J Neurophysiol (January 13, 2010). doi:10.1152/jn.00459.2009

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. Rouiller [1]

[(1)] Unit of Physiology and Program in Neurosciences, Department of Medicine, Faculty of Sciences, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland.

[(2)] Dept. of Neurosurgery, Neurosurgery Clinic, University Hospital of Lausanne, Rue du Bugnon, CH-1011 Lausanne, Switzerland.

Running title: Interhemispheric interaction after unilateral motor cortex lesion

Keywords: primates, recovery, ipsilateral hand, interhemispheric interaction.

Text pages: 39
Words: 13792 words, including abstract (244 words), introduction (816 words), methods (2584 words), results (2744 words), discussion (3062 words), references and Figure legends.
Tables: 1
Figures: 8 (plus six supplementary Figures)

Address for correspondence: Prof. Eric M. Rouiller, Unit of Physiology, Department of Medicine, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland. Phone: 0041 26 300 86 09. Fax: 0041 26 300 96 75. E-mail: Eric.Rouiller@unifr.ch

Abstract

Manual dexterity, a prerogative of primates, is under the control of the corticospinal (CS) tract. As 90-95% of CS axons decussate, it is assumed that this control is exerted essentially on the contralateral hand. 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 post-lesion 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 post-lesion, using our standard behavioural 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 on the long-term (100-300 days post-lesion) in 6 out of 10 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 post-lesion 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 hemi-paralysis, for instance after a unilateral stroke affecting motor control, there is wide variability in the extent of recovery of motor control that depends on several parameters (e.g. the precise location and extent of the lesion, type and rapidity of intervention after the cerebral vascular accident, type of rehabilitative therapy; see e.g. van der Lee et al. 1999; Jorgensen et al. 1999a; Nudo et al. 2001; Zemke et al. 2003; Ward and Cohen, 2004; Masiero and Carraro, 2008; Oujamaa et al. 2009). A cursory evaluation may lead to the prediction that, when there is good functional recovery of the contralesional hand, the patient uses the hand affected by the lesion in a sustained manner. In contrast, when there is poor recovery of the contralesional hand, the patient relies more, if not exclusively, on the ipsilesional hand (unaffected by the lesion) to accomplish most tasks, thus possibly acquiring, through experience, enhanced capabilities in the ipsilesional hand, as compared to the pre-lesion situation or to normal subjects (e.g. Nakayama et al. 1994; Jorgensen et al. 1999b; Liepert et al. 2000a; Cauraugh and Summers, 2005).

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

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

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

constraints of the therapeutic protocols applied to some of the monkeys, the time windows during which behavioural assessment took place were not the same across monkeys. In contrast to human studies, our model of experimental motor cortex lesion in the macaque monkey allows us to use each animal as its own control to compare the manual performance before and after the lesion. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by local (Swiss) veterinary authorities.

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: i) anti-Nogo-A antibody treatment; ii) cell therapy with injection of autologous adult progenitor cells, collected from the same animal in the prefrontal cortex (see Brunet et al. 2005). The anti-Nogo-A antibody treatment was tested on monkeys with motor cortex lesions as it was found to significantly enhance functional recovery and sprouting of corticospinal axons after cervical cord injury in macaques (Freund et al. 2006, 2007, 2009). The anti-Nogo-A antibody paradigm was tested on a sub-group of three monkeys (Mk-VA, Mk-SL, Mk-MO) and compared with a subgroup of four monkeys also subjected to a unilateral lesion of the motor cortex but that did not receive any treatment (Mk-CE, Mk-JU, Mk-GE and Mk-RO; see Table 1). Three additional monkeys (Table 1) were included in the pilot cell therapy project, two monkeys (Mk-JO and Mk-JA) received an implantation of autologous adult progenitors cells in the vicinity of the cortical lesion, whereas one monkey (Mk-AV) served as sham control animal (infusion of vehicle only). The present study does not address the issue of the efficacy of the treatments; the therapeutic effects of the two treatments on the contralesional hand will be reported elsewhere.

Behavioural 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 behavioural tests focused on fine finger movements in order to track the functional recovery (see also Pizzimenti et al. 2007; Murata et al. 2008; Darling et al., 2009 for recent contributions). Monkeys were trained to perform our “modified Brinkman board” test (e.g. Rouiller et al. 1998; Liu and Rouiller 1999; Schmidlin et

al. 2004; Freund et al. 2006, 2009), that requires a reach and grasp motor sequence to retrieve small food pellets from wells while using the precision grip (opposition of the thumb and index finger). Food pellets were made of dried banana powder or glucose powder, compressed in a round shape of about 4 mm in diameter. This test of hand motor capacity was performed on a perspex board (10 cm x 20 cm) containing 50 randomly distributed slots, each filled with a food pellet at the beginning of the test. The dimension of the slots was 15 mm long, 8 mm wide and 6 mm deep. Twenty-five slots were oriented vertically and twenty-five slots horizontally. As outlined in detail in a recent report (Freund et al., 2009), retrieval from horizontal slots was more challenging as it required a postural adaptation of the hand (specifically a forearm rotation) in addition to the precision grip, whereas for the vertical slots, the precision grip can be performed with the hand in its natural posture (in pronation of the forearm). The monkeys were not food deprived: the pellets, which served as positive reinforcement during the tests, were the animals' first access to food in the morning. At the end of the tests, the monkeys received additional food (cereals, fruits). The body weight of the monkeys was checked before each behavioural session. The monkeys had free access to water in the animal room.

Individual testing sessions typically lasted about 60 minutes: they included the time to transfer the monkeys to the primate chair and their transport to and from the animal room to the laboratory, as well as delivery of the additional food at the end of the session. An initial pre-lesion training phase was necessary to bring the monkeys to a stable level of performance that corresponded to a plateau in the reach and grasp score (represented by the red horizontal lines in Fig. 2A). The pre-lesion plateau, which was achieved within a time frame ranging from 20 to 128 days before the lesion depending on the specific experimental protocol for each monkey (Table 1), was used to establish the median value of the pre-lesion score (Fig. 2A). As the goal of the present study was to assess the long-term effect of the unilateral motor cortex lesion on the ipsilesional hand, the post-lesion behavioural 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 2 to 5 sessions per week during several months before and after the cortical lesion. For each daily session, the entire modified Brinkman board task was performed once with each hand, corresponding to 50 pellets retrieved by the left hand and 50 pellets retrieved by the right hand, in other words 100 pellets in total when the monkey was successful for all slots

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

Following the modified Brinkman board task, within the 60 minutes of the behavioural session, the monkeys were also tested with other reach and grasp tasks, such as the rotating Brinkman board task, the hidden Brinkman board task as well as the reach and grasp drawer task (see Freund et al., 2006 and web site above). However, the modified Brinkman board task was the only test performed systematically by all monkeys and on each behavioural session. The inclusion of these other (closely related) tasks in the behavioural 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 behavioural session were always identical for both hands. In other words, there was no attempt to favour practice with the contralesional hand.

Surgery

After the monkeys reached a stable pre-lesion performance level (a stable number of pellets retrieved in the first 30 seconds during each session), they were implanted unilaterally

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

Electrophysiology: intracortical microstimulation (ICMS)

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

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

Permanent lesion of M1 hand representation with ibotenic acid

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

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

Data analysis

Within the pre- and post-lesion time frame of behavioural 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 pre-lesion period was used to establish the reach and grasp performance of reference, indicated by the median value (red horizontal lines in Fig. 2A). Post-lesion, behavioural 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 (pre-lesion versus long-term post-lesion) allowed a quantitative assessment of the effect of the lesion several months thereafter. The behavioural data were analyzed statistically using an unpaired non-parametric Mann-Whitney test. A similar analysis was conducted on the second behavioural parameter, the contact time. The pre-lesion data used to address the issue of hand dominance (see section 6 in the results) were analyzed using a paired comparison of daily scores obtained for the left and the right hand (paired t-test or Wilcoxon test). The statistical analysis and the related graphs were obtained using the software SigmaStat 3.5 and SigmaPlot 10.0.

At the end of the experiments, the animals were sacrificed with an overdose of pentobarbital sodium (90 mg/kg body weight, i.p.). Transcardiac perfusion with 0.9% saline (500 ml) was followed by fixative (4000 ml of 4% phosphate-buffered paraformaldehyde). The brains were placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection for 3-5 days. Frontal sections (50 μm thick) of the brain were prepared and collected in five series. One series of sections was Nissl stained with cresyl violet whereas a second series was processed to visualize the marker SMI-32, as previously described (Liu et al. 2002; Wannier et al. 2005; Beaud et al. 2008). The epitope recognized by the SMI-32 antibody lies on non-phosphorylated regions of neurofilament protein and is only expressed by specific categories of neurons (Campbell and Morrison 1989; Tsang et al. 2006). The two series of sections were then used to reconstruct on consecutive sections the position and extent of the permanent lesion in the cerebral cortex, especially using the SMI-32 stained sections (Fig. 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

1) Unilateral lesion of the motor cortex

The unilateral lesion of the motor cortex was produced by infusion of ibotenic acid at multiple sites defined by intracortical microstimulation (ICMS; see Supplementary Fig. 1). Most ICMS sites selected for infusion of ibotenic acid were located in the rostral bank of the central sulcus, where most of the hand is represented in the primary motor cortex (supplementary Fig. 1; Fig. 1B middle and right sections). As ibotenic acid was also injected at a few sites more rostrally, the lesion also extended onto the part of the motor cortex at the brain surface (left section in Fig. 1B). The infusion of ibotenic acid at multiple sites did not produce a uniform lesion, but rather several distinct zones, the areas of which were added in order to compute the total volume of the lesion in the gray matter (Table 1). The total volume of the lesion is used to correlate with the behavioural data in order 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 up to 10 mm. In one monkey (Mk-SL), the lesion spread medially to the sub-cortical white matter (Fig. 1A). There was also limited, but lesser, sub-cortical 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 section.

