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MECANISME DE RECUPERATION FONCTIONNELLE SUITE A UNE LESION UNILATERALE DU CORTEX MOTEUR PRIMAIRE CHEZ LE MACAQUE: CONNEXIONS ET REORGANISATION DES AIRES CORTICALES

THESE

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«L’idéal de la vie n’est pas l’espoir de devenir parfait,

C’est la volonté d’être toujours meilleur»

Ralph Waldo Emerson

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Liste des abréviations

AD	Noyau Antérieur Dorsal
AV	Noyau Antérieur Ventral
AIP	Aire Intra-Pariétale
BDA	Biotin Dextran Amine
Cg	Gyrus Cingulaire
CeM	Noyau Centro Médial
CL	Noyau Central Latéral
CM	Noyau Centro Médian
CMA	Aire Motrice Cingulaire
CMAr	Aire Motrice Cingulaire rostrale
CMAc	Aire Motrice Cingulaire caudale
CMAd	Aire Motrice Cingulaire dorsale
CMAv	Aire Motrice Cingulaire ventrale
EEG	Electroencéphalographie
GABA	Acide GammaAminoButyrique
ICMS	Micro-Stimulation Intra Corticale
IL	Noyau Intra Laminaire
IRMf	Imagerie par Résonnance Magnétique fonctionnelle
LD	Noyau Latéral Dorsal
LP	Noyau Latéral Postérieur
mAB	Anticorps monoclonal
MAG	Myelin Associated Glycoprotein
MEG	Magnétoencéphalographie
M1	Cortex Moteur Primaire
MD	Noyau Médio Dorsal
OMgp	Oligodendrocyte Myelin Glycoprotein
OMS	Organisation Mondiale de la Santé
PC	Noyau Para Central
PFc	Cortex Préfrontal
PM	Cortex Prémoteur
PMd	Cortex Prémoteur dorsal

PMv	Cortex Prémoteur ventral
PMd-r	Cortex Prémoteur dorsal rostral
PMv-r	Cortex Prémoteur ventral rostral
PMd-c	Cortex Prémoteur dorsal caudal
PMv-c	Cortex Prémoteur ventral caudal
PU	Noyau Pulvinar
R	Noyau Réticulaire
Rh	Noyau Rhomboide
S1-S2	Cortex Somatosensoriel primaire et secondaire
SMA	Aire Motrice Supplémentaire
SMI-32	Anticorps monoclonal à protéine neurofilament
SNC	Système Nerveux Central
VA	Noyau Ventro Antérieur
VM	Noyau Ventro Médial
VL	Noyau Ventro Latéral
VLa	Noyau Ventro Latéral antérieur
VLp	Noyau Ventro Latéral postérieur
VP	Noyau Ventro Postérieur
VPL	Noyau Ventro Postérieur Latéral
VPM	Noyau Ventro Postérieur Médian
TMS	Stimulation Magnétique Transcranienne
ZI	Zone Incerta

Résumé

Chez les mammifères adultes, les fibres nerveuses du système nerveux central, localisées dans le cerveau et la moelle épinière, sont normalement incapables de se régénérer après une lésion. Le système nerveux périphérique des mammifères comme le système nerveux central des vertébrés inférieurs sont capables, à la suite d'une axotomie (interruption nerveuse), de pousser et de se reconnecter avec les organes cibles, en d'autres termes il présente des capacités de récupération fonctionnelle considérables si les lésions sont modérées. Ces fibres nerveuses sont ainsi capables de former des connexions fonctionnelles avec leurs tissus cibles, c'est-à-dire la capacité intrinsèque de se régénérer à condition d'être placées dans un environnement approprié. Des molécules peuvent promouvoir la repousse des fibres nerveuses, alors que d'autres l'inhibent au contraire. Ces molécules qui sont capables d'empêcher la croissance des axones in vitro ont été découvertes dans le SNC intact dans les années 1990 et sur le site de lésions. Parmi ces molécules, Nogo a été identifié comme une des composantes majeures de la myéline capable d'inhiber la croissance axonale dans le système nerveux central lésé chez l'adulte. Plusieurs autres inhibiteurs ont été identifiés, comme les protéines MAG (myelin associated glycoprotein) ou OMgp (oligodendrocyte myelin glycoprotein). La compréhension des mécanismes d'action de ces molécules en général, et de Nogo en particulier, génère aujourd'hui des espoirs quant au développement de nouvelles thérapies.

Suite aux études qui ont été faites sur l'effet de l'anticorps anti-Nogo-A suite à la lésion de la moelle épinière ou du cortex moteur chez les rats, une étude préliminaire chez des macaques adultes ayant subi une lésion unilatérale du cortex moteur primaire dans le laboratoire du Professeur Rouiller (thèse A. Wyss) suggère que l'anticorps anti-Nogo-A augmente la récupération des fonctions motrices perdues lors de la lésion pour la main contralatérale de ces animaux.

S'intégrant dans ce contexte de recherche, le présent travail de thèse vise à étendre ces données préliminaires et surtout élucider quelques mécanismes de l'effet de l'anticorps anti-Nogo-A sur la récupération de la dextérité manuelle suite à une lésion unilatérale du cortex moteur primaire. Dans une première étude, l'effet de la lésion unilatérale du cortex moteur primaire sur la main ipsilésionnelle a été investiguée, une question controversée dans la littérature (**chapitre I**). Le **chapitre II** est consacré à une étude électrophysiologique basée sur la technique de microstimulation intracorticale (ICMS) avant et après la lésion lorsque les macaques adultes atteignent un niveau stable de récupération avec les tests comportementaux

de «Brinkman modifié», permettant l'établissement des cartes corticales pouvant aider à déterminer si le cortex moteur est modifié et réorganisé. Le **chapitre III** permet d'évaluer le rôle de M1 et de PM dans la récupération des fonctions motrices après lésion de M1 et suite à l'injection de muscimol dans trois aires différentes du cortex moteur.

Après l'achèvement des investigations comportementales et électrophysiologiques, un traceur rétrograde (BDA) a été injecté dans le cortex prémoteur chez trois groupes d'animaux (intacts, non traités et traités) pour des analyses anatomiques sur les connexions callosales d'une part (**chapitre IV**) et thalamocorticales (**chapitre V**). Les résultats ont permis de montrer qu'il y'avait une augmentation des cellules marquées au BDA dans l'hémisphère intact opposé à la lésion chez les singes traités comparés à ceux qui n'ont pas été traités, ce qui suggère qu'un renforcement des entrées provenant de l'hémisphère intact vers le cortex prémoteur ipsilésionnel pourrait contribuer à une meilleure récupération fonctionnelle suite à la lésion unilatérale du cortex moteur primaire.

En résumé, les résultats présentés dans cette thèse offrent une nouvelle perspective quant aux capacités de réparation du système nerveux central adulte suite à une lésion du cortex moteur primaire. Ils montrent que le SNC est capable de se réorganiser suite à la lésion, une réorganisation pouvant favoriser la récupération fonctionnelle. Cette dernière est par ailleurs susceptible d'être renforcée par un traitement avec l'anticorps anti-Nogo-A. Ces résultats contribuent à une meilleure compréhension des mécanismes de récupération fonctionnnelle suite à une lésion unilatérale de M1.

Summary

In adult mammals, the nerve fibers of the central nervous system, localized in the brain and the spinal cord, are normally incapable to regenerate after a lesion. The peripheral nervous system of mammals as the central nervous system of the lower vertebrates are capable, following an axotomy (nervous interruption), to grow and to re-connect with the target organs, in other words it presents considerable capacity of functional recuvery if the lesions are moderate.

Nerve fibers are capable of forming functional connections with their target tissues, an intrinsic capacity to regenerate on the condition of being placed in an appropriate environment. Some molecules can promote the regrowth of nerve fibers, while others inhibit it. These inhibiting molecules, which are capable of preventing the growth of the axons in vitro, were discovered in the intact CNS in the 1990s and on the site of lesion. Among these molecules, Nogo was identified as one of the major constituents of the myelin capable of inhibiting the axonal growth in the lesioned adult central nervous system. Several other inhibitors were identified, such as proteins MAG (myelin associated glycoprotein) or OMgp (oligodendrocyte myelin glycoprotein). The understanding of the mechanisms of action of these molecules in general, and Nogo in particular, generates today hopes as for the development of new therapies today.

Following studies which were made on the effect of the anti-Nogo-A antibody following the lesion of the spinal cord or the motor cortex in rats, a preliminary study in adult macaques subjected to an unilateral lesion of the primary motor cortex in the laboratory of Professor Rouiller (Ph.D. thesis A. Wyss) suggested that the anti-Nogo-A antibody increased the recovery of the motor functions lost during the lesion on the contralateral hand of these animals.

The present thesis aims at extending these preliminary data and to elucidate some of the mechanisms of the effect of the anti-Nogo-A antibody on the recovery of the manual dexterity following an unilateral lesion of the primary motor cortex. In a first study, a still debated issue in the literature was addressed, namely the effect of unilateral lesion of the primary motor cortex on the ipsilesionnal hand (**Chapter I**). **Chapter II** is dedicated to an electrophysiological study based on the technique of intracortical microstimulation (ICMS), before and after the lesion when the adult macaques reached a stable level of the behavioral tests of “modified Brinkman” allowing the establishment of the cortical maps, which can help to determine whether the motor cortex is modified and reorganized. **Chapter III** allows

estimating the role of M1 and PM in the recovery of the motor functions after M1 lesion and following the injection of muscimol in three different areas of the motor cortex.

After the completion of the behavioral and electrophysiological investigations, a retrograde tracer (BDA) has been injected in the premotor cortex of three groups of animals (intact, untreated and treated) for anatomical analyses of the callosal connections (**Chapter IV**) and thalamocortical projections (**Chapter V**). The results allowed showing that there was an increase of BDA stained cells in the intact hemisphere, opposite to the lesion, in the treated monkeys as compared with those who were not treated, suggesting that an increase of inputs from the intact hemisphere may contribute to an enhanced functional recovery.

In summary, the results presented in this thesis offer a new perspective as for the capacities of repair of the adult central nervous system following the lesion of the primary motor cortex; they show that CNS is capable to be reorganized following the lesion, a reorganization possibly underlying functional restitution. The latter is likely to be enhanced with a treatment based on the anti-Nogo-A antibody. These results contribute to a better understanding of the mechanisms of functional recovery following an unilateral lesion of M1.

1 Introduction générale

Les maladies neurodégénératives forment un sous-groupe de maladie dégénérative qui affecte le fonctionnement du cerveau ou, plus généralement, le système nerveux de façon progressive au cours de son évolution. Celles-ci peuvent être plus ou moins longues (de quelques semaines à plusieurs années). Les maladies neurodégénératives peuvent être causées par la mort et la détérioration des neurones, ou d'autres cellules du système nerveux ou encore par la rupture de la gaine de myéline qui recouvre les axones des neurones qui se connectent les uns aux autres. La conséquence pour le malade est donc une altération progressive, souvent irréversible, des fonctions nerveuses qui peuvent conduire à son décès. Le cerveau et la moelle épinière peuvent être touchés par des lésions diffuses ou limitées à certaines zones spécifiques. En fonction des régions du système nerveux atteintes par la maladie, les troubles pourront affecter la motricité, le langage, la mémoire, la perception, la cognition..... Une fois qu'ils sont endommagés ou détruits, les neurones ne peuvent pas être régénérés. Cependant, d'autres parties du cerveau peuvent en partie reprendre les fonctions des neurones perdus, mais les cellules neuronales ne peuvent pas être remplacées. C'est l'une des raisons pour laquelle les maladies neurodégénératives sont des conditions de santé graves.

