motor systems in hemiplegic stroke patients: a positron emission tomography study. *Stroke* 30: 1510–1516, 1999.
- Netz J, Lammers T, Hömberg V.** Reorganization of motor output in the non-affected hemisphere after stroke. *Brain* 120: 1579–1586, 1997.
- Nowak DA, Grefkes C, Dafotakis M, Kust J, Karbe H, Fink GR.** Dexterity is impaired at both hands following unilateral subcortical middle cerebral artery stroke. *Eur J Neurosci* 25: 3173–3184, 2007.
- Nudo RJ, Milliken GW.** Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiol* 75: 2144–2149, 1996.
- Nudo RJ, Plautz EJ, Frost SB.** Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* 24: 1000–1019, 2001.
- Nudo RJ, Wise BM, SiFuentes F, Milliken GW.** Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science* 272: 1791–1794, 1996.
- Oujamaa L, Relave I, Froger J, Mottet D, Pelissier JY.** Rehabilitation of arm function after stroke. Literature review. *Ann Phys Rehabil Med* 52: 269–293, 2009.
- Park MC, Belhaj-Saïf A, Cheney PD.** Properties of primary motor cortex output to forelimb muscles in rhesus macaques. *J Neurophysiol* 92: 2968–2984, 2004.
- Park MC, Belhaj-Saïf A, Gordon M, Cheney PD.** Consistent features in the forelimb representation of primary motor cortex in rhesus macaques. *J Neurosci* 21: 2784–2792, 2001.
- Pizzimenti MA, Darling WG, Rotella DL, McNeal DW, Herrick JL, Ge J, Stilwell-Morecraft KS, Morecraft RJ.** Measurement of reaching kinematics and prehensile dexterity in nonhuman primates. *J Neurophysiol* 98: 1015–1029, 2007.
- Plautz EJ, Barbay S, Frost SB, Friel KM, Dancause N, Zoubina EV, Stowe AM, Quaney BM, Nudo RJ.** Post-infarct cortical plasticity and behavioral recovery using concurrent cortical stimulation and rehabilitative training: a feasibility study in primates. *Neurol Res* 25: 801–810, 2003.
- Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik PA, Krakauer JW.** Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci USA* 106: 1590–1595, 2009.
- Riddle CN, Edgley SA, Baker SN.** Direct and indirect connections with upper limb motoneurons from the primate reticulospinal tract. *J Neurosci* 29: 4993–4999, 2009.
- Rouiller EM, Babalian A, Kazennikov O, Moret V, Yu X-H, Wiesendanger M.** Transcallosal connections of the distal forelimb representations of the primary and supplementary motor cortical areas in macaque monkeys. *Exp Brain Res* 102: 227–243, 1994.
- Rouiller EM, Yu XH, Moret V, Tempini A, Wiesendanger M, Liang F.** Dexterity in adult monkeys following early lesion of the motor cortical hand area: the role of cortex adjacent to the lesion. *Eur J Neurosci* 10: 729–740, 1998.
- Sawaki L, Butler AJ, Xiaoyan L, Wassenaar PA, Mohammad YM, Blanton S, Sathian K, Nichols-Larsen DS, Wolf SL, Good DC, Wittenberg GF.** Constraint-induced movement therapy results in increased motor map area in subjects 3 to 9 months after stroke. *Neurorehabil Neural Repair* 22: 505–513, 2008.
- Schaechter JD, Kraft E, Hilliard TS, Dijkhuizen RM, Benner T, Fincklestein SP, Rosen BR, Cramer SC.** Motor recovery and cortical reorganization after constraint-induced movement therapy in stroke patients: a preliminary study. *Neurorehabil Neural Repair* 16: 326–338, 2002.
- Schaechter JD, Perdue KL.** Enhanced cortical activation in the contralesional hemisphere of chronic stroke patients in response to motor skill challenge. *Cereb Cortex* 18: 638–647, 2008.
- Schmidlin E, Wannier T, Bloch J, Belhaj-Saïf A, Wyss A, Rouiller EM.** Reduction of the hand representation in the ipsilateral primary motor cortex following unilateral section of the corticospinal tract at cervical level in monkeys (Abstract). *BMC Neurosci* 6: 56, 2005.