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

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

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

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

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

The data shown in Figure 2 for three representative monkeys suggest that, when the contralesional hand recovered well from the lesion (e.g. Mk-JA), the long-term post-lesion performance in the ipsilesional hand was enhanced post-lesion on the long-term. In contrast

(Mk-JU), in the case of poor recovery of the contralesional hand, the manual performance in the ipsilalesional hand is not affected, maintaining a level of performance that is close to or slightly worse than the pre-lesion performance. In between these two extreme cases (Mk-JU and Mk-JA), in the monkey with an intermediate recovery of the contralesional hand (Mk-MO), the ipsilesional hand also exhibited enhanced long-term post-lesion performance, but to a somewhat lesser degree than in Mk-JA, although the pre- versus post-lesion difference was nevertheless statistically significant. As shown in Figure 2A, the above 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 Figure 2 was found to be true when considering the other seven monkeys included in the present study (Fig. 3). Four out of the seven monkeys in Figure 3 also showed a long-term enhancement of manual performance (total score) in the ipsilesional hand (Mk-VA, Mk-RO, Mk-JO, Mk-AV), as evidenced by a better post-lesion than pre-lesion retrieval score. In the other three monkeys (Mk-SL, Mk-GE, Mk-CE), the long-term performance in the ipsilesional hand remained at the same level of performance as pre-lesion (Fig. 3). Note that the latter three monkeys exhibited relatively incomplete contralesional recovery of their manual dexterity post-lesion, as compared to the relatively better recovery of the contralesional hand in the other four monkeys (Fig. 3).

Overall (Figs. 2 and 3), long-term post-lesion enhancement of reach and grasp performance in the ipsilesional hand was found in six out of ten monkeys, as assessed by the total retrieval score in the modified Brinkman board task. In these six monkeys, this enhancement was associated with relatively good recovery of the contralesional hand. To better analyze the dependency between the two hands, the long-term post-lesion manual performance in the ipsilesional hand (expressed in % of pre-lesion score) was plotted as a function of the percent of recovery of the contralesional hand (Fig. 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 data are based on an analysis of manual performance as assessed by the total retrieval score (sum of vertical and horizontal slots) in the modified Brinkman board task.

As the synergy of movements is somewhat different for the vertical and horizontal slots (Freund et al. 2009), it is of interest to analyze the same data considering the vertical and horizontal slots separately (Figs. 5-6; Supplementary Figs. 2-3). 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 lead to the same conclusion as the total score data. Interestingly, contralesional hand performance for Mk-MO was poor when retrieving pellets from the horizontal slots, and this was associated with insignificant long-term enhancement of manual performance in the ipsilesional hand (Fig. 5). 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 reveal a correlation between long-term reach and grasp performance in the ipsilesional hand and the percent of recovery of the contralesional hand, although the correlation was less pronounced than for the total score (Fig. 6). Nevertheless, the correlation was statistically significant for both the vertical slots ($p<0.01$) and the horizontal slots ($p<0.05$).

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

The above data indicate that the long-term enhancement of manual performance in the ipsilesional hand is strongly correlated with the degree of recovery for the contralesional hand (Figs. 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 in percent of the pre-lesion score) was plotted as function of the volume of the lesion, expressed in mm^3, encompassing primarily the gray matter in M1 and, to a lesser extent, gray matter in S1 in some monkeys (see also Table 1). As shown in Figure 7 (top panel), there is a significant inverse correlation ($r=-0.735$; $p<0.01$) between the long-term performance in the ipsilesional hand and the volume of the lesion (in the motor cortex and S1). Clearly, the six monkeys with a significant long-term enhancement of manual performance of the ipsilesional hand (filled symbols in Fig. 7 top panel) had a smaller lesion than the other four monkeys. For a more comprehensive description of the relationship

between the 3 relevant parameters, the bottom panel of Figure 7 shows a 3D plot of the long-term performance in the ipsilesional hand versus the percent of recovery of the contralesional hand and the volume of the cortical lesion.

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

The above pellet retrieval score data take into account the entire sequence of movements to collect the pellets (including reaching, withdrawing). In contrast, the contact time parameter is restricted to the time of contact between the fingers and the pellet while it is in the slot (the retrieval time). The contact time reflects specifically the grasping capability during execution of the precision grip, and thus may be a more precise measure of manual dexterity. The contact time was measured during each session for the first five vertical slots and the first five horizontal slots. The data were then cumulated for the pre-lesion plateau period and for the long-term post-lesion period during the same time windows as for the pellet retrieval score data (Table 1). The contact time data are presented similar as the pellet retrieval score data (see Fig. 5 and Supplementary Figs. 1 and 2), in the form of box and whisker plots and analyzed statistically using the Mann-Whitney test (Supplementary Figs. 4 and 5).

The median contact time for the contralesional hand was largely in line with the retrieval score data. The majority of monkeys for which the long-term retrieval score for the contralesional hand remained significantly lower post-lesion, as compared to pre-lesion, exhibited a consistent long lasting increase in contact time (i.e. more time was needed to grasp the pellet). This was true for the vertical slots for Mk-MO, Mk-JU, Mk-SL, Mk-JO, Mk-CE and Mk-GE (Supplementary Fig. 4) whereas, for the horizontal slots, this was true for Mk-JU, Mk-SL, Mk-JO, Mk-GE and Mk-CE (Supplementary Fig. 5). One monkey (Mk-VA) showed contact times post-lesion which did not increase or even decreased as compared to pre-lesion, inconsistent with a poor post-lesion pellet retrieval score over the long term. The three monkeys (Mk-JA, Mk-RO and Mk-AV) with a complete recovery of retrieval score (>95%) for the contralesional hand exhibited a contact time which was not statistically different pre- versus post-lesion, or was even shorter post-lesion (Supplementary Figs. 4 and 5).

As far as the ipsilesional hand is concerned, the contact time data showed less difference between the pre-lesion and the long-term post-lesion periods than did the retrieval score data. An enhancement of post-lesion ipsilesional hand manual dexterity over the long-

term, evidenced by a decrease in contact time, was observed in four monkeys for the vertical slots (Mk-JA, Mk-RO, Mk-VA and Mk-CE; Supplementary Fig. 4) and in two monkeys for the horizontal slots (Mk-VA and Mk-JO; Supplementary Fig. 5). 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 (Supplementary Fig. 6), for both the vertical slots (r=0.579) and the horizontal slots (r=0.349). However, these correlations for the contact time were only a trend as they were not statistically significant (p>0.05).

5) Differences with clinical studies

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

6) Hand dominance for the Modified Brinkman board?

The data presented in Figure 8 are also pertinent to address the issue of whether intact monkeys have a dominant hand when performing the modified Brinkman board task, as assessed by the total retrieval score. In other words, is pre-lesion performance different for

the left hand versus the right hand? Comparing the total number of pellets retrieved for the 12 intact monkeys shown in the left hand panel of Figure 8 reveals that there was no significant difference in left hand versus right hand performance for 9 out the 12 monkeys (paired t-test or Wilcoxon test: $p>0.05$; range 0.088 – 0.885). In the other 3 intact monkeys, the pre-lesion total retrieval score was significantly higher for one hand as compared to the other hand ($p<0.05$), with a better score for the right hand in 2 monkeys and for the left hand in 1 monkey. Comparing the total number of pellets retrieved pre-lesion by the left or the right hand in the group of 10 monkeys included in the present study was 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 hand ($p<0.05$), whereas in the other 7 monkeys there was no significant difference (the p value was greater than 0.05, ranging from 0.108 to 0.898). In the three monkeys exhibiting hand dominance pre-lesion, two monkeys had a better score for the left hand and one for the right hand. In summary, in a total population of 22 monkeys, only six animals exhibited hand dominance (three for the left hand and three for the right hand). It can thus be concluded that, for the modified Brinkman board task, there was no clear and systematic hand dominance, at least as revealed by the total retrieval score.

Discussion

Based on the retrieval score data and the contact time data, but to a lesser extent for the latter (see below), the results of the present study are consistent with our hypothesis that, after unilateral motor cortex lesion, long-term manual performance in the ipsilesional hand co-varies with the extent of post-lesion recovery of the contralesional hand. This is, to the best of our knowledge, an original observation as most previous studies on unilateral motor cortex lesions focused on the recovery of the contralesional hand and the behavioural assessment was limited to the period immediately following the lesion until a performance plateau was reached. The ipsilesional effect observed here appeared, in some cases, only several months post-lesion, although there is no systematic relationship between the extent of enhancement of manual performance and the time frame in which it occurs (Table 1).

As expected, the extent of recovery in the contralesional hand is inversely correlated with the lesion volume (Fig. 7, bottom panel). As 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 panel). This result contrasts with the observation in rats of a post-lesion facilitation of motor skill learning in the non-affected hand, an augmentation that parallels increasing lesion size, within a certain range, and as observed 20 days post-lesion (Allred and Jones 2004). This discrepancy may be related to the different time points (i.e. several months post-lesion in our monkeys) and the very different organization of the corticospinal system between rodents and primates.

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

Limitations of interpretation

The interpretation of our results concerning a co-variation 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 behavioural 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 non-human 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 (around 40%). In the present study, as a result of the two treatments (anti-Nogo-A antibody; autologous progenitor cells' therapy), some monkeys with a fairly large lesion exhibited a substantial recovery, clearly above 40% (Mk-SL, Mk-MO, Mk-JO). A possible direct effect of the treatments on the enhancement of manual performance in the ipsilesional hand over the long-term after a unilateral lesion of the motor cortex is difficult to evaluate. Among the treated monkeys (n=5), two animals showed a marked enhancement of manual performance with their ipsilesional hand, whereas the other three treated monkeys did not (this depends on the slot orientation; Figs. 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 the treatments applied to some of the monkeys. The bottom panel of Figure 7 emphasizes the interdependency between the three parameters (extent of recovery in the contralesional hand; manual performance in the ipsilesional hand over the long-term; volume of cortical lesion), as well as the limitations of interpretation due to the presence of multiple variables in the present study.

Spread of the lesion to cortical areas adjacent to M1.