Les lésions du système nerveux central chez l'adulte, d'origine traumatique ou vasculaire sont extrêmement dévastatrices du fait de la persistance de graves séquelles, résultant également d'une dégénerescence des cellules nerveuses. Ces séquelles sont dues à la très faible capacité du système nerveux central de l'adulte à régénérer et à être plastique après une lésion. En particulier, les lésions du cortex moteur primaire et de la moelle épinière aboutissent à une perte de la fonction motrice.

Dans le cadre de ce présent travail, nous nous limiterons aux handicaps moteurs causés par une lésion du cortex moteur primaire et aux mécanismes qui peuvent permettre la récupération des fonctions.

1.1 Organisation générale du système moteur

Organisation anatomo-fonctionnelle

Le cortex moteur (au sens large) représente la partie du cortex cérébral dans laquelle les neurones montrent une modification de leur activité en relation avec un mouvement volontaire préparé, programmé et exécuté par le sujet. Les aires motrices corticales correspondent pour la plupart d'entre elles aussi à des zones dont la stimulation électrique ou magnétique fait apparaître des mouvements controlatéraux localisés. Il est subdivisé en quatre

régions principales qui sont réciproquement interconnectées (He et al., 1993; Stepniewska et al., 1993): le cortex moteur primaire (M1) situé dans le gyrus précentral, le cortex premoteur (PM) situé rostralement par rapport à M1, l'aire motrice supplémentaire (SMA) située près de la ligne médiane, et enfin l'aire motrice cingulaire (CMA) située dans le gyrus cingulaire. Ces subdivisions sont basées sur des critères anatomiques et physiologiques (Wiesendanger and Wiesendanger, 1985a; Matelli et al., 1985; Luppino et al., 1990; Matelli et al., 1991; Kurata, 1991; Matsuzaka et al., 1992; Luppino et al., 1993; Tanji, 1994; Kurata and Hoffman, 1994; Kurata, 1994; Tanné et al., 1995; Picard and Strick, 1996; Matelli and Luppino, 1996; Caminiti et al., 1996; Passingham, 1996; Preuss et al., 1996; Rizzolatti et al.,1996; Shima and Tanji, 1998; Nakamura et al., 1998; Nakamura et al., 1999; Ikeda et al., 1999; Gabernet et al., 1999).

La Figure 1 montre les subdivisions modernes du cortex frontal chez le singe. La première est basée surtout sur les propriétés fonctionnelles des différentes parties du cortex moteur, et la deuxième, proposée par Matelli et ses collaborateurs (1985; 1991), est basée sur les donnés cytoarchitectoniques et histochimiques, ainsi que sur les propriétés fonctionnelles et hodologiques. La nomenclature adoptée par Matelli et ses collaborateurs (1985; 1991) provient de celle utilisée par von Economo (1925).

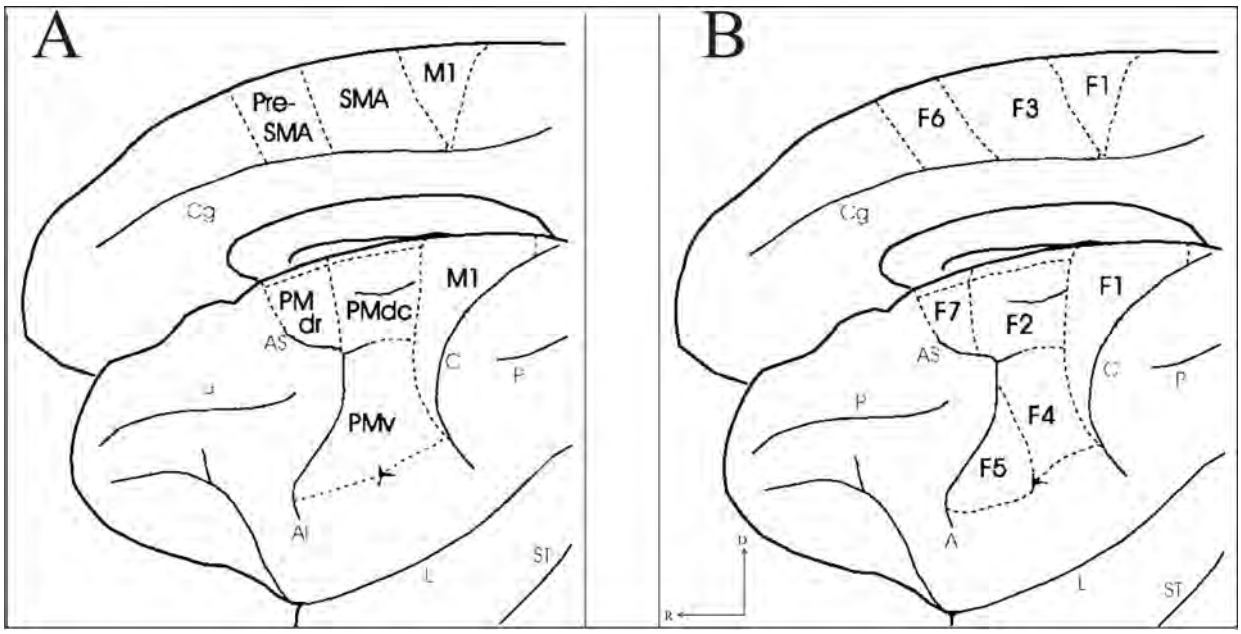


Figure 1: Subdivisions modernes du cortex frontal chez le macaque (Luppino et Rizzolatti, 2000), A: sur la base des propriétés fonctionnelles du cortex moteur, B: sur la base des données cytoarchitectoniques et histochimiques, telles qu'elles apparaissent sur une vue latérale de la face externe de l'hémisphère gauche et la face interne de l'hémisphère droite. R=rostral; D=dorsal. Voir liste des abréviations (page 7).

Le cortex moteur primaire (M1)

Le cortex moteur primaire ou aire motrice primaire (M1) est responsable du contrôle des mouvements fins et précis. L'aire M1 correspond à l'aire 4 de Brodmann, située sur la paroi antérieure du sillon central. Il est nommé F1 d'après la nomenclature introduite par Matelli et ses collaborateurs (1985), ainsi que Rizzolatti et ses collaborateurs (1998). Il est caractérisé histologiquement par la présence de grandes cellules pyramidales principalement dans la couche V (cellules de Betz). Il contient des neurones qui projettent directement vers la moelle épinière, formant environ 50% de la projection corticospinale. Le cortex moteur primaire reçoit des informations principalement de l'aire prémotrice ou cortex prémoteur (PM), et de l'aire motrice supplémentaire (SMA). Une lésion du cortex moteur se traduit par une paralysie (déficit moteur complet) ou une parésie (déficit moteur incomplet). Des lésions de l'aire motrice primaire (comme celles que provoque un accident vasculaire cérébral) entraînent la paralysie des muscles squelettiques régis par cette aire. Si la lésion touche l'hémisphère droit, le côté gauche du corps est paralysé, et vice versa. Toutefois, seuls les mouvements volontaires sont impossibles, les muscles demeurant aptes aux contractions réflexes dont la plupart sont commandés par des centres de la moelle épinière.

Le cortex pré moteur

Le cortex pré moteur (PM) est impliqué dans le contrôle des mouvements en réponse aux stimuli sensoriels aussi bien que la préparation motrice (Godschalk et al., 1985; Weinrich and Wise, 1982; Wise and Mauritz, 1985), il permet d'avoir un contrôle des postures par l'intermédiaire d'une régulation de la musculature axiale et proximale. Chez le macaque, le cortex prémoteur correspond à la région latérale (Godschalk et al., 1984; Rizzolatti et al., 1983; Schell and Strick, 1984), il est subdivisé en deux parties principales: le cortex premoteur ventral (PMv) et le cortex premoteur dorsal (PMd) (Kurata, 1991).

Le cortex premoteur dorsal (PMd) est formé par deux aires: les aires F7 et F2 aussi appelées respectivement partie rostrale du cortex prémoteur dorsal (PMd-r) and partie caudale du cortex premoteur dorsal (PMd-c). Il est possible de produire des mouvements par stimulation électrique expérimentale de F2 (PMd-c), ce qui a permis d'établir une organisation somatotopique grossière, avec une représentation du bras et du pied localisée respectivement du coté ventral et dorsal de la cavité supérieure précentrale. Toutefois, le seuil d'intensité d'une stimulation électrique requise pour évoquer un mouvement est plus grand dans PMd que dans M1 (Kurata and Tanji, 1986; Godschalk et al., 1995; Raos et al., 2003). Les études de Luppino et Rizzolatti (2000) montrent que F2 (PMd-c) n'est pas simplement

impliqué dans l'exécution motrice mais joue aussi un rôle dans certains aspects de la préparation motrice (Wise et al., 1997). PMd-c est une source des projections corticospinales et il est connecté à M1. Par contre, les propriétés fonctionnelles de F7 (PMd-r) sont moins connues, à l'exception de sa partie dorsale qui contient le champ visuel supplémentaire (SEF). PMd-r n'est pas une source de projections corticospinales et il est connecté à PMd-c et Pre-SMA (F6), mais pas à M1. En résumé, les lésions expérimentales, l'inactivation réversible et les études d'enregistrement simple chez le primate ont montré que PMd joue un rôle majeur dans les processus sensorimoteurs nécessaires pour la sélection et l'exécution des mouvements déclenchés par des signaux sensoriels (Passingham, 1985, 1988; Kurata et al., 1994; Wise et al., 1997).

Le cortex prémoteur ventral (PMv), quant à lui, est aussi subdivisé en deux parties: les aires F5 et F4, respectivement appelées PMv-r et PMv-c. Sur la base de stimulations électriques produisant des mouvements, il a été démontré que F4 (PMv-c) contient une représentation du bras, du cou et des mouvements de la face. C'est la source de projections corticospinales et il est directement connecté à M1. F5 (PMv-r) peut aussi être stimulé électriquement pour produire des mouvements, reflétant une représentation des mouvements de la main et de la bouche. PMv-r reçoit une projection modeste de la partie ventrale du cortex préfrontal dorsolateral. Ses principales connexions sont avec l'aire pariétale située à l'intérieur du sillon intra-pariétal, l'aire intra-pariétale antérieure (AIP) et le cortex préfrontal (PFc). L'aire F5 est fortement connectée avec M1, et elle est aussi l'origine d'une projection corticospinale. Les seuils d'intensité pouvant évoquer des mouvements par stimulation électrique intracorticale dans PMv sont plus bas que ceux observé dans PMd (Gentilucci et al., 1988; Rizzolatti et al., 1988; Godschalk et al., 1995; Preuss et al., 1996). La destruction totale ou partielle de PM entraine la perte des habilités motrices qui y sont programmées, sans diminuer la force des muscles squelettiques ni la capacité d'accomplir des mouvements individuels.

L'aire motrice supplémentaire

L'aire motrice supplémentaire (SMA) joue un rôle fondamental dans la planification et l'initiation du mouvement, surtout pour les séquences motrices ayant un niveau de complexité important. Il est subdivisé cytoarchitectoniquement en une zone rostrale (pre-SMA or area F6) et une zone caudale (SMA-propre or F3) (Luppino et al., 1991; Matsuzaka et al., 1992; Picard and Strick, 1996, 2001). Ses fonctions sont considérées être essentiellement exécutives, il

participe avec le cortex moteur primaire dans l'exécution des mouvements, particulièrement pour les mouvements proximaux et axiaux (Luppino and Rizzolatti, 2000).