- Schmidlin E, Wannier T, Bloch J, Rouiller EM.** Progressive plastic changes in the hand representation of the primary motor cortex parallel incomplete recovery from a unilateral section of the corticospinal tract at cervical level in monkeys. *Brain Res* 1017: 172–183, 2004.
- Seitz RJ, Hofflich P, Binkofski F, Tellmann L, Herzog H, Freund HJ.** Role of the premotor cortex in recovery from middle cerebral artery infarction. *Arch Neurol* 55: 1081–1088, 1998.
- Serrien DJ, Strens LHA, Cassidy MJ, Thompson AJ, Brown P.** Functional significance of the ipsilateral hemisphere during movement of the affected hand after stroke. *Exp Neurol* 190: 425–432, 2004.
- Sunderland A.** Recovery of ipsilateral dexterity after stroke. *Stroke* 31: 430–433, 2000.
- Swayne OB, Rothwell JC, Ward NS, Greenwood RJ.** Stages of motor output reorganization after hemispheric stroke suggested by longitudinal studies of cortical physiology. *Cereb Cortex* 18: 1909–1922, 2008.
- Takeda K, Gomi Y, Imai I, Shimoda N, Hiwatari M, Kato H.** Shift of motor activation areas during recovery from hemiparesis after cerebral infarction: a longitudinal study with near-infrared spectroscopy. *Neurosci Res* 59: 136–144, 2007.
- Tsang YM, Chiong F, Kuznetsov D, Kasarskis E, Geula C.** Motor neurons are rich in non-phosphorylated neurofilaments: cross-species comparison and alteration in ALS. *Brain Res* 861: 45–58, 2006.
- van der Lee JH, Wagenaar RC, Lankhorst GJ, Vogelaar TW, Deville WL, Bouter LM.** Forced use of the upper extremity in chronic stroke patients: results from a single-blind randomized clinical trial. *Stroke* 30: 2369–2375, 1999.
- Wannier T, Schmidlin E, Bloch J, Rouiller EM.** A unilateral section of the corticospinal tract at cervical level in primate does not lead to measurable cell loss in motor cortex. *J Neurotrauma* 22: 703–717, 2005.
- Ward NS, Brown MM, Thompson AJ, Frackowiak RSJ.** Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain* 126: 1430–1448, 2003.
- Ward NS, Brown MM, Thompson AJ, Frackowiak RSJ.** The influence of time after stroke on brain activations during a motor task. *Ann Neurol* 55: 829–834, 2004.
- Ward NS, Cohen LG.** Mechanisms underlying recovery of motor function after stroke. *Arch Neurol* 61: 1844–1848, 2004.
- Ward NS, Frackowiak RSJ.** Age-related changes in the neural correlates of motor performance. *Brain* 126: 873–888, 2003.
- Ward NS, Newton JM, Swayne OB, Lee L, Frackowiak RSJ, Thompson AJ, Greenwood RJ, Rothwell JC.** The relationship between brain activity and peak grip force is modulated by corticospinal system integrity after subcortical stroke. *Eur J Neurosci* 25: 1865–1873, 2007.

- Weiller C, Ramsay SC, Wise RJ, Friston KJ, Frackowiak RS.** Individual patterns of functional reorganization in the human cerebral cortex after capsular infarction. *Ann Neurol* 33: 181–189, 1993.
- Werhahn KJ, Conforto AB, Kadom N, Hallett M, Cohen LG.** Contribution of the ipsilateral motor cortex to recovery after chronic stroke. *Ann Neurol* 54: 464–472, 2003.
- Wolf SL, Winstein CJ, Miller JP, Taub E, Uswatte G, Morris D, Giuliani C, Light KE, Nichols-Larsen D.** Effect of constraint-induced movement therapy on upper extremity function 3 to 9 months after stroke: the EXCITE randomized clinical trial. *J Am Med Assoc* 296: 2095–2104, 2006.
- Zemke AC, Heagerty PJ, Lee C, Cramer SC.** Motor cortex organization after stroke is related to side of stroke and level of recovery. *Stroke* 34: e23–e28, 2003.