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

instance, in two monkeys with comparably reduced post-lesion performance in the ipsilesional hand (Mk-CE and Mk-JU), one had a part of the somatosensory cortex lesioned (10 mm^3) whereas the other monkey did not. At the other extreme, one monkey with enhancement of ipsilesional hand's performance (119%) had a lesion encroaching on the somatosensory cortex, whereas in another monkey (124% performance), the post-central gyrus was not affected by the ibotenic acid infusion. There was also no systematic relationship between the extent of the recovery of the contralesional hand and the presence/absence or size of lesion affecting the somatosensory cortex. The reasons for this are likely two-fold. First, a lesion of the somatosensory cortex does not necessarily affect the hand representation and, second, the monkeys were over-trained on this task, suggesting that the contribution of the somatosensory cortex may be less crucial than during training or during early phases of regular practice or immediately after the lesion. As far as the spread of the lesion in PM is concerned, there is also no correlation with the long-term enhancement of ipsilesional manual performance. In the group of monkeys with spread in PM, some exhibited behavioural enhancement (Mk-JA, Mk-AV), whereas others did not (Mk-CE, Mk-JU, Mk-SL). Note however that the extent of the lesion in PM and in the somatosensory cortex was included in the total volume of the lesion in gray matter considered in the analysis of correlation with the behavioural 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 as compared to 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 (Supplementary Fig. 6). How can it be

explained that contact time data are not fully corroborating with the retrieval score data? To address this question, the strategy used by the monkey to perform the modified Brinkman board was investigated. To assess one facet of the strategy, the cumulative distance between consecutive slots was determined, both pre-lesion and post-lesion. If monkeys visit the slots in a systematic manner (e.g. starting at a given extremity of the board and then moving progressively towards the other extremity of the board), then the cumulative distance is smaller than in case of random spatial choice of the slots. For each monkey, the difference of cumulative distance between consecutive slots (post-lesion minus pre-lesion) was calculated. For the ipsilesional hand, there was a significant inverse correlation between the difference of cumulative distance and the long-term manual performance (not shown). In other words, the monkeys that did not exhibit enhancement of manual performance in the ipsilesional hand had a post-lesion strategy in which they visited slots more randomly. In contrast, monkeys with long-term enhancement of the ipsilesional hand visited the slots in a more ordered sequence pre- and post-lesion. When visiting the slots randomly, subjects exhibit some hesitation before moving to the next slot, resulting in fewer pellets retrieved in 30 seconds. It can be tentatively concluded that the enhancement of manual performance reflects more an improvement of strategy than a better manual dexterity per se. As a consequence, the correlation with the contact time was weaker than with the retrieval score which includes the entire temporal course of the trial, including some strategic aspects. Finally, there was some disparity across monkeys, ranging from a reliable correlation between retrieval score and contact time to an absence of correlation between the two parameters.

Comparison with functional recovery in human subjects

From a clinical point of view, a consequence of the present study may be that an efficient therapy aimed at improving the motor control of the contralesional hand, for instance after stroke, is not only pertinent for the affected hand, but also for the fine control of the ipsilesional hand over the long-term, in particular for frequently performed motor sequences. Along this line, constraint induced therapy (e.g. Miltner et al. 1999; Liepert et al. 2000b; Schaechter et al. 2002; Wolf et al. 2006; Sawaki et al. 2008) aimed at immobilizing the non-affected limb to force the use of the affected limb appears to make sense, not only for enhancing the recovery of the contralesional hand by practice, but also for long-term manual performance in the non-affected hand. It has been argued that constraint-induced therapy

should not be imposed too early during the recovery phase, nor should it be too severe in order to avoid 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. Luft et al. 2004b; Altschuler et al.1999).

Potential mechanisms: cortical contribution

Two potential mechanisms will be presented for the observed correlation between the extent of contralesional recovery and the ipsilesional manual performance over the long-term, starting here at the level of the cerebral cortex (see next section for potential subcortical mechanisms). In human subjects, transient unilateral disruption of the motor cortex with repetitive transcranial magnetic stimulation (TMS) increased excitability of the unaffected motor cortex, resulting in improved motor learning with the hand ipsilateral to the motor cortex disrupted with TMS (Kobayashi et al. 2009). These observations were interpreted in terms of inter-hemispheric competition. Suppression of motor control in M1 on one side may transcallosally disinhibit the contralateral motor cortex, leading to an increase of corticospinal drive onto the motoneurons controlling the muscles of the hand ipsilateral to the lesioned or transiently disrupted motor cortex (e.g. Hummel and Cohen, 2006; Reis et al. 2009). A major difference with the present study is that these observations in humans were conducted immediately after the inactivation (i.e. TMS disruption), whereas the present enhancement of the ipsilesional hand in monkeys was observed over the long-term (several months post-lesion). Furthermore comparison between stroke in humans and the present data in monkeys with restricted lesion focused on M1 is limited by the absence of focal lesion in M1 in humans. Nevertheless, is interhemispheric competition a relevant concept to interpret, at least in part, the present data derived from a restricted unilateral lesion centered on the hand representation in motor cortex? The lesion of the hand representation which is primarily in M1 is expected to have only a minor impact on the callosal connectivity because, as compared to other body representations in M1 or to premotor areas, the hand representation in M1 is only weakly connected with the opposite hemisphere (Jenny, 1979; Rouiller et al. 1994). A possible role played by the callosal projection thus concerns other body representations in M1 or other motor cortical areas (PM, SMA) at the origin of stronger callosal projections.

Consistent with a wider recruitment of motor cortical areas, when a movement sequence is executed with more difficulty (e.g. during aging: Ward and Frackowiak 2003; Heunincks et al. 2008; or e.g. after poor recovery from stroke: Ward et al. 2003, 2004), a more widespread brain area is activated as compared to young human subjects or to patients exhibiting better recovery. The increase of brain activity in the lesioned hemisphere, in the case of poor recovery with persisting motor deficit may be associated with a long lasting increase in callosal inhibition of the intact hemisphere, thus preventing a refinement of motor control on the ipsilesional hand over the long-term. This interpretation however in terms of level of activity in one or the other hemispheres related to the degree of recovery, may actually be complicated by the observation that, as compared to intact human subjects, brain activation is lower in patients with cortical lesion but higher in patients with subcortical lesion (Luft et al. 2004a; Murase et al. 2004; Duque et al. 2005).

Potential mechanisms: subcortical contribution

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

the motoneurons) and disynaptic reticulospinal pathways (46% of the motoneurons), indicating that the reticulospinal system may contribute to an enhancement of the motor performance of the ipsilesional hand after unilateral motor cortex lesion.

Hand dominance and pertinence of the non-human primate model

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

Acknowledgments

The authors wish to thank the technical assistance of Véronique Moret, Christine Roulin, Françoise Tinguely, Christiane Marti, Laurence Grollimund and Catherine Pythoud (histology and behavioural evaluations), Julie Savidan and Alexandra Meszaros (lesion reconstruction), Josef Corpataux, Laurent Bossy, Bernard Bapst and Bernard Morandi (animal house keeping), André Gaillard (mechanics), Bernard Aebischer (electronics), Laurent Monney (informatics). The data derived from the spinal cord injured monkeys (see Figure 8) were collected with the contribution of Drs P. Freund, E. Schmidlin, T. Wannier, A. Mir and M. Schwab. Thanks are due to Dr. E. Schmidlin and Juliane Aebischer for their contribution to some experiments. The authors thank Mrs Murielle Rouiller-Schaer and Dr. Pamela Banta-Lavenex for editing the English language.

Grant Sponsors: Swiss National Science Foundation, grants No 31-61857.00, 310000-110005 (EMR), No 3100A0-103924 (JB), Novartis Foundation; The National Centre of Competence in Research (NCCR) on "Neural plasticity and repair", and the Christopher Reeves Foundation (Springfield, NJ). The antibodies were provided by Novartis Pharma.

References

Allred RP and 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 and Ramachandran VS. Rehabilitation of hemiparesis after stroke with a mirror. *The lancet* 353: 2035-2036, 1999.

Beaud ML, Schmidlin E, Wannier T, Freund P, Bloch J, Mir A, Schwab ME and 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. *BMC Neurosci* 9: 5, 2008.

Belhaj-Saïf A and 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 and Bloch J. Primate adult brain cell autotransplantation, a new tool for brain repair? *Experimental Neurology* 196: 195-198, 2005.

Calautti C and Baron JC. Functional neuroimaging studies of motor recovery after stroke in adults: a review. *Stroke* 34: 1553-1566, 2003.

Campbell MJ and 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.

Capitanio JP and 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 and 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 and 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 and Donnan GA. Motor impairment and recovery in the upper limb after stroke: behavioral and neuroanatomical correlates. *Stroke* 36: 625-629, 2005.

Cauraugh JH and 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 and 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 and 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, S. C., Nelles, G., Benson, R. R., Kaplan, J. D., Parker, R. A., Kwong, K. K., Kennedy, D. N., Finklestein, S. P., and Rosen, B. R. 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 and 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 and 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 and Morecraft RJ. Measurement of reaching kinematics and prehensile dexterity in nonhuman primates. *Exp Neurol* 220: 90-108, 2009.

Davidson AG and 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 and 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 and 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 and 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 and 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 and 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 and Rouiller EM. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. *Nature Med* 12: 790-792, 2006.

Freund P, Wannier T, Schmidlin E, Bloch J, Mir A, Schwab ME and 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.

Freund P, Schmidlin E, Wannier T, Bloch J, Mir A, Schwab ME and 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.

Fridman EA, Hanakawa T, Chung M, Hummel F, Leiguarda RC and Cohen LG. Reorganization of the human ipsilesional premotor cortex after stroke. *Brain* 127: 747-758, 2004.

Frost SB, Barbay S, Friel KM, Plautz EJ and 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 and Cole J. Recovery of skilled motor functions after small repeated lesions of motor cortex in macaque. *J Neurophysiol* 13: 137-148, 1950.

Hermsdörfer J, Ulrich S, Marquardt C, Goldenberg G and Mai N. Prehension with the ipsilesional hand after unilateral brain damage. *Cortex* 35: 139-161, 1999a.

Hermsdörfer J, Laimgruber K, Kerkhoff G, Mai N and 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 and Goldenberg G . Ipsilesional deficits during fast diadochokinetic hand movements following unilateral brain damage. *Neuropsychologia* 40: 2100-2115, 2002.