Les études hodologiques ont aussi démontré que SMA-propre est une source de denses projections corticospinales organisées topographiquement, il envoie des projections denses sur le cortex moteur primaire (M1) et projette aussi directement sur la moelle épinière (Muakkassa and Strick, 1979; Ghosh et al., 1987; Dum and Strick, 1991a,b, 1996, 2005; Galea and Darian-Smith, 1994; He et al., 1995; Rouiller et al., 1996; Hatanaka et al., 2001; Wang et al., 2001; Maier et al., 2002). Les études de Luppino and Rizzolati (2000) basées sur des expériences de microstimulation intracorticale chez le singe ont montré que SMA-propre (F3) est électriquement excitable, avec un courant d'intensité faible (Hummelsheim et al., 1986), ce qui a permis d'établir la présence d'une représentation complète des mouvements du corps. SMA-propre semble être impliqué dans le contrôle des séquences de mouvements (Orgogozo and Larsen, 1979; Roland et al., 1980; Tanji and Shima, 1994). Les mouvements distaux, lorsque évoqués, sont souvent observés en association avec des mouvements proximaux. Les enregistrements neuronaux unitaires ont montré que les neurones de SMA-propre présentent fréquemment des réponses somatosensorielles.

Au contraire de F3 (SMA-propre), F6 (pre-SMA) n'a pas de connexions importantes avec M1 et ne projette pas sur la moelle épinière (Dum and Strick, 1991a,b, 1996, 2005; Tokuno and Tanji, 1993; Galea and Darian-Smith, 1994; Lu et al., 1994; He et al., 1995; Hatanaka et al., 2001; Wang et al., 2001). Par contre pre-SMA est densément interconnecté avec différentes régions du cortex préfrontal (Luppino et al., 1990, 1993; Bates et Goldman-Rakic, 1993; Lu et al., 1994; Wang, 2005). Etonnamment, pre-SMA ne semble pas être densément connecté avec SMA-propre (Luppino et al., 1990, 1993; Wang et al., 2001). Les études de Luppino et Rizzolati (2000) montrent que pre-SMA est faiblement excitable par microstimulation intracorticale. Les réponses motrices peuvent être évoquées seulement avec des intensités de courants élevés, et elles consistent en des mouvements lents et complexes limités au bras. Les enregistrements unitaires de neurones ont montré que dans pre-SMA, à la différence de SMA-propre, les réponses visuelles, sont communes tandis que les réponses somatosensorielles sont rares. Pre-SMA est la source d'une projection corticospinale modeste, n'a pas de connexion directe avec M1 et est connectée avec de nombreuses aires prémotrices rostrales et caudales. Pre-SMA serait donc impliqué dans les séquences de mouvements d'apprentissage (mémoire procédurale).

En résumé, SMA-propre peut jouer un rôle important dans le contrôle de la posture et particulièrement dans l'ajustement postural précédant les mouvements volontaires, tandis que,

pre-SMA semble être un point nodal dans la transmission des informations limbiques et préfrontales à d'autres aires motrices. Fonctionnellement, pre-SMA semble être associé à la sélection et la préparation de mouvement et particulièrement au contrôle des actions en termes de décision de quand commencer un mouvement selon les contraintes externes et la motivation. De par ses propriétés, pre-SMA semble jouer un rôle important dans l'organisation des séquences de mouvements complexes. Il est important de noter que SMA-propre et pre-SMA de l'homme sont considérés comme des homologues de F3 et F6 du singe, respectivement.

L'aire motrice cingulaire (CMA)

L'aire motrice cingulaire est une région du cortex moteur impliquée dans le mouvement volontaire. Cette région peut être subdivisée en deux parties : une partie rostrale (CMAr), qui a un rôle important dans le contrôle cognitif des mouvements et une autre partie caudale (CMAc) directement impliquée dans leur exécution (Dum and Strick, 1991). CMAc est à son tour subdivisé en deux parties: une partie ventrale (CMAv) et une partie dorsale (CMAd) (Dum and Strick, 1991; Picard and Strick, 1996). Ces trois régions (CMAr, CMAv, CMAd) envoient des projections directes sur la moelle épinière (Biber et al., 1978; Dum and Strick, 1991a, 1996; Galea and Darian-Smith, 1994; He et al., 1993, 1995; Hutchins et al., 1988; Keizer and Kuypers, 1989; Macpherson et al., 1982; Morecraft et al., 1997; Murray and Coulter, 1981; Nudo and Masterton, 1990; Toyoshima and Sakai, 1982) et elles sont réciproquement connectées au cortex prémoteur, (Barbas and Pandya, 1987; Deacon, 1992; Ghosh and Gattera, 1995; Godschalk et al., 1984; Kunzle, 1978; Kurata, 1991; Matelli et al., 1986; Morecraft and Van Hoesen, 1993) et au cortex moteur primaire (Dum and Strick, 1991a; Leichnetz, 1986; Morecraft et al., 1997; Morecraft and Van Hoesen, 1992; Nimchinsky et al., 1996). Cette région corticale occupe ainsi une position stratégique lui permettant d'intégrer des informations d'ordre émotionnel dans le cadre des processus décisionnels et de jouer en retour un rôle majeur dans la planification de l'action (Paus, 2001). CMAr semble jouer un rôle particulier dans la commande au plus haut niveau du comportement moteur, ceci par le fait que cette aire reçoit des projections importantes et directes du cortex préfrontal et du système limbique (aire 23c, 24c) (Luppino et al., 1990, Morecraft and Van Hoesen, 1993, 1998). Par contre, CMAd et CMAv semblent jouer un rôle dans l'exécution de mouvements, particulièrement dans l'orientation des mouvements des membres.

1.2 Organisation somatotopique

En 1870, Fritsch et Hitzig (Fritsch and Hitzig 1870) montrèrent que la stimulation électrique du cortex frontal du singe et du chien évoquait le mouvement des pattes du coté opposé. La découverte de l'excitabilité électrique du cerveau permit par la suite l'exploration des fonctions corticales. Puis Charles Sherrington (1898) a montré que, chez le primate, la stimulation électrique la plus efficace est celle délivrée à la région prérolandique (aire 4 de Brodmann) qui garde depuis le nom de aire motrice primaire. Penfield et Boldrey ont pu stimuler électriquement diverses régions du cortex cérébral lors d'opération neurochirurgicales chez l'humain, révélant les premières cartographies fonctionnelles humaines (Penfield and Boldrey, 1937). Ils ont alors mis en évidence une région corticale spécialisée dans la motricité, mais également la présence, à l'intérieur de cette région, d'une représentation de chaque région du corps. De là est né l'homunculus de Penfield, qu'il a présenté avec Rasmussen en 1950 (Penfield and Rasmussen, 1950).

La stimulation électrique a donc été à l’origine du concept de la somatotopie (Penfield et Rasmussen, 1950). Ainsi donc, chaque partie du corps est représentée sur le cortex moteur primaire telle une carte (Fig. 2). Il existe une organisation somatotopique du cortex moteur. Par exemple, la surface correspondant à la représentation de la face et des mains est plus étendue que celle du tronc car la musculature de ces zones exige un contrôle moteur particulièrement fin. Cette meilleure dextérité et rapidité du mouvement des mains et de la face confère à l’homme la parole et l’usage d’outil. La carte somatotopique n’est pas immuable, elle peut se modifier dans le temps suite à une lésion, une expérience ou un entraînement (Nudo et al., 1992; Nudo et al., 1996a,b; Nudo et al., 2001; Dancause et al., 2006b).

La notion d'homoncule moteur suppose que l'aire motrice primaire constitue une projection systématique du corps et que certains neurones corticaux correspondent spécifiquement aux muscles qu'ils commandent. On sait maintenant que cette conception n'est pas tout à fait exacte. La recherche sur le fonctionnement de l'aire motrice primaire indique en effet qu'un muscle donné est régi par de nombreux points du cortex et que chaque neurone moteur cortical envoie des influx nerveux à plus d'un muscle. Autrement dit, les neurones moteurs corticaux régissent des muscles qui fonctionnent en synergie (Schieber, 1991; 1995).

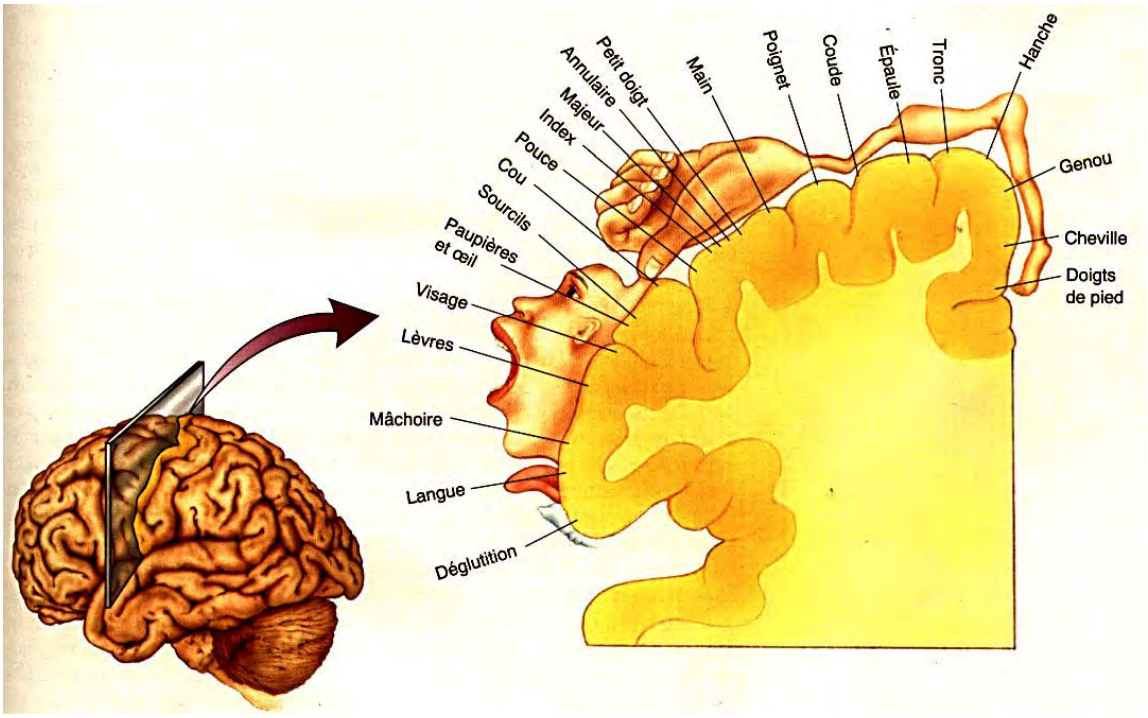


Figure 2: Représentation somatotopique des muscles dans le gyrus précentral contrôlant différentes parties du corps suite à de faibles stimulations électriques dans le cortex moteur primaire humain (aire 4), selon l'Homunculus de Penfield et Rasmussen (1950). http://archimede.bibl.ulaval.ca/archimede/fichiers/23445/ch01.html.