Heuninckx S, Wenderoth N and 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 and 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 and Matthews PM. The role of ipsilateral premotor cortex in hand movement after stroke. *Proceedings of the National Academy of Sciences of the United States of America* 99: 14518-14523, 2002.

Jorgensen HS, Reith J, Nakayama H, Kammersgaard LP, Raaschou HO and Olsen TS. What determines good recovery in patients with the most severe strokes? The Copenhagen stroke study. *Stroke* 30: 2008-2012, 1999a.

Jorgensen HS, Nakayama H, Raaschou HO and Olsen TS. Stroke. Neurologic and functional recovery the Copenhagen Stroke Study. *Phys Med Rehabil Clin N Am* 10: 887-906, 1999b.

Kermadi I, Liu Y, Tempini A and Rouiller EM. Effects of reversible inactivation of the supplementary motor area (SMA) on unimanual grasp and bimanual pull and grasp performance in monkeys. *Somat and Motor Res* 14: 268-280, 1997.

Kobayashi M, Theoret H and Pascual-Leone A. Suppression of ipsilateral motor cortex facilitates motor skill learning. *Eur J Neurosci* 29: 833-836, 2009.

Kozlowski DA, James DC and Schallert T. Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. *J Neurosci* 16: 4776-4786, 1996.

Leasure JL and Schallert T. Consequences of forced disuse of the impaired forelimb after unilateral cortical injury. *Behav Brain Res* 150: 83-91, 2004.

Lemon RN and Griffiths J. Comparing the function of the corticospinal system in different species: Organizational differences for motor specialization? *Muscle Nerve* 32: 261-279, 2005.

Lemon RN. Descending pathways in motor control. *Annu Rev Neurosci* 31: 195-218, 2008.

Liepert J, Bauder H, Miltner WHR, Taub E and Weiller C. Treatment-induced cortical reorganization after stroke in humans. *Stroke* 31: 1210-1216, 2000a.

Liepert J, Hamzei F and Weiller C . Motor cortex disinhibition of the unaffected hemisphere after acute stroke. *Muscle Nerve* 23: 1761-1763, 2000b.

Liu J, Morel A, Wannier T and 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 and 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, Waller S, Forrester L, Smith GV, Whitall J, Macko RF, Schulz JB and Hanley DF. Lesion location alters brain activation in chronically impaired stroke survivors. *Neuroimage* 21: 924-935, 2004a.

Luft AR, McCombe-Waller S, Whitall J, Forrester LW, Macko R, Sorkin JD, Schulz JB, Goldberg AP and Hanley DF. Repetitive bilateral arm training and motor cortex activation in chronic stroke: a randomized controlled trial. *JAMA* 292: 1853-1861, 2004b.

Masiero S and 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 and 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 and 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 and 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 and 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 and 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, Jorgensen HS, Raaschou HO and 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 and 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 and 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 and Fink GR. Dexterity is impaired at both hands following unilateral subcortical middle cerebral artery stroke. *Eur J Neurosci* 25: 3173-3184, 2007.

Nudo RJ and 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, Wise BM, SiFuentes F and Milliken GW. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science* 272: 1791-1794, 1996.

Nudo RJ, Plautz EJ and Frost SB. Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* 24: 1000-1019, 2001.

Oujamaa L, Relave I, Froger J, Mottet D and Pelissier JY. Rehabilitation of arm function after stroke. Literature review. *Ann Phys Rehabil Med* 52: 269-293, 2009.

Park MC, Belhaj-Saïf A, Gordon M and Cheney PD. Consistent features in the forelimb representation of primary motor cortex in rhesus macaques. *J Neurosci* 21: 2784-2792, 2001.

Park MC, Belhaj-Saïf A and Cheney PD. Properties of primary motor cortex output to forelimb muscles in rhesus macaques. *J Neurophysiol* 92: 2968-2984, 2004.

Pizzimenti MA, Darling WG, Rotella DL, McNeal DW, Herrick JL, Ge J, Stilwell-Morecraft KS and 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 and 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 and Krakauer JW. Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci U S A* 106: 1590-1595, 2009.

Riddle CN, Edgley SA and 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 and 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 and 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 and 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, Finklestein SP, Rosen BR and 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 and 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 and 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 Research* 1017: 172-183, 2004.

Schmidlin E, Wannier T, Bloch J, Belhaj-Saïf A, Wyss A and 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. *BMC Neuroscience* 6:56: 2005.

Seitz RJ, Hoflich P, Binkofski F, Tellmann L, Herzog H and 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 and Brown P. Functional significance of the ipsilateral hemisphere during movement of the affected hand after stroke. *Experimental Neurology* 190: 425-432, 2004.

Sunderland, A. Recovery of Ipsilateral Dexterity After Stroke. Stroke 31: 430-433, 2000.

Swayne OB, Rothwell JC, Ward NS and Greenwood RJ. Stages of Motor Output Reorganization after Hemispheric Stroke Suggested by Longitudinal Studies of Cortical Physiology. *Cereb Cortex* 2008.

Takeda K, Gomi Y, Imai I, Shimoda N, Hiwatari M and 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 and Geula C. Motor neurons are rich in non-phosphorilated 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 and 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 and Rouiller EM. A unilateral section of the corticospinal tract at cervical level in primate does not lead to measurable cell loss in motor cortex. *Journal of Neurotrauma* 22: 703-717, 2005.

Ward NS, Brown MM, Thompson AJ and Frackowiak RSJ. Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain* 126: 1430-1448, 2003.

Ward NS and Frackowiak RSJ. Age-related changes in the neural correlates of motor performance. *Brain* 126: 873-888, 2003.

Ward NS and Cohen LG. Mechanisms underlying recovery of motor function after stroke. *Archives of Neurology* 61: 1844-1848, 2004.

Ward NS, Brown MM, Thompson AJ and Frackowiak RS. The influence of time after stroke on brain activations during a motor task. *Ann Neurol* 55: 829-834, 2004.

Ward NS, Newton JM, Swayne OB, Lee L, Frackowiak RS, Thompson AJ, Greenwood RJ and 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 and 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 and 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 and Nichols-Larsen D. Effect of constraint-induced movement therapy on upper extremity function 3 to 9 months after stroke: the EXCITE randomized clinical trial. *JAMA* 296: 2095-2104, 2006.

Zemke AC, Heagerty PJ, Lee C and Cramer SC. Motor cortex organization after stroke is related to side of stroke and level of recovery. *Stroke* 34: e23-e28, 2003.

Legend to Figures

Figure 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 ten monkeys, five (top panel) were control animals for two pilot treatment studies, three were treated with anti-Nogo-A antibody (bottom panel), and two were subjected to an autologous adult progenitor cell therapy (see methods and Table 1).

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

Figure 2

A: Number of pellets retrieved in 30 seconds as a function of time (days) in the modified Brinkman board task for three monkeys (Mk-JU top; Mk- MO middle and Mk-JA bottom). The numbers of retrieved pellets (scores) are indicated separately for the vertical wells (blue

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

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

Figure 3

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

Figure 4

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

Figure 5

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

Figure 6

Same data as in Figure 4 (correlation between long-term post-lesional ipsilesional hand performance and % of recovery of the contralesional hand) for the two slots orientations (vertical and horizontal) analyzed separately. Filled symbols are for the monkeys exhibiting significant long-term enhancement of ipsilesional hand performance (see Figs. 5 and Supplementary Figs. 1 and 2), whereas there was no enhancement for the ipisilesional hand in the monkeys represented by open symbols.

Figure 7

Top panel: The post-lesion long-term ipsilesional hand performance (compared to pre-lesion) is plotted as a function of the volume of the motor cortex lesion (corresponding to the volume of gray matter affected by the lesion in the motor cortex and in the primary somatosensory cortex; see Table 1 for more detail). The dashed line indicates a statistically significant inverse correlation between these 2 parameters ($p<0.01$). Center inset box gives the symbol code referring to the three groups of monkeys (see text). Filled symbols are for the monkeys

exhibiting a significant long-term enhancement of ipsilesional hand performance (see Figs. 2 and 3). Open symbols are for the monkeys without such enhancement of ipsilesional hand performance.

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

Figure 8

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

Supplementary Figure 1

A: Intracortical microstimulation (ICMS) map in Mk-SL as seen from the surface, in which each circular symbol represents the site of each electrode penetration. The black curve represents the approximate location of the central sulcus. The size of the circles indicates the lowest ICMS threshold (in microAmps) obtained along the corresponding electrode penetration (see table on the bottom right of the panel). The color of the circles represents the body region where the ICMS at threshold elicited a movement on the contralateral side. Finger movements were obtained at the lowest threshold along the electrode penetrations depicted in yellow. The grid (in mm) represents the coordinate system used in the chamber that was chronically implanted in the left hemisphere to register the rostro-caudal and medio-lateral positions of the electrode penetrations (separated by 1 mm from each others).

B: On the surface map shown in panel A, the electrode penetration represented by the dashed green line is represented by a single circle, as the electrode penetration is perpendicular to the cortical layer (only one ICMS site with a low threshold, in principle at the depth corresponding to layer V: green arrow). The electrode penetration represented by the dashed red line is also represented on the surface by a single circle (in panel A), although the penetration is roughly parallel to the cortical layers in the rostral bank of the central sulcus. As a consequence, the surface map does not show the multiple ICMS sites where low threshold can be obtained (red arrows), where the electrode tip is close to corticopsinal neurons in layer V. In order to generate a more complete representation of the hand area in the motor cortex, the rostral bank of the central sulcus was “unfolded”, with a rotation to the right (thick blue arrow) using the top of the central sulcus as axis of rotation (small blue arrow). As a consequence, each ICMS site along the penetration represented by the red dashed line appears on the unfolded map, yielding a more realistic extent for the hand area (yellow circles in panel C). The thin blue line indicates the general orientation of the cortical layers, switching from horizontal to roughly vertical in the rostral bank of the central sulcus.

C: In the unfolded map, ICMS sites eliciting a movement at 30 microAmps of stimulation are represented (as a consequence all symbols have the same size). The red crosses are for the ICMS sites selected for ibotenic acid infusion, corresponding to the sites where low thresholds were observed (usually below 10 microAmps). When two adjacent ICMS sites (separated by 1 mm) had the same threshold, the ibotenic acid was injected at a depth in between these 2

sites. Two adjacent sites of infusion (crosses next to each other) on the map are actually separated along the rostra-caudal axis by 1 mm. The black curve shows the approximate location of the central sulcus, whereas the dashed black curve shows the approximate location of the fundus of the central sulcus.

Supplementary Figure 2

Same data as in Figure 3 (7 monkeys) but the number of pellets retrieved (score) is considered for the vertical slots only. Same conventions as in Figure 2B.

Supplementary Figure 3

Same data as in Figure 3 (7 monkeys) but the number of pellets retrieved (score) is considered for the horizontal slots only. Same conventions as in Figure 2B.

Supplementary Figure 4

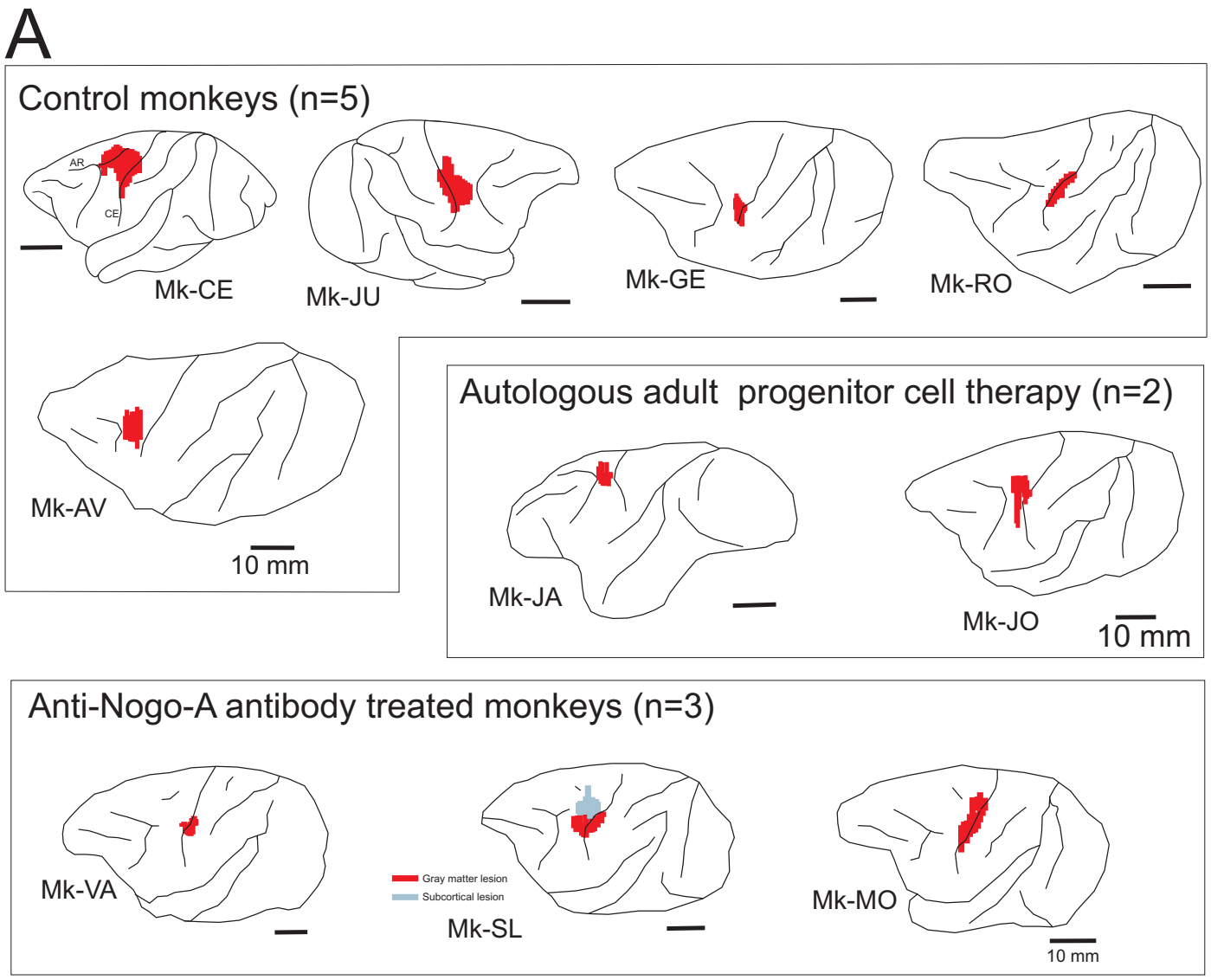
Contact time data for the ten monkeys involved in the study, derived from the first five vertical slots visited by the monkey. The data are presented in the form of box and whisker plots (see legend of Fig. 2), showing the distribution of contact times obtained from the ipsilesional hand (ipsi) or the contralesional hand (contra), pre-lesion (pre) or post-lesion (post), respectively. The statistics are for the comparison of pre- versus post-lesion contact times for each hand (Mann and Whitney test). Short contact time means good manual dexterity.

Supplementary Figure 5

Contact time data for the ten monkeys involved in the study, derived from the first five horizontal slots visited by the monkey. Same conventions as in Supplementary Figure 4.

Supplementary Figure 6

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


A
Control monkeys (n=5)
AR
CE
Mk-CE
Mk-JU
Mk-GE
Mk-RO
Mk-AV
10 mm
Autologous adult progenitor cell therapy (n=2)
Mk-JA
Mk-JO
10 mm
Anti-Nogo-A antibody treated monkeys (n=3)
Mk-VA
Gray matter lesion
Subcortical lesion
Mk-SL
Mk-MO
10 mm