1.3 Organisation cellulaire

L'étude histologique du cortex cérébral a commencé très tôt. Les types cellulaires ont été mis en évidence dès le XIX^{e} siècle. De grands noms ont été associés à ces découvertes, tels que les prix Nobel Santiago Ramon y Cajal et Camillo Golgi. Le cortex cérébral est une mince couche de tissu nerveux dont la surface est d'environ 2 200 cm^{2} (la surface d'un carré de 47 x 47 cm) et dont l'épaisseur varie entre 3 et 4,5 mm suivant les régions. Son volume est d'environ 600 cm^{3}. Il contient entre 10^{9} et 10^{10} neurones et un nombre plus important encore (mais inconnu) de cellules gliales. Au microscope, le néocortex humain apparaît subdivisé en six couches (Fig. 3), avec cependant des variations entre différentes régions. Les couches sont numérotées depuis la surface. On distingue dans l'ordre:

✓ La couche I (couche moléculaire). Elle contient des axones et des dendrites. Les neurones des couches internes y envoient des dendrites courtes et orientées perpendiculairement à la surface du cortex et des axones longs orientés parallèlement à cette surface (Jones and Powell, 1970). On y trouve aussi quelques neurones de Cajal-Retzius et des neurones étoilés.

✓ La couche II (couche granulaire externe), contient de petits neurones dits «neurones granulaires». Elle reçoit les afférences d'autres aires du cortex. On parle en ce qui la concerne de connexions cortico-corticales afférentes.

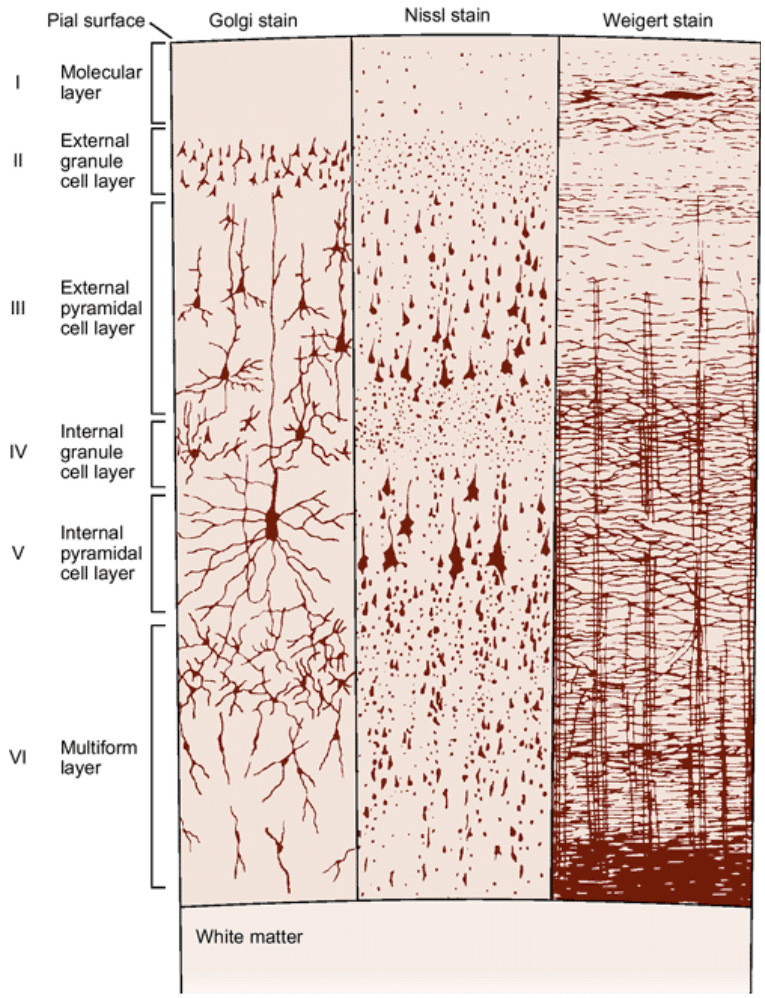
✓ La couche III (couche pyramidale externe), constituée de cellules pyramidales, émet des connexions vers d'autres zones du cortex cérébral. Il s'agit ici de connexions cortico-corticales efférentes.

✓ La couche IV (couche granulaire interne), elle contient des neurones étoilés et pyramidaux. C'est par cette couche que les informations ascendantes (en général du thalamus) entrent dans le cortex. Elle reçoit aussi des afférences en provenance de l'autre hémisphère cérébral.

✓ La couche V (couche pyramidale interne), contient essentiellement des neurones pyramidaux dont les dendrites apicales se projettent soit dans la couche moléculaire (couche I) soit dans la couche granulaire interne (couche IV). C'est également une couche envoyant des connexions efférentes qui sortent du cortex, par exemple des projections sous-corticales vers les motoneurones, mais aussi des projections cortico-corticales.

✓ La couche VI (couche polymorphe), c’est la couche la plus interne à l'état adulte. Elle envoie des prolongements axonaux en direction du thalamus permettant une rétroaction sur les entrées du cortex cérébral. Elle est à l'origine aussi des fibres commissurales et des fibres d'association. Ces couches corticales ne sont pas un simple empilement de neurones. Les neurones s'organisent en unités fonctionnelles prenant la forme de colonnes perpendiculaires à la surface du cortex, chacune assurant une fonction précise.

A ce stade, il faut relever que le cortex moteur, contrairement aux autres régions du cortex cérébral, est dépourvu de la couche IV, ce qui explique son appelation de «cortex agranulaire».



<u>Figure 3</u>: Disposition et densité des neurones du cortex cérébral dans les différentes couches. L'apparence du cortex dépend de la coloration utilisée. La coloration de Golgi révèle les corps cellulaires des neurones et les dendrites. La coloration de Nissl montre les corps cellulaires et les dendrites proximaux et la coloration de Weigert pour les fibres myélinisées révèle la distribution axonale (Heimer, 1994).

1.4 Afférences et efférences du cortex moteur

Le cortex moteur est un haut lieu de convergence. Les afférences du cortex moteur proviennent à partir des hémisphères ipsilatéral et contralatéral des autres aires corticales (afférences cortico-corticales) tel que le cortex préfrontal (aires adjacentes du cortex moteur, aires motrices homologues de l'hémisphère controlatéral, via le corps calleux), somesthésique, visuel et auditif via le cortex pariétal. Ses afférences sont aussi sous-cortico-corticales, d'origine thalamique, par lequel passent les efferences des noyaux gris centraux et du cervelet.

Il existe trois efférences majeures du cortex moteur:

✓ la première, et la plus importante, est la projection motrice du cortex sur la moelle épinière (faisceau corticospinal ou pyramidal) formé par les axones des neurones pyramidaux principalement de la couche V, dont 60% sont issus du cortex moteur primaire, 30% du cortex prémoteur et préfrontal et 10% du cortex pariétal. Le faisceau corticospinal constitue des projections directes des neurones corticaux vers les motoneurones α (particulièrement vers les motoneurones contrôlant les mouvements des doigts), les motoneurones gamma et les interneurones.

✓ La projection motrice sur les noyaux gris centraux (corticostriatale): voie sous-cortico-corticale (projection sur le striatum) formée par des axones destinés au noyau caudé et au putamen.

✓ La projection motrice du cortex sur le tronc cérébral avec des axones destinés au noyau rouge, à la formation réticulée et aux noyaux du pont.

✓ Une quatrième efférence, moins importante, du cortex moteur envoie des projections vers des aires motrices controlatérales via le corps calleux.

La totalité du cortex moteur émet des fibres qui descendent en forme de couronne et vont jusqu'à la moelle (faisceau corticospinal et cortico-rubrospinal) ou se projettent sur le tronc cérébral (faisceau cortico-réticulaire ou faisceau vestibulospinal) ou les noyaux gris centraux (faisceau sous-cortico-cortical).

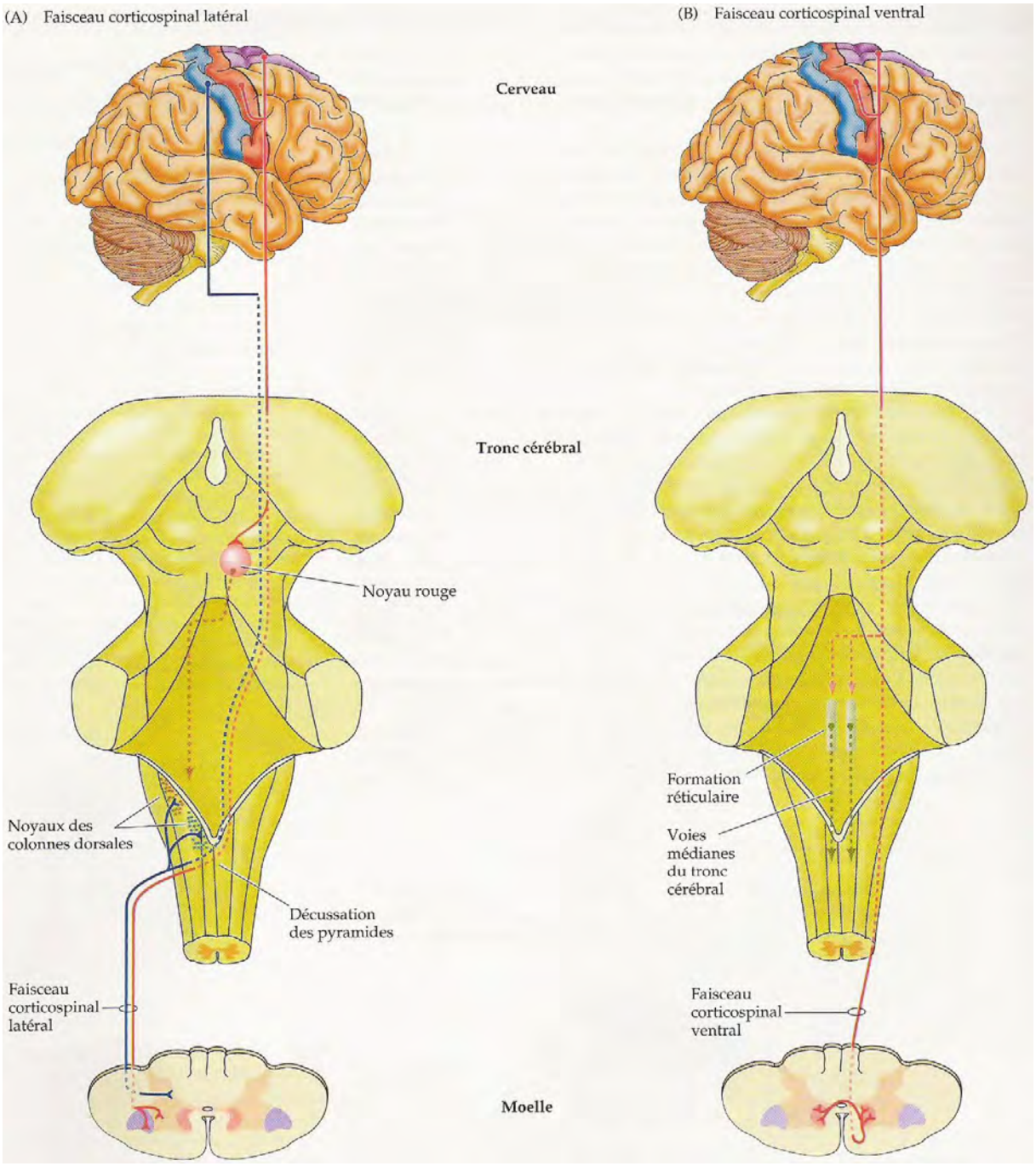
1.5 Les voies motrices descendantes

Les voies motrices permettent à partir des aires corticales et via la moelle épinière de transmettre l'information motrice aux muscles, que ce soit pour des mouvements volontaires, des mouvements posturaux ou des réflexes antigravitationnels. Mais les voies empruntées ne sont pas les mêmes. Pour cela, les axones des neurones du cerveau empruntent deux systèmes majeurs pour atteindre la moelle épinière: un système latéral (voies corticospinale et rubrospinale) véhiculant les commandes motrices volontaires et un système ventromédian (voies tectospinale, vestibulospinale et réticulospinale) acheminant les commandes posturales et les réflexes antigravitationnels. La capacité à réaliser de manière précise une opposition entre le pouce et l'index, aussi bien la réalisation des mouvements indépendants de nos doigts, est sous control direct de la voie corticospinale. Dans le cadre du présent travail, nous nous limiterons seulement au système moteur latéral composé de la voie corticospinale et de la voie rubrospinale, car plus impliqué dans la réalisation des mouvements volontaires de la musculature distale.