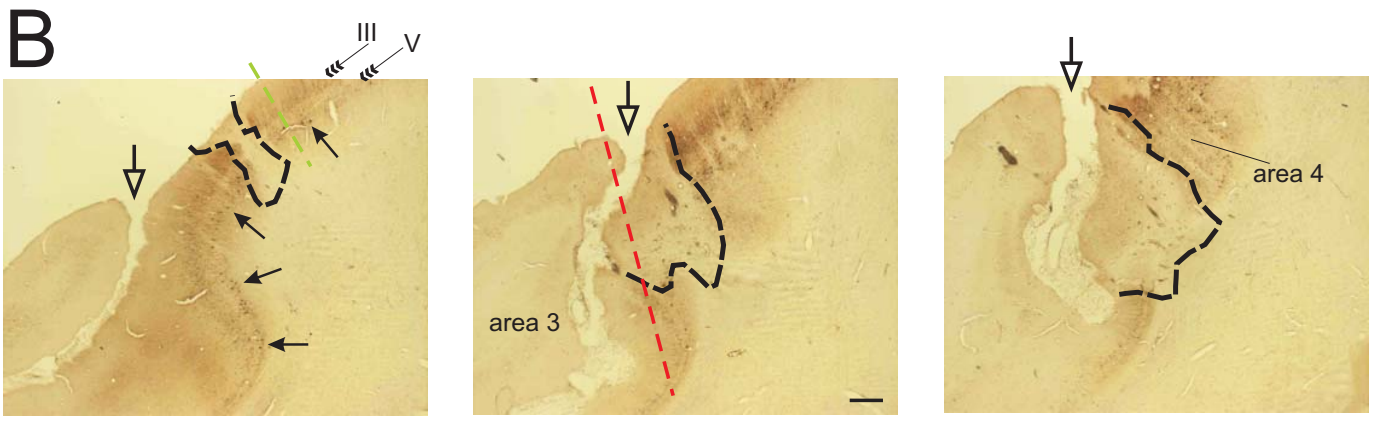

B
III
V
area 3
area 4

Fig. 1

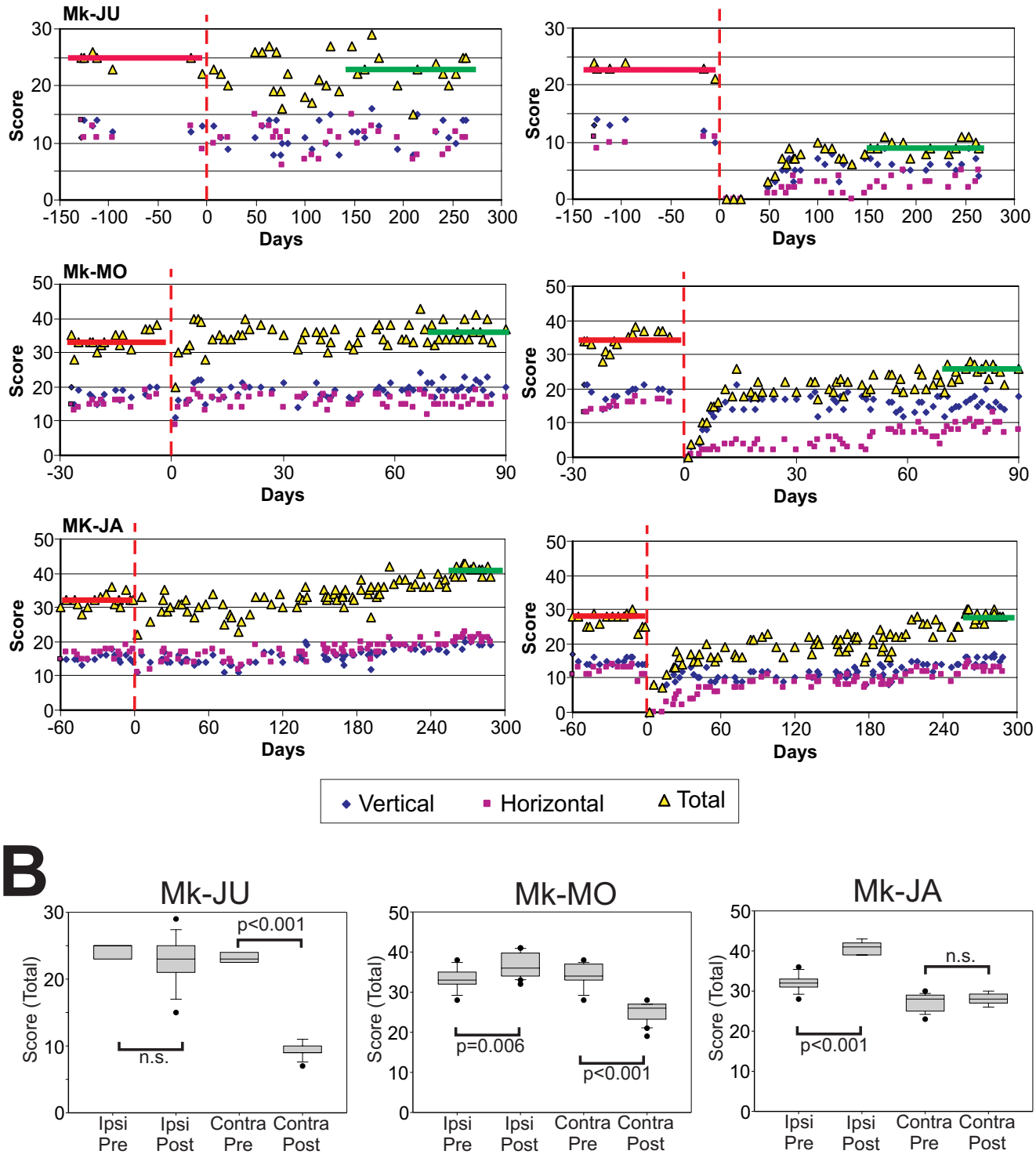

A
Ipsilesional hand
Contralesional hand
Mk-JU
Mk-MO
MK-JA
Score
Days
Vertical
Horizontal
Total
B
Mk-JU
Mk-MO
Mk-JA
Score (Total)
p<0.001
n.s.
p=0.006
p<0.001
p<0.001
n.s.
Ipsi Pre
Ipsi Post
Contra Pre
Contra Post

Fig. 2

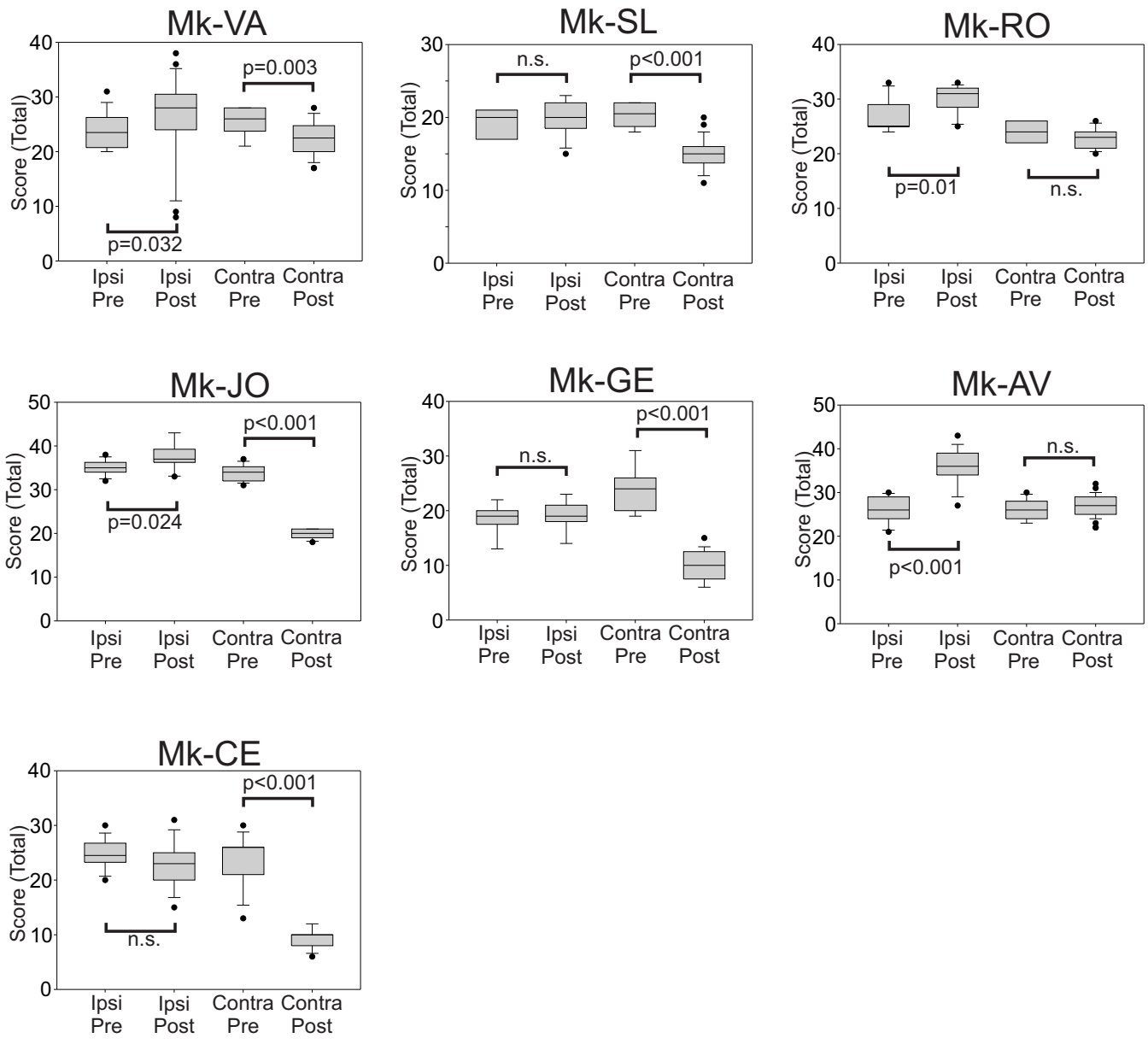

Mk-VA
Score (Total)
p=0.003
p=0.032
Ipsi Pre
Ipsi Post
Contra Pre
Contra Post
Mk-SL
n.s.
p<0.001
Mk-RO
p=0.01
n.s.
Mk-JO
p<0.001
p=0.024
Mk-GE
n.s.
p<0.001
Mk-AV
n.s.
p<0.001
Mk-CE
p<0.001
n.s.

Fig. 3

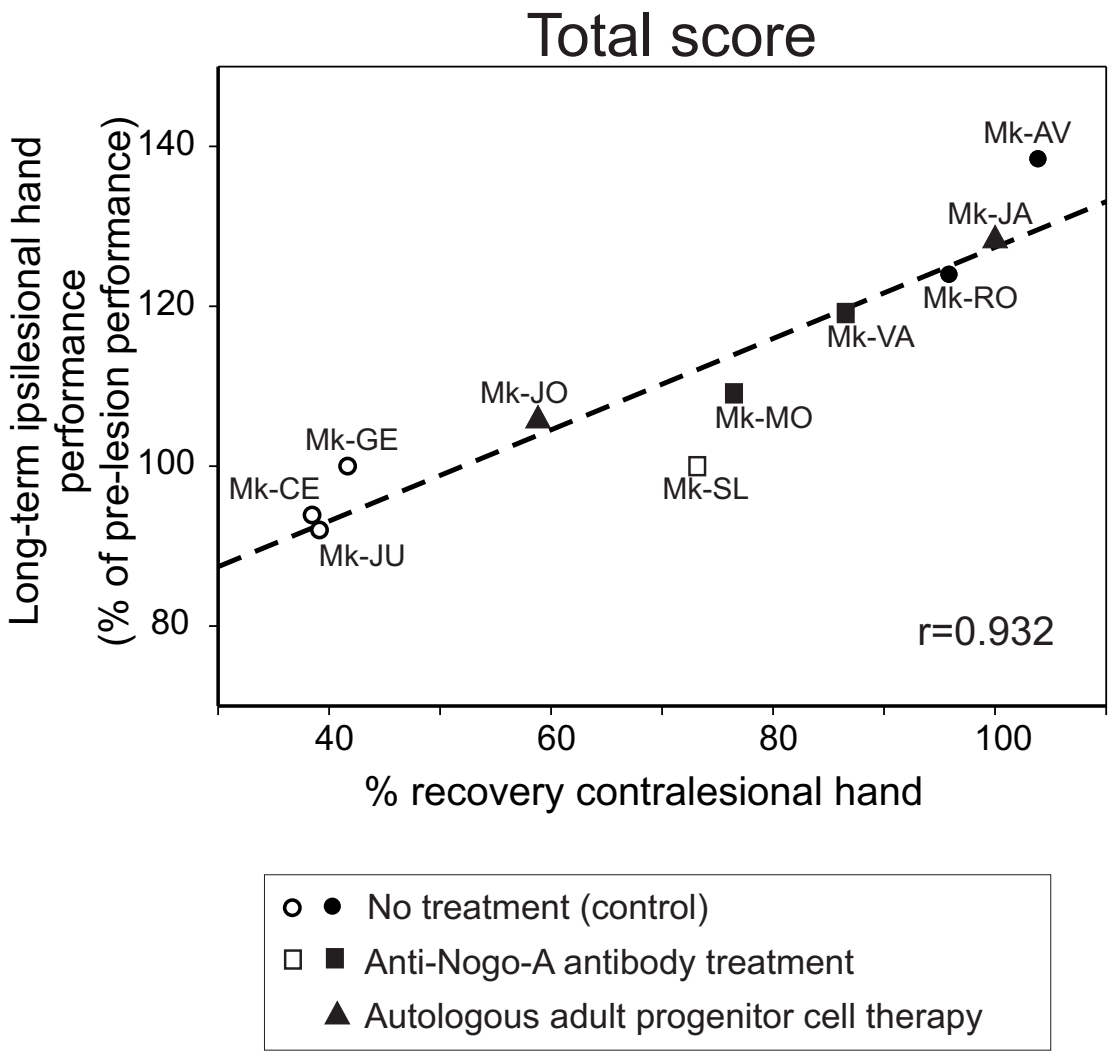

Total score
Long-term ipsilesional hand performance (% of pre-lesion performance)
140
120
100
80
40
60
80
100
% recovery contralesional hand
Mk-AV
Mk-JA
Mk-RO
Mk-VA
Mk-JO
Mk-MO
Mk-GE
Mk-CE
Mk-SL
Mk-JU
r=0.932
No treatment (control)
Anti-Nogo-A antibody treatment
Autologous adult progenitor cell therapy