La voie corticospinale

C’est la voie la plus importante des voies motrices descendantes. C’est un nouveau système phylogénétique qui apparaît premièrement chez les mammifères et se développe principalement chez les primates (Shapovalov, 1975; Phillips and Porter, 1977; Kuypers, 1981; Porter and Lemon, 1993). Elle prend son origine au niveau du cortex cérébral et représente, dans le système nerveux, la voie la plus longue et l’une des plus importantes, quantitativement (10^6 axones). Les axones issus du cortex passent par la capsule interne, reliant le télencéphale au thalamus, traversent le mésencéphale et le pont, et se réunissent pour former un faisceau dense à l’allure d’une pyramide au niveau du bulbe **(faisceau pyramidal)**. A la jonction entre le bulbe et la moelle épinière, le faisceau pyramidal présente une décussation, c'est-à-dire que les axones du cortex moteur droit vont traverser la ligne médiane puis cheminer dans la colonne latérale gauche de la moelle épinière en formant le **faisceau corticospinal latéral ou croisé** (et inversement pour les axones du cortex moteur gauche) (Fig. 4A). Les axones du faisceau corticospinal latéral se terminent dans la partie dorsolatérale de la corne ventrale et dans la substance grise intermédiaire, où se trouvent localisés les motoneurones et les interneurones de commande des muscles distaux (Fig. 5), ils représentent 90% des axones du cortex moteur (Galea and Darian-Smith. 1997a; Rouiller et al., 1996; Lacroix et al., 2004). Les 10% des axones du cortex moteur restants ne vont pas subir de décussation entre le bulbe et la moelle, mais vont descendre directement dans le cordon médio-ventral ipsilatéral de la moelle en formant le faisceau pyramidal direct, sans croiser la ligne médiane au niveau bulbaire. Ces neurones forment le **faisceau corticospinal ventral** (Fig. 4B) qui se termine dans la partie médiane de la corne antérieure ou ventrale où se trouvent des motoneurones de commande des muscles axiaux et proximaux (Fig. 5).

Les fonctions possibles de la voie corticospinale sont: contrôle descendant des afférences, incluant la nociception (Cheema et al., 1984; Wall and Lidierth, 1997), sélection, déclenchement et gain du contrôle des réflexes spinaux (Evarts and Tanji, 1976), excitation directe et indirecte des motoneurones (Porter and Lemon, 1993), inhibition des motoneurones (Jankowska et al., 1976; Maier et al., 1998), contrôle autonomique (Bacon and Smith, 1993).



<u>Figure 4</u>: Représentation de la voie corticospinale. A: Faisceau corticospinal latéral, B: faisceau corticospinal ventral. (Figure tirée de «Neurosciences», Purves, Augustine, Fitzpatrick, Katz, LaMantia, McNamara, 1998).

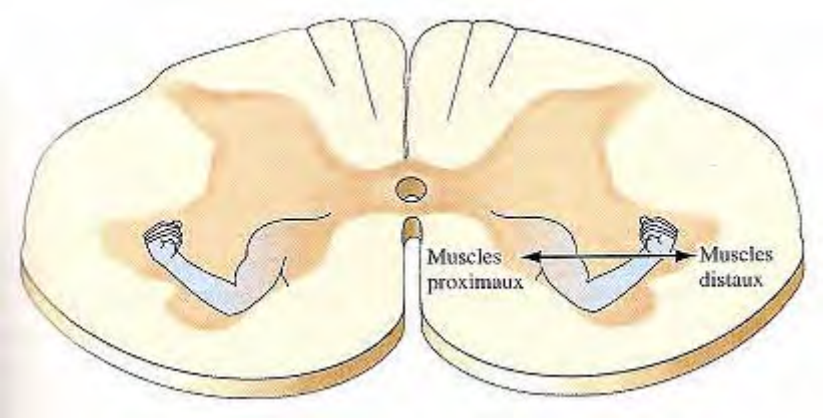


Figure 5: Organisation somatotopique des motoneurones α sur une coupe transversale de la corne ventrale de la moelle cervicale. Les motoneurones innervant la musculature axiale sont situés en position médiane, ceux qui innervent la musculature distale occupent une position plus latérale. http://www.ncbi.nlm.nih.gov/books/NBK10816/figure/A1092/

Le système corticomotoneuronal (CM)

Si la main est habile, c'est notamment parce qu'elle est contrôlée par un système neurologique particulier : le système corticomotoneuronal (Lemon, 1993, 2008). Ce système est spécifique chez le singe et chez l'homme, et il permet un contrôle moteur très précis des doigts (dextérité manuelle), comme l'action d'opposer le pouce avec chaque doigt successivement. En général, la dextérité manuelle se développe en parallèle à l'établissement des connexions corticomotoneuronales monosynaptiques (Bernhard et al., 1953; Heffner and Masterton, 1975). Chez les rongeurs, la majorité des axones de la voie corticospinale descend dans le funiculus dorsal et se termine principalement dans la corne dorsale de la moelle épinière, cette voie corticospinale présente des différences anatomiques majeures avec ceux des chats et des singes (Kuypers, 1981; Casale et al., 1988). Des études neuroanatomiques chez les primates ont montré que la majorité des terminaisons des fibres corticospinales ne sont pas distribuées dans la couche IX mais dans la zone intermédiaire de la substance grise de la moelle épinière (Kuypers, 1981; Ralston and Ralston, 1985; Bortoff and Strick, 1993), ou les interneurones de types variés sont localisés. Cependant, il a été démontré que la dextérité manuelle chez le primate pouvait aussi se réaliser via la voie corticomotoneuronale indirecte (Sasaki et al., 2004). Nishimura et Tadashi (2007) ont montré que si les connexions corticomotoneuronales directes étaient interrompues, la voie corticomotoneuronale indirecte pouvait transmettre la commande pour contrôler les mouvements fins des doigts. Bernhard et Bohm (1954) ont suggéré que les connexions corticomotoneuronales pourraient être d'une importance particulière pour le contrôle des mouvements fins des doigts, et qu'elles

pourraient aussi impliquer des muscles proximaux. Le système corticomotoneuronal contribue aussi au control de la force lors de la pince de précision (Maier et al., 1993). Bien que plusieurs accents ont été placés sur le rôle du système corticomotoneuronal dans le contrôle des mouvements des doigts (Bortoff and Strick, 1993; Lemon, 1993), il faudrait se rappeler que Bernhard et Bohm (1954) ont montré que les réponses corticomotoneuronales monosynaptiques pourraient être enregistrées au niveau des nerfs des membres supérieurs et antérieurs. Il y a des preuves abondantes que plusieurs muscles des membres supérieurs et antérieurs reçoivent des projections corticomotoneuronales (Jankowska et al., 1975; Rothwell et al., 1991; Palmer and Ashby, 1992; Nielsen and Petersen, 1995; Nielsen et al., 1995). Kuypers (1978) a montré une fonction plus générale de la projection corticospinale, il a suggéré qu'elle a fourni la capacité pour le «fractionnement» des mouvements et le contrôle de petits groupes de muscles de manière hautement sélective. Ainsi, le système corticomotoneuronal pourrait contribuer au contrôle moteur «analytique» de base décrit premièrement par Sherrington (1898).

La voie rubrospinale

La voie rubrospinale est une des composantes du système moteur latéral. Elle prend son origine au niveau du noyau rouge situé dans le mésencéphale. Le noyau rouge reçoit des informations afférentes du cortex frontal. La voie rubrospinale intervient dans la motricité et la coordination de muscles distaux des membres inférieurs et supérieurs. Les axones issus du noyau rouge décussent au niveau du pont et rejoignent ceux du faisceau corticospinal au niveau de la colonne latérale (région latérale de la corne ventrale et de la zone intermédiaire) de la moelle épinière (Fig. 6). La voie rubrospinale participe, avec la voie corticospinale directe, au contrôle moteur des bras (ou des pattes antérieures). Le noyau rouge reçoit des informations du cortex frontal, une région corticale qui contribue aussi massivement au faisceau corticospinal. La voie rubrospinale émane d'une population de neurones de grande taille occupant le pole caudal du noyau rouge (noyau rouge magnocellulaire) et qui ne présente qu'une faible fraction du nombre total de neurones du noyau; chez l'humain, le noyau rouge n'a que très peu de neurones de grande taille. Au cours de l'évolution des primates, cette voie rubrospinale a été largement remplacée par la voie corticospinale directe. Alors que la voie rubrospinale contribue de facon importante au contrôle moteur chez beaucoup d'espèces de mammifères (par exemple les rongeurs), son rôle chez l'homme apparaît des plus réduit car le noyau rouge ne comporte presque exclusivement que des neurones de petite taille (noyau rouge parvocellulaire) qui ne projettent d'aucune facon sur la

moelle épinière, la plupart de ces fonctions ayant été prises en charge par la voie corticospinale.

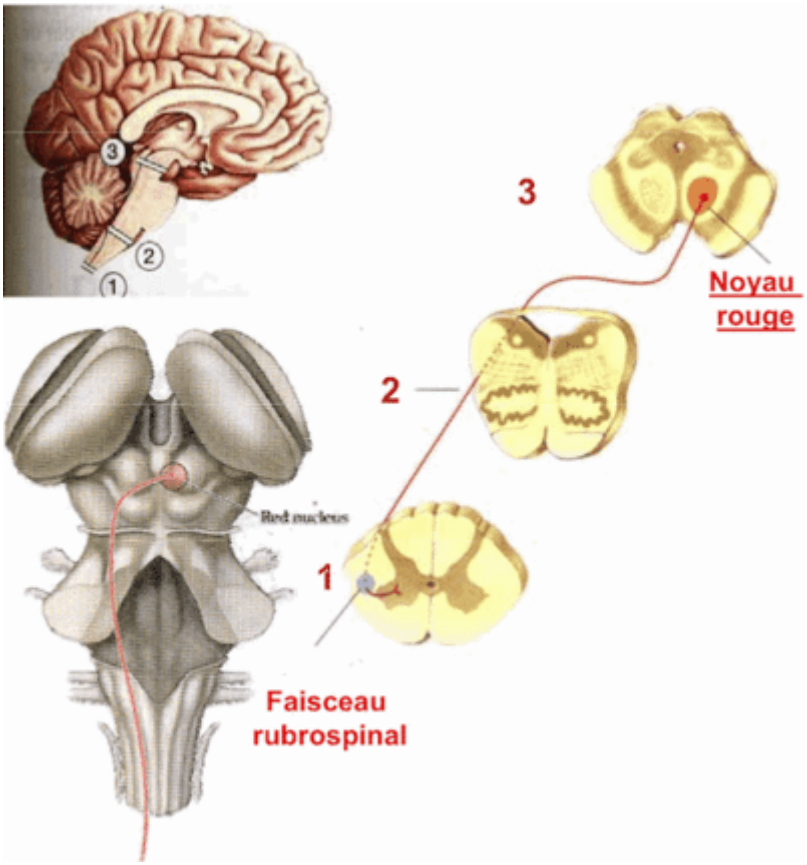


Figure 6: Représentation du faisceau rubrospinal.
http://www.peterdeli.com/uni/wiki/images/0/0d/Système_latéral-la_voie_rubrospinale.gif

1.6 Connectivité du cortex moteur

Dans le cadre du présent travail, du fait que notre étude porte sur le cortex moteur et en particulier sur le cortex moteur primaire, nous nous limiterons à étudier les connexions cortico-corticales du cortex frontal intrahémisphériques aussi que interhémisphériques via le corps calleux (aires adjacentes du cortex, aires motrices homologues de l'hémisphère controlatéral) et les connexions sous cortico-corticales d'origine thalamique du fait de l'importance du thalamus comme relais dans le traitement de l'information.

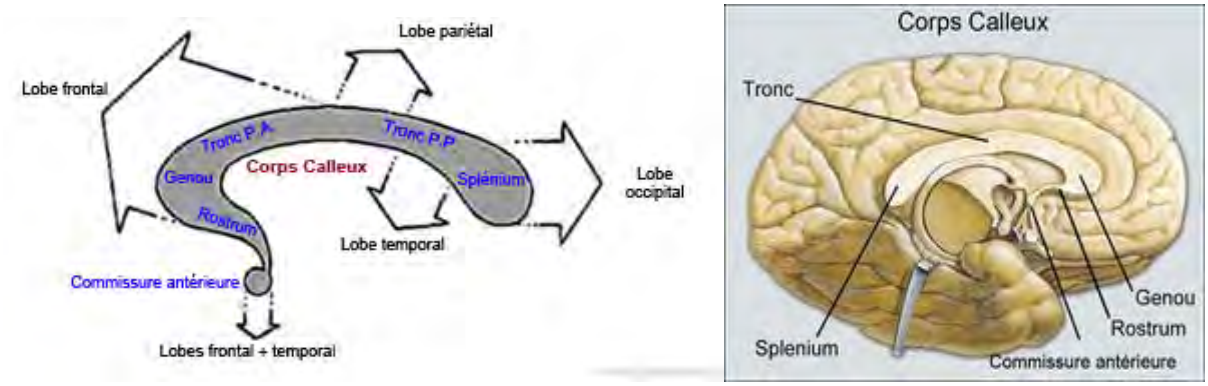
Connectivité cortico-corticale

Connectivité interhémisphérique

Anatomie du corps calleux

Le corps calleux est une commissure (moyen d'union entre deux parties) transversale du cerveau. C'est un faisceau d'axones (fibre nerveuse qui correspond au prolongement long, mince et cylindrique du corps cellulaire d'un neurone), interconnectant les deux hémisphères cérébraux. Le corps calleux assure donc le transfert d'informations entre les deux hémisphères et ainsi leur coordination. Les autres commissures sont le fornix, le cingulum et la commissure blanche antérieure. Le corps calleux est le plus important faisceau de fibres blanches reliant les deux hémisphères, comportant environ 200 à 800 millions de neurones (Koppel and Innocenti, 1983; Aboitiz, 1992), ce qui reste peu en rapport des milliards de neurones que comporte le cerveau, mais énorme, en rapport avec d'autres regroupements de fibres nerveuses. On estime que moins de 3% des neurones du cortex envoient des prolongements calleux. Le corps calleux permet aux deux hémisphères de communiquer de manière relativement rapide : le délai inter-hémisphérique est de 20 à 50 ms. Le corps calleux relie, entre autres, chacun des lobes des deux hémisphères avec son homologue dans l'autre hémisphère et est composé, d'avant en arrière, de 4 parties (Clarke, 2003a). (Fig. 7A et B):

1. Le Rostrum : Appelé aussi l'isthme ou le bec, il relie essentiellement les 2 lobes frontaux et une partie du lobe temporal.
2. Le Genou : C'est la partie la plus arrondie, il permet essentiellement le lien entre les 2 lobes frontaux. Le tiers antérieur connecte les aires frontales non connectées par le rostrum tandis que les deux-tiers postérieurs se composent en avant de fibres inter-temporales, et en arrière de fibres inter-pariétales.
3. Le Tronc : Partie la plus plate du corps calleux. La partie antérieure continue de relier les lobes frontaux entre eux, mais aussi les lobes pariétaux. La partie postérieure finit de relier les lobes temporaux.
4. Le Splénium : Appelé aussi le bourrelet, il relie les lobes occipitaux. Le corps calleux n'est pas homogène, il est plus développé au niveau de l'avant (cortex frontal) qu'en arrière



<u>Figure 7</u>: Anatomie du corps calleux. <u>Gauche</u>: Connexions entre les différents lobes. <u>Droite</u>: Subdivisions anatomiques du corps calleux

http://www.psychoweb.fr/images/stories/article-neuropsychologie/anatomie/corps-calleux-shema-connexion.jpg

http://www.psychoweb.fr/images/stories/article-neuropsychologie/anatomie/corps-calleux-splenium-rostrum.jpg

Connexions interhémisphériques

Le Corps Calleux est composé de deux types de fibres: (Fig. 8):

✓ Les fibres homotypiques qui relient des cortex de même nature (la majorité des fibres). Il s'agit essentiellement des cortex associatifs sensoriels, des cortex prémoteurs et des aires motrices supplémentaires. Les neurones prennent naissance dans les couches supragranulaires, en particulier la couche III, et se terminent dans la couche IV (Habib and Pelletier, 1994). L'étude, chez l'animal, de leur trajet grâce aux techniques de marquage cellulaire a montré que les axones n'ont pas forcément de projection point par point. Dans le cortex visuel, par exemple, la plupart d'entre eux ont des terminaisons ramifiées sur quelques millimètres carrés de cortex contralatéral, correspondant à des groupes de neurones intégrés dans des colonnes dont la fonction est similaire à celle de la colonne d'origine, par exemple codant pour une orientation identique (Innocenti, 1994). Les aires sensorielles primaires, et peut-être la partie apicale des lobes frontaux, sont quasiment dépourvues de connexions calleuses.

✓ Les fibres hétérotypiques qui relient des cortex non homologues (la vision au langage par exemple).

Les fibres du Corps Calleux sont, au centre du cerveau, transversales, mais irradient en tous sens vers le cortex lorsqu'elles pénètrent dans les hémisphères. Les radiations antérieures du corps calleux sont souvent dénommées forceps mineur et les radiations postérieures forceps majeur**.** Les fibres du splenium, en se portant en bas et en dehors, forment le tapetum, contournant la partie externe du ventricule latéral. Il existe, proches du Corps Calleux, deux

autres commissures plus restreintes : la commissure antérieure qui relie les 2 lobes frontaux et la commissure hippocampique qui relie notamment les 2 hippocampes.

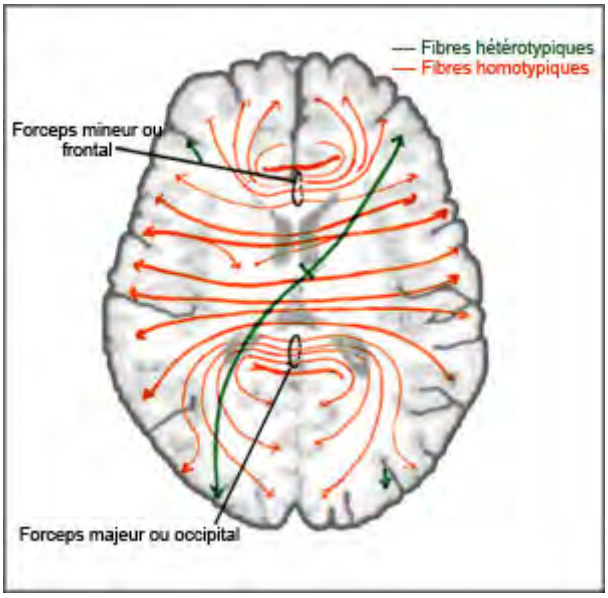


Figure 8: Interconnexions homotypiques et hétérotypiques du corps calleux.
http://www.psychoweb.fr/images/stories/article-neuropsychologie/anatomie/interconnexion-homotypiques-et-heterotypiques.jpg

Rôles fonctionnel des connexions callosales

Par l'intermédiaire du corps calleux, une grande partie des influx nerveux générés par un hémisphère gagne l'hémisphère opposé. Les résultats de la callosotomie expérimentale chez l'animal et thérapeutique chez l'homme ou encore les syndromes de déconnexion spontanée, démontrent que ces influx permettent d'une part de transmettre certains types d'informations d'un hémisphère à l'autre et, d'autre part, de coordonner les réponses de chaque hémisphère lors de leur activation simultanée. Les rôles fonctionnels des connexions callosales sont divers. En premier lieu, le corps calleux assure le transfert d'informations sensorimotrices d'un hémisphère à un autre, il permet aussi à chaque hémisphère cérébral d'exercer en permanence une action soit excitatrice, soit inhibitrice (selon les situations) sur l'hémisphère cérébral opposé. La fonction excitatrice implique l'activation des deux hémisphères mais aussi le partage de l'information entre les deux hémisphères alors que la fonction inhibitrice permet ainsi de maintenir une indépendance des processus entre les hémisphères et leur séparation, en inhibant l'un quand l'autre est engagé dans une tâche pour laquelle il est spécialisé.

Connectivité callosale du cortex moteur

Les études de Jenny (1979) ont montré l'existence de deux types de connexions cortico-corticales au niveau de la région de la main de l'aire 4 du cortex moteur: ce sont les connexions dirigées vers les aires homotopiques et non-homotopiques (hétérotopiques) du cortex. Bien que la plupart des connexions interhémisphériques du cortex moteur soient dirigées vers des aires homotopiques contralatérales, des projections additionnelles non-homotopiques ont été aussi observées. La première projection non-homotopique observée était dirigée vers la partie latérale du cortex adjacent au sillon précentral, la seconde projection non-homotopique provient de la surface médiale du gyrus frontal ou de la partie supérieure du sillon cingulaire. La dernière projection non-homotopique provient de la partie postérieure du sillon arqué. La région de la main du cortex moteur primaire (M1) reçoit des connexions callosales importantes des aires PM et M1. Dans la région homotopique de M1, des cellules marquées étaient groupées dans des zones limitées, M1 reçoit aussi des connexions callosales faibles des aires SMA et CMA (principalement de CMAd) (Rouiller et al., 1994).

Le cortex prémoteur est fortement connecté avec son homologue dans l'hémisphère opposé. Les 4 aires du cortex PM (PMd-r, PMd-c, PMv-r et PMv-c) reçoivent des connexions callosales homotopiques et ont des connexions distinctes avec les aires heterotopiques (Boussaoud et al., 2005). PMd-r reçoit les connexions les plus importantes de PMd-r, de la partie dorsale du cortex préfrontal, de Pré-SMA et de CMA-r, des connexions limitées ont été aussi observées dans PMv-r, PMd-c et CMA-v (Marconi et al., 2003; Boussaoud et al., 2005). PMd-c est quant à lui densément connecté avec PMd-c, PMd-r et Pré-SMA. Des connexions modérées ou faibles ont été observées dans CMA, SMA et M1 (Marconi et al., 2003; Boussaoud et al., 2005). La majeure partie des connexions callosales de PMv-r proviennent de PMv-r, des connexions additionnelles ont été également observées dans Pré-SMA, CMA-r et dans la partie ventrale du cortex préfrontal (PFc), alors que les connexions callosales les plus intenses de PMv-c ont été observées dans PMv-r et PMv-c, de faibles connexions callosale ont été également observées dans Pré-SMA, CMA-r et M1. Mais dans certains cas, des connexions ont été observées dans PMd-r, SMA, CMA-v, CMA-d et PMd-c.