Fig. 4

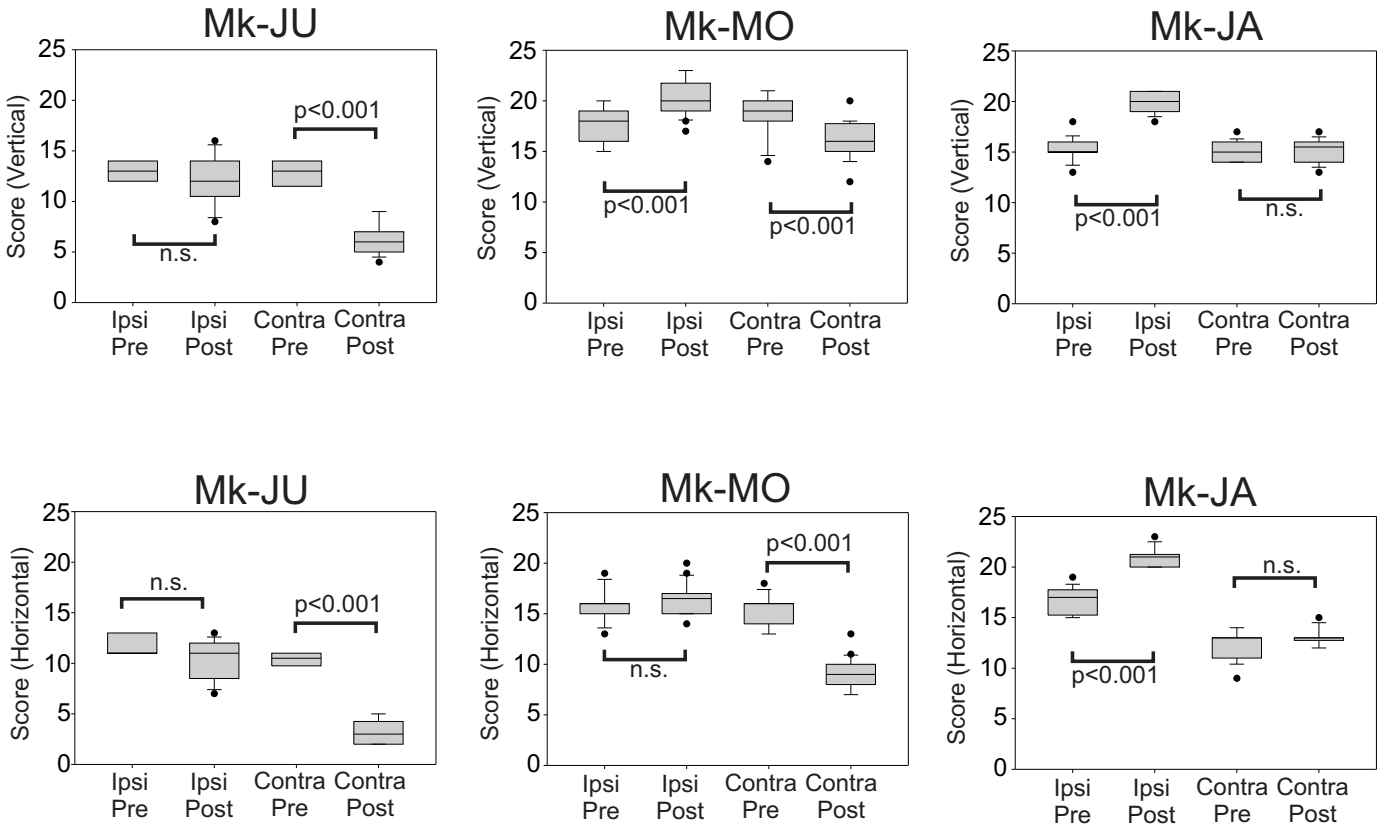

Mk-JU
Mk-MO
Mk-JA
Score (Vertical)
Score (Horizontal)
25
20
15
10
5
0
n.s.
p<0.001
Ipsi Pre
Ipsi Post
Contra Pre
Contra Post

Fig. 5

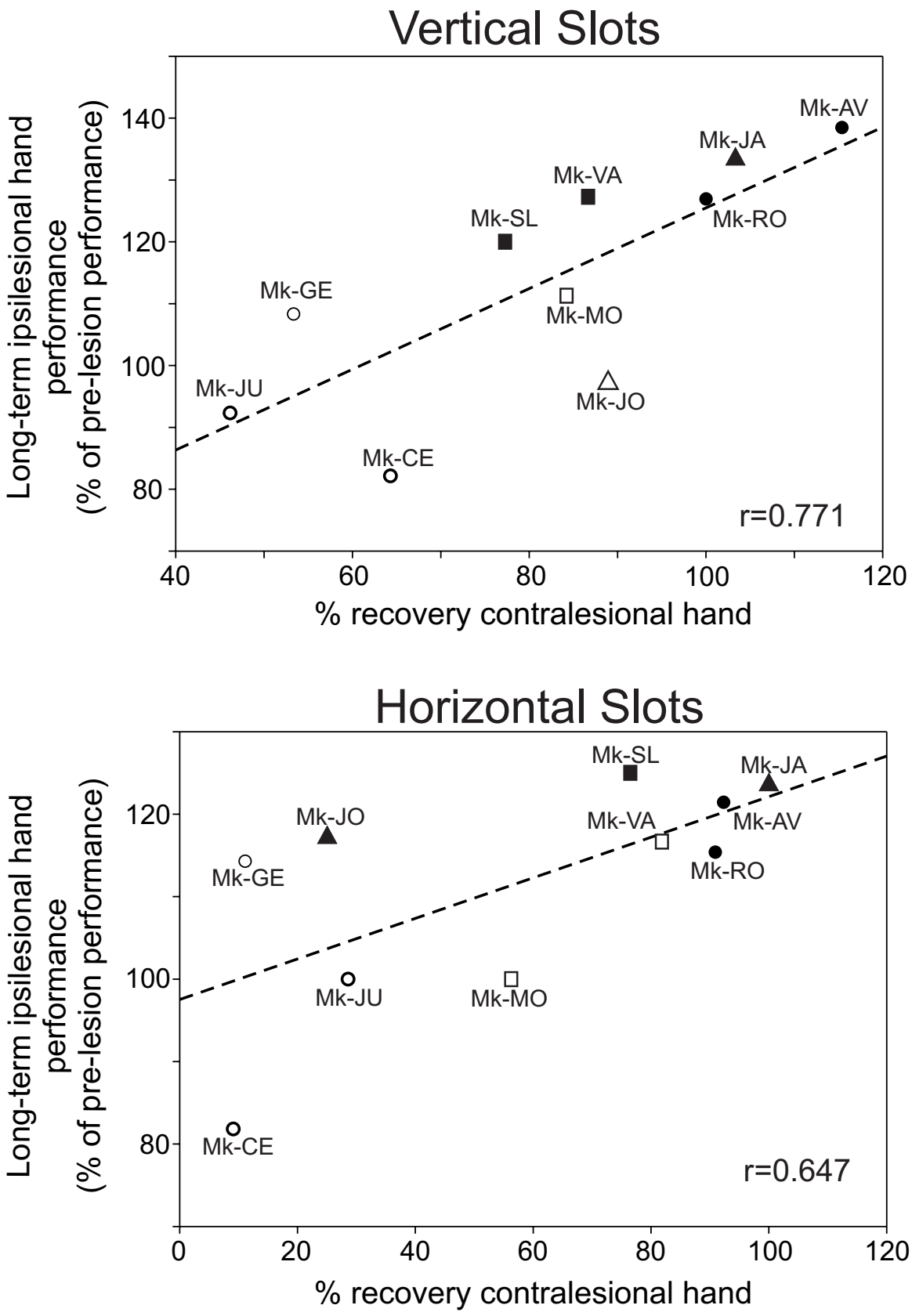
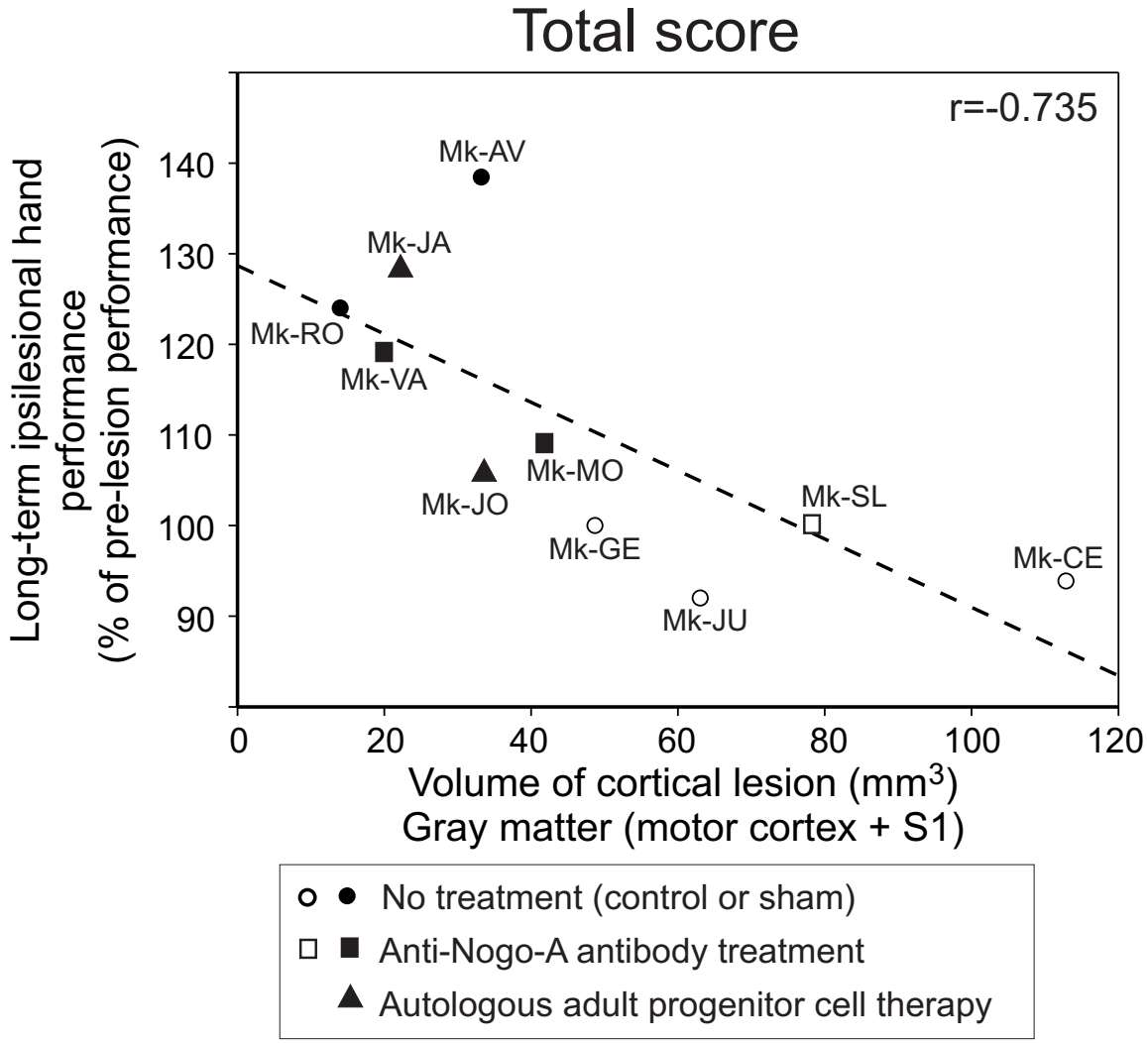