Les principales sources de projections callosales de Pré-SMA et SMA proviennent de leurs homologues dans l'hémisphère opposé (McGuire et al., 1991; Rouiller et al., 1994; Liu et al., 2002). Pré-SMA et SMA ont des projections callosales avec CMA, mais les projections sont plus grandes dans la partie rostrale (CMA-r) que dans la partie caudale (CMA-d et CMA-v) (Liu et al., 2002), mais aussi des projections avec PM. On note que Pré-SMA a plus de

connexions avec PMd-r qu'avec PMd-c. Pré-SMA est aussi connecté à Cg, PFc. SMA est quant à lui largement connecté à PMd-c, peu de connexions ont été observées dans la partie rostrale de PM (PMd-r et PMv-r). Les travaux de Rouiller et ses collaborateurs (1994) ont également montré des connexions importantes de SMA avec CMA (CMA-r, CMA-v et CMA-d), de faibles connexions avec le cortex PM et quasiment pas de connexions avec M1.

Connectivité intrahémisphérique entre les différentes aires du cortex

La majeure partie des connexions de M1 s'effectue avec les aires corticales du lobe frontal (Ghosh et al., 1987; Muakkassa et Strick, 1979; Godschalk et al., 1984). Ces afférences en provenance des aires motrices non primaires (aires prémotrices dorsale et ventrales et aire motrice supplémentaire) respectent l'organisation somatotopique. En plus des afférences frontales, M1 reçoit des projections denses des aires somatosensorielles (aires 3a, 1, 2 de Brodmann, et aire somatosensorielle secondaire) et des connexions moins importantes en provenance de l'aire 3b de Brodmann, du cortex pariétal postérieur et du cortex cingulaire (Ghosh et al., 1987; Godschalk et al., 1984). M1 ne reçoit pas d'afférences directes en provenance des régions préfrontales et temporales.

Le cortex prémoteur est quant à lui connecté à un grand nombre d'aires corticales des lobes frontal et pariétal, mais pas ou peu avec les lobes temporal et occipital. Une des caractéristiques des aires prémotrices est qu'elles projettent sur l'aire motrice primaire (Dum et Strick, 1991). Ainsi, toutes les subdivisions du cortex prémoteur, à l'exception de PMd-r selon Barbas et Pandya (1987), projettent sur M1. Ces projections s'organisent entre des régions contenant la même représentation somatotopique (Godschalk et al., 1984) et montrent une ségrégation assez nette des territoires de projection des parties ventrale (PMv) et dorsale (PMd) sur M1 (Barbas et Pandya, 1987). Les connexions intrinsèques au cortex prémoteur sont nombreuses mais sont, en raison des controverses sur le découpage anatomique de PM, difficiles à résumer. Matelli et ses collaborateurs (1984) ont montré que ces connexions ne suivent pas une organisation somatotopique mais tendraient au contraire à connecter des aires de représentation somatotopique différentes. Ainsi, l'aire de la main de PMv-r se connecterait à l'aire de la bouche de PMv-r et l'aire de la jambe de PMd-c. Plusieurs études (Kurata, 1991; Barbas et Pandya, 1987; Ghosh et Gattera, 1995) ont montré que les connexions entre les parties rostrale et caudale de PMd sont denses. A l'inverse, les connexions entre PMd et PMv sont plutôt rares (Ghosh et Gattera, 1995). Les auteurs, dans cette même étude, confirment que des représentations somatotopiques différentes sont connectées au sein de chacune des parties dorsale et ventrale de PM.

Les cortex PMd et PMv sont connectés avec toutes les aires motrices supplémentaires (SMA, pré- SMA) et cingulaires mais avec une topographie différente. PMd-c projette sur PMd-r, pré- SMA, SMA et l'aire motrice cingulaire (CMA) et reçoit des projections de PMd-r et des aires 23 et 24 du gyrus cingulaire. Quelques cellules marquées par un traceur rétrograde injecté dans PMd-c ont aussi été trouvées dans PMv (Kurata, 1991). PMd-r semble être la partie du cortex prémoteur la moins connectée aux aires motrices. Elle partage des connexions réciproques avec PMd-c, pré-SMA et l'aire cingulaire 24 (Barbas et Pandya, 1987; Kurata, 1991) et reçoit des afférences de FEF. PM-v, outre sa riche connectivité intrinsèque, présente principalement des connexions topographiquement organisées avec SMA (Matelli et al., 1986). Les études portant sur les connexions de PMv (principalement sa partie rostrale) (Kurata, 1991; Barbas et Pandya, 1987; Ghosh et Gattera, 1995) ont montré l'existence des efférences vers PMd-r, pré-SMA et l'aire 24 et des afférences de SMA, pré-SMA et du cortex cingulaire. D'après Ghosh et Gattera (1995), toutes les aires prémotrices reçoivent des afférences de SEF. PMd-c est la seule partie du cortex prémoteur sans relations avec le cortex préfrontal. PMd-r est interconnecté avec les aires 46, 9 et 8 du cortex préfrontal dorsolatéral (Barbas et Pandya, 1987; Lu et al. 1994; Ghosh et Gattera, 1995).

Connectivité thalamocorticale

Le thalamus (*chambre à coucher*) est une structure anatomique paire de substance grise cérébrale diencéphalique, c'est la plus volumineuse structure du diencéphale. C'est un important centre relais sensoriel, comprenant de nombreux noyaux étroitement interconnectés avec le cortex cérébral. En dehors des voies olfactives, toutes les voies sensorielles qui projettent sur le cortex cérébral font auparavant relais dans le thalamus. Le thalamus véhicule également presque toutes les autres entrées vers le cortex : motrices, limbiques et modulatrices liées à la vigilance Il est composé de nombreux noyaux établissant des connexions réciproques avec l'ensemble du cortex cérébral. Les deux thalamus sont situés de part et d'autre du IIIème ventricule dont ils constituent les parois latérales. Situé en position intermédiaire entre cortex et tronc cérébral, le thalamus a principalement une fonction de relais et d'intégration des afférences sensorielles, mais intervient également dans la motricité involontaire (extrapyramidale) et la régulation cérébelleuse du mouvement, la régulation de la conscience, de la vigilance et du sommeil. Il distribue ainsi donc les informations qu'il reçoit vers les aires corticales appropriées (Henderson et al., 2000; Banati et al., 2000; Bagary et al., 2002). Point important, les noyaux thalamiques reçoivent, en plus de leurs afférences sous-corticales, des connexions excitatrices réciproques des zones du cortex cérébral sur lesquelles

ils projettent. A cet égard, la densité des projections cortico-thalamiques est supérieure à celle des projections thalamo-corticales, ce qui met en lumière le rôle crucial du cortex cérébral pour un contrôle en retour («feedback») sur les entrées sensorielles.

Anatomie du thalamus

Le thalamus est constitué de deux masses ovoïdes jumelles, situées au dessus de la région sous-thalamique et de l'hypothalamus, avec lequel il forme la paroi latérale du troisième ventricule. Les deux parties du thalamus se rejoignent à travers le troisième ventricule par la commissure grise ou adhérence interthalamique. Latéralement au thalamus se trouve le bras postérieur de la capsule interne et en position antérieure se situe le noyau caudé. Le pôle antérieur du thalamus s'étend jusqu'au trou de Monro et sa face dorsale forme une partie du plancher du corps du ventricule latéral. Le pôle caudal du thalamus est bordé par le mésencéphale. Le thalamus est composé en majorité de substance grise qui s'organise en de nombreux noyaux. La lame médullaire interne, en forme de Y, est une fine couche de substance blanche constituée des connexions afférentes et efférentes des noyaux thalamiques.

Subdivision du thalamus

Les toutes premières études développementales et comparatives ont permis de diviser le thalamus en trois parties: l'épithalamus, le thalamus ventral et le thalamus dorsal (Droogleever-Fortuyn, 1912; Herrick, 1918; Clark, 1932a).

✓ L'épithalamus comprend les parties antérieure et postérieure des noyaux paraventriculaires et habenulaires. Il a des affinités plus proches avec l'hypothalamus qu'avec le cortex cérébral. L'épithalamus n'envoie ni ne reçoit des fibres vers/du cortex cérébral bien qu'il y ait des évidences en faveur de la présence de cellules projetant corticalement dans le noyau paraventriculaire.

✓ Le thalamus ventral comprend le noyau réticulaire, le noyau géniculé ventro-latéral, la zona incerta et le noyau du domaine de forel le plus souvent oublié. Bien que recevant les fibres du cortex cérébral et d'une partie des ganglions de la base, le thalamus ventral n'envoie pas d'axones à n'importe lequel de ces sites. Les travaux de Nicolelis et ses collaborateurs (1992) ont toutefois montré la présence d'une population de cellules projetant corticallement dans la zona incerta.

✓ Le thalamus dorsal, partie la plus importante, comprend plusieurs groupes de noyaux nucléaires: noyaux antérieurs, noyaux médians et noyaux latéraux (Fig. 9A). Ces grands groupes sont subdivisés en plusieurs noyaux individuels (Fig. 9B). Il envoie des fibres vers, et

reçoit des fibres du cortex cérébral et du striatum (noyau caudé, putamen et noyau acumbens). Les projections vers et à partir du cortex incluent non seulement le neocortex, mais aussi le paleocortex et l'archicortex de la formation hippocampale (Shipley and Sorenson, 1975; Wyss et al., 1979; Insausti et al., 1987; Wouterlood et al., 1990). Les noyaux thalamiques sont classés en deux groupes : les noyaux relais (spécifiques) et les noyaux non-spécifiques. La plupart des noyaux du thalamus sont des noyaux relais qui reçoivent des entrées de nombreuses voies ascendantes et projettent sur le cortex. Ils sont également connectés de façon réciproque aux aires corticales. Les projections vers le cortex peuvent se faire soit de façon diffuse, soit être localisées à des régions spécifiques, motrices ou sensitives. Les noyaux «non spécifiques» (noyaux intralaminaires) envoient des projections diffuses dans de vastes régions corticales. Dans le cadre du présent travail, nous nous limiterons aux noyaux du thalamus dorsal du fait que ces noyaux envoient et reçoivent des fibres du cortex cérébral.

Le groupe des noyaux latéraux se situe sur la partie ventrale du thalamus et se subdivisent en deux groupes: le groupe des noyaux ventraux formé du noyau ventral antérieur (VA), du noyau ventral latéral (VL), du noyau ventral postérieur (VP) subdivisé en deux sous noyaux latéral (VPL) et médian (VPM), et du noyau ventral médian (VM). Le groupe des noyaux latéraux et du pulvinar formé du noyau latéral postérieur (LP), du pulvinar (PU) et du complexe du corps géniculé (latéral et médian).

Le groupe des noyaux antérieurs se situe sur la partie la plus antérieure du thalamus. Il contient le noyau antéroventral (AV), le noyau antéromédian (AM), le noyau antérodorsal (AD) et le noyau lateral dorsal (LD).

Le groupe des noyaux médians se situe sur la partie dorsale médiane du thalamus. Il contient essentiellement le noyau médiodorsal (MD) et d'autres formations plus petites.

Les noyaux thalamiques de la ligne médiane forment un petit groupe de noyaux additionnels qui bordent le troisième ventricule.

Les noyaux intralaminaires forment plusieurs groupes cellulaires situés au sein de la lame médullaire interne. Ils sont formés des noyaux centro-latéral (CL), des noyaux centro-médian (CM) et des noyaux paracentral (PC).