Vertical Slots
Long-term ipsilesional hand performance (% of pre-lesion performance)
% recovery contralesional hand
Mk-AV
Mk-JA
Mk-VA
Mk-SL
Mk-RO
Mk-GE
Mk-MO
Mk-JU
Mk-JO
Mk-CE
r=0.771
Horizontal Slots
Long-term ipsilesional hand performance (% of pre-lesion performance)
% recovery contralesional hand
Mk-SL
Mk-JA
Mk-JO
Mk-VA
Mk-AV
Mk-GE
Mk-RO
Mk-JU
Mk-MO
Mk-CE
r=0.647
No treatment (control)
Anti-Nogo-A antibody treatment
Autologous adult progenitor cell therapy

Fig. 6


Total score
r=-0.735
Long-term ipsilesional hand performance (% of pre-lesion performance)
Volume of cortical lesion (mm3) Gray matter (motor cortex + S1)
Mk-AV
Mk-JA
Mk-RO
Mk-VA
Mk-MO
Mk-JO
Mk-SL
Mk-GE
Mk-CE
Mk-JU
No treatment (control or sham)
Anti-Nogo-A antibody treatment
Autologous adult progenitor cell therapy

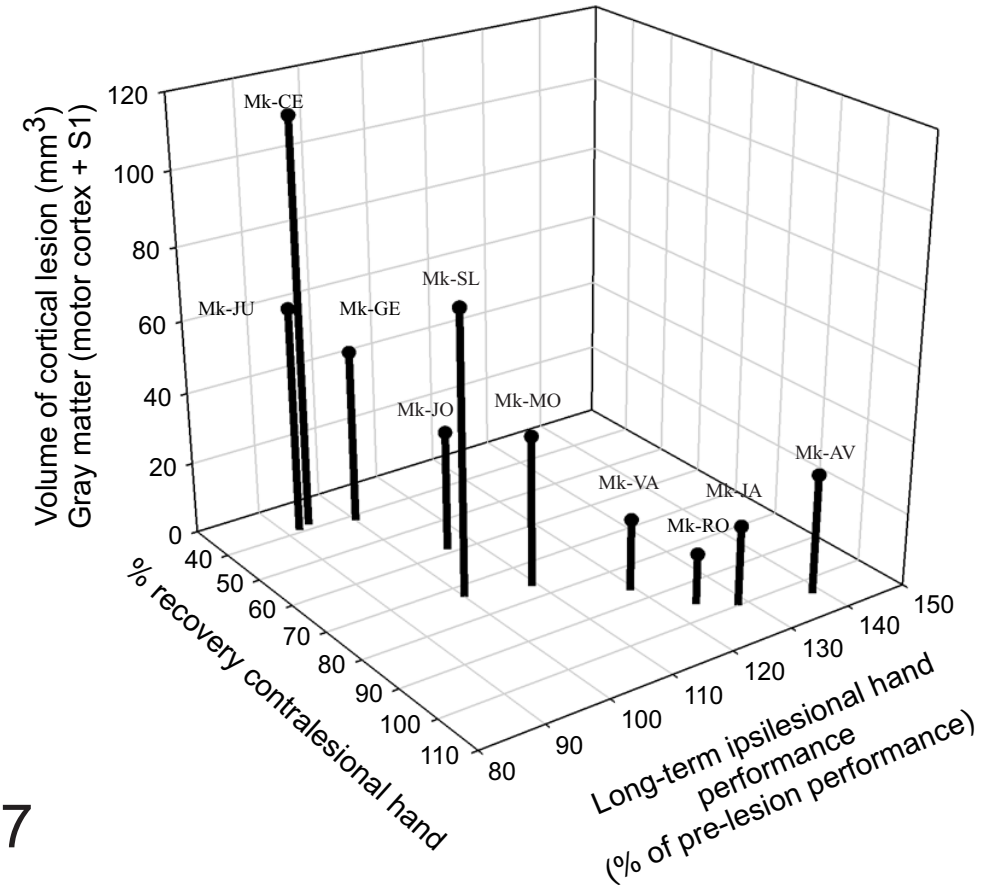

Volume of cortical lesion (mm3) Gray matter (motor cortex + S1)
% recovery contralesional hand
Long-term ipsilesional hand performance (% of pre-lesion performance)
Mk-CE
Mk-JU
Mk-GE
Mk-SL
Mk-JO
Mk-MO
Mk-VA
Mk-JA
Mk-AV
Mk-RO

Fig. 7

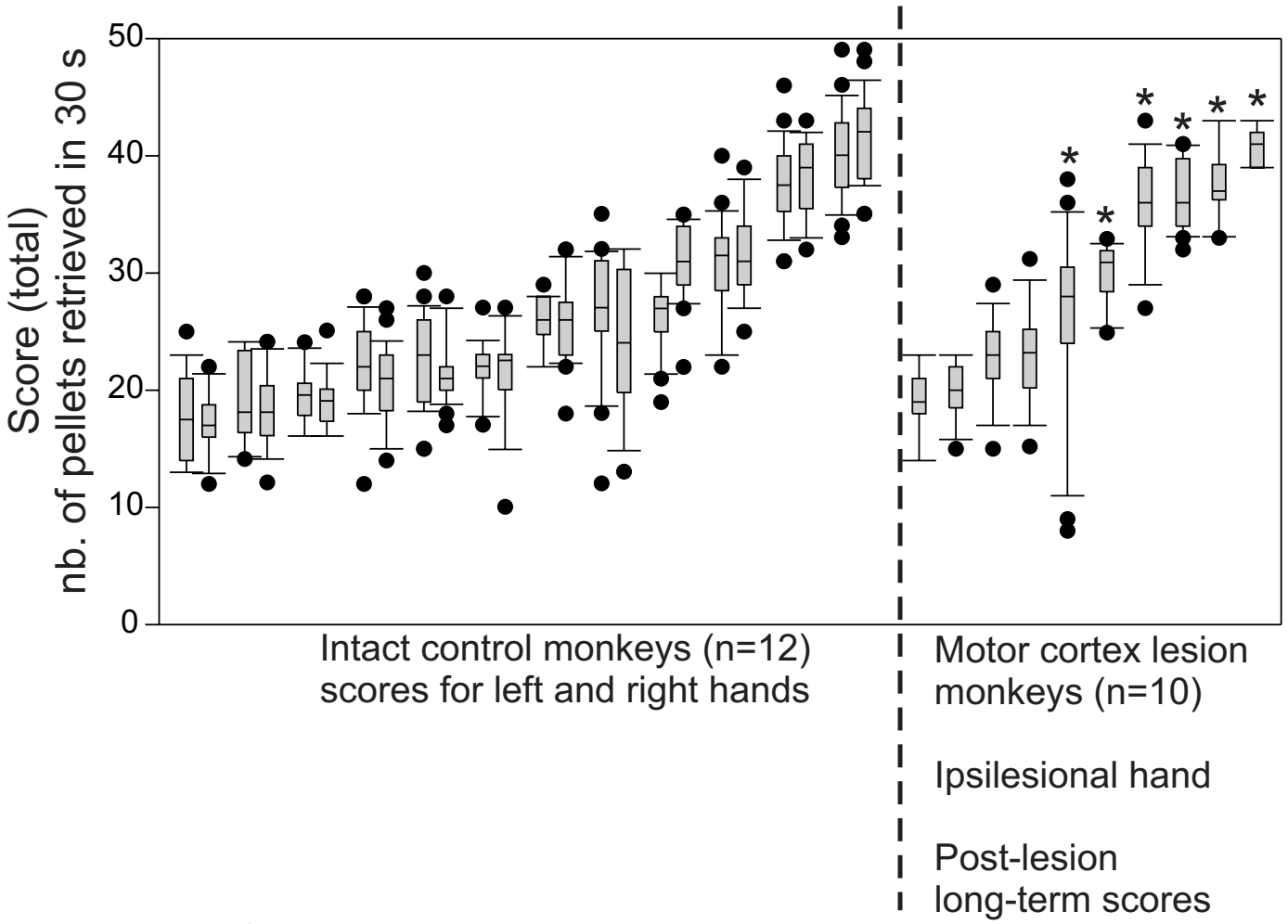

Score (total)
nb. of pellets retrieved in 30 s
50
40
30
20
10
0
*
Intact control monkeys (n=12)
scores for left and right hands
Motor cortex lesion
monkeys (n=10)
Ipsilesional hand
Post-lesion
long-term scores

Fig. 8

Table 1: List of monkeys subjected to permanent primary motor cortex lesion and included in the present study with identification code.

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

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