Une fine couche cellulaire appelée noyau réticulé se situe sur la face latérale du thalamus, entre la lame médullaire externe (composée de fibres thalamocorticales et corticothalamiques) et la capsule interne.

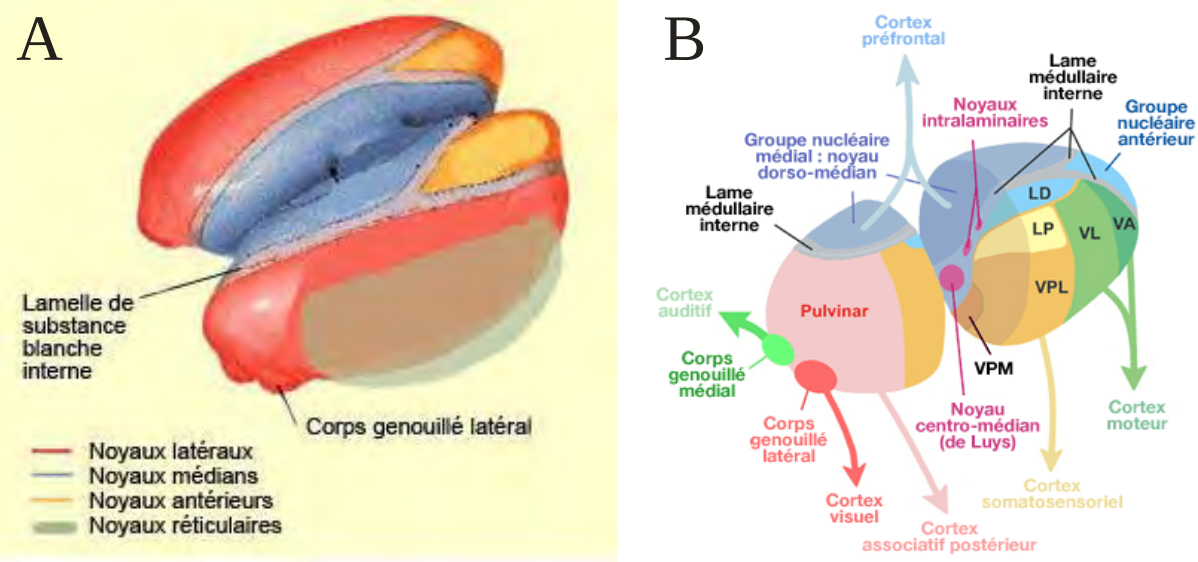


Figure 9: Subdivisions du thalamus. A: les principaux noyaux du thalamus, B: les sous-noyaux du thalamus et leurs connexions avec le cortex cérébral.

http://www.vetopsy.fr/anat/neuro/encephale/dien/pulvinar.php

Organisation fonctionnelle des différents noyaux

Le groupe de noyaux antérieurs reçoit les fibres afférentes du corps mamillaire et se projette principalement sur le gyrus cingulaire. Il fait partie du système limbique et intervient dans les aspects émotionnels du comportement et de la mémoire.

Le noyau ventral antérieur (VA) reçoit des fibres afférentes du globus pallidus et de la substance noire et se projette principalement dans les régions motrices du lobe frontal, en particulier le cortex prémoteur (aire 6 de Brodmann). Il a une fonction motrice.

Le noyau ventral latéral (VL) reçoit des fibres afférentes du pallidum, de la substance noire et du cervelet. Il se projette sur les aires motrices du lobe frontal et sur le cortex moteur. Sa fonction est également motrice.

Le noyau ventral postérieur (VP) quant à lui reçoit les terminaisons des voies ascendantes somesthésiques issues de la moelle épinière et du tronc cérébral: faisceaux spinothalamiques et trigéminothalamiques, lemnisque median. Ces fibres se terminent dans le noyau ventral postérieur selon une organisation somatotopique précise : la partie externe du noyau ou noyau ventro-postérieur latéral (VPL) reçoit les informations des faisceaux spinothalamiques et du lemnisque médian ; sa partie interne ou noyau ventro-postérieur médian (VPM) reçoit les informations du faisceau trigéminothalamique, des informations gustatives issues des noyaux gustatifs bulbaires et des informations vestibulaires. Il se projette

sur le cortex somatosensoriel primaire du lobe pariétal et a une fonction de sensations somatiques (somesthésie).

Le corps génouillé médian reçoit des fibres afférentes du colliculus inférieur et se projette sur le cortex auditif primaire (lobe temporal). Il fait partie du système auditif.

Le corps génouillé latéral quant à lui reçoit des fibres afférentes du tractus optique et se projette sur le cortex visuel primaire (lobe occipital ipsilatéral). Il joue le rôle de relais thalamique pour la vision.

Le noyau latéral dorsal a des connexions réciproques avec le gyrus cingulaire. Il fait partie du système limbique; intervient dans les aspects émotionnels du comportement et de la mémoire.

Le noyau latéral postérieur et le pulvinar reçoivent des afférences du colliculus supérieur et pour le pulvinar, des lobes temporal, pariétal et occipital; pour le noyau latéral postérieur: du lobe pariétal. Ils se projettent sur les aires associatives du cortex temporo-pariéto-occipital. Le noyau latéral postérieur et le pulvinar fonctionnent ensemble. Le pulvinar serait un centre d'interprétation de l'image qui jouerait un rôle important dans l'attention visuelle et dans la perception du mouvement.

Le noyau médio-dorsal reçoit des afférences provenant de l’hypothalamus, de l’amygdale, d’autres noyaux thalamiques (dont les noyaux intralaminaires et les noyaux latéraux), du cortex olfactif, des noyaux gris centraux. Il se projette sur le cortex préfrontal et contribue au contrôle de l’humeur et des émotions.

Les noyaux intralaminaires (inclus dans la lame médullaire interne du thalamus), comprennent: le noyau centro-médian de Luys, et le noyau parafasciculaire, le noyau paracentral, le noyau central latéral. Ils reçoivent des fibres afférentes ascendantes de la formation réticulée du tronc cérébral, des faisceaux spinothalamiques et trigéminothalamiques, du cervelet, des noyaux gris centraux et se projettent vers de larges régions du cortex cérébral, vers le noyau caudé et le putamen (noyaux gris centraux).

Le noyau réticulaire reçoit des afférences du cortex et des noyaux thalamiques (relais et intralaminaires) et de la réticulée activatrice ascendante du tronc cérébral et projette des fibres inhibitrices sur les noyaux du thalamus. Le noyau réticulaire a pour fonction la modulation de l’activité thalamique. Les noyaux de la ligne médiane reçoivent des afférences de la formation réticulée du tronc cérébral, de l’hypothalamus, de l’amygdale et projettent sur le télencéphale basal. C’est l’une des voies du système limbique.

Projection / connectivité thalamocorticale

Parmi les noyaux du thalamus dorsal, il y a clairement un certain nombre de types de connections différentes avec le cortex (Jones, 1981a, 1985; Macchi, 1983, 1988, 1993, Macchi and Jones, 1997). Les projections du thalamus sur les aires motrices chez les primates ont été étudiées principalement chez le singe macaque (Schell and Strick, 1984; Strick, 1985; 1986; Wiesendanger and Wiesendanger, 1985; Ghosh et al., 1987; Jones, 1987; Matelli et al., 1989; Darian-Smith et al., 1990a,b; Nakano et al., 1992; 1993; Inase and Tanji, 1994; Kurata, 1994; Rouiller et al., 1994; Shindo et al., 1995; Matelli and Luppino, 1996; Rouiller et al., 1999; Kurata, 2005; Morel et al., 2005) et peu d'informations sont disponibles concernant les connexions thalamiques pour d'autres primates. Les études anatomiques avec injection de traceurs rétrogrades dans les différentes aires du cortex moteur ont permis de déterminer les projections du thalamus sur ces aires motrices corticales.

Les travaux de Fang et ses collaborateurs (2006) sur les primates prosimiens (singe hibou; *Otolemur garnetti*) ont montré que M1 est largement connecté aux noyaux ventro-latéral antérieur (VLa) et postérieur (VLp), ainsi qu'au noyau intralaminaire (IL), des connexions ayant été également observées dans le noyau LP. Peu de connexions ont été observées dans les noyaux VA et VM. Les travaux de Macchi et Jones (1997), sur les macaques, ont montré que les noyaux VLp et VLa sont les sources principales des projections sur le cortex moteur primaire, VA et VM fournissent peu de projections, si bien qu'ils sont à peine qualifiés de noyaux moteur du thalamus. Certaines études montrent que VLa projette principalement dans la partie rostrale de M1 chez le macaque (Matelli et al., 1989, Nakano et al., 1993). Les projections du noyau intralaminaire sur M1 chez les macaques sont similaires à ceux observées chez les singes hibou. Les projections les plus importantes proviennent de CL (Strick, 1975; Schell and Strick, 1984; Leichnetz, 1986; Darian-Smith et al., 1990), tandis que PC montre de faibles projections (Leichneitz, 1986) ou pas de projections (Matelli et al., 1989; Darian-Smith et al., 1990) vers M1. PC projette probablement de manière plus dense vers le cortex premoteur et préfrontal que vers M1 (Ilinsky et al., 1985). Des connexions de M1 avec d'autres noyaux thalamiques ont cependant été également observées, notament dans les noyaux MD, VM, VP et PU, avec des noyaux marqués en quantité très réduite suggérant que ces noyaux projettent très peu vers M1.

Le cortex premoteur dorsal (PMd) reçoit principalement des connexions avec des densités égales des noyaux VL (antérieur et postérieur). VM a aussi quelques connexions avec PMd, et une troisième source de projection sur PMd provient du noyau intralaminaire (IL).

Des projections importantes vers PMd ont été observées dans VA, principalement dans la partie rostrale.

Le cortex prémoteur ventral (PMv) reçoit également la majeure partie de ses projections thalamocorticales des noyaux VL avec une densité plus importante au niveau du noyau postérieur (VLp) par rapport au noyau antérieur (VLa). Peu de projections (ou pas) ont été observées dans VA. D'autres projections moins importantes ont été observées au niveau des noyaux IL et MD. De manière générale, les projections du thalamus vers PM (PMd et PMv) proviennent en majorité des noyaux VL (VLa et VLp) (Rouiller et al., 1999; Morel et al., 2005). Les noyaux intralaminaires (principalement CL) ont des projections significatives sur toutes les régions motrices chez les singes hibou, avec des connexions plus denses sur la partie rostrale de PMd et clairsemées sur la partie caudale de M1 (Stepniewska et al., 2007). Des projections assez importantes avec le cortex moteur et premoteur ont été aussi observées chez les macaques (Schell and Strick, 1984; Matelli et al., 1989; Darian-Smith et al., 1990a; Nakano et al., 1993; Rouiller et al., 1994, 1999; Morel et al., 2005). De plus, le noyau CM projette aussi sur PM (Matelli et al., 1989; Nakano et al., 1993; Rouiller et al., 1999; Morel et al., 2005), tandis que les singes hibou ne semblent pas avoir de telles connexions (Stepniewska et al., 2007). Le pulvinar montre aussi de faible projection vers PM, dans certains cas chez les singes hibou (Stepniewska et al., 1994b) ou chez les macaques (Matelli et al., 1989; Rouiller et al., 1999; Morel et al., 2005). La majeure partie des projections du thalamus sur SMA provient des noyaux VL avec une prédominance dans le noyau VLp, alors que d'autres projections proviennent des noyaux ventromedian (VM) et des noyaux IL, tandis que peu de projections proviennent des noyaux centromedian (CM) et MD. La Figure 10 montre les projections du thalamus sur le cortex moteur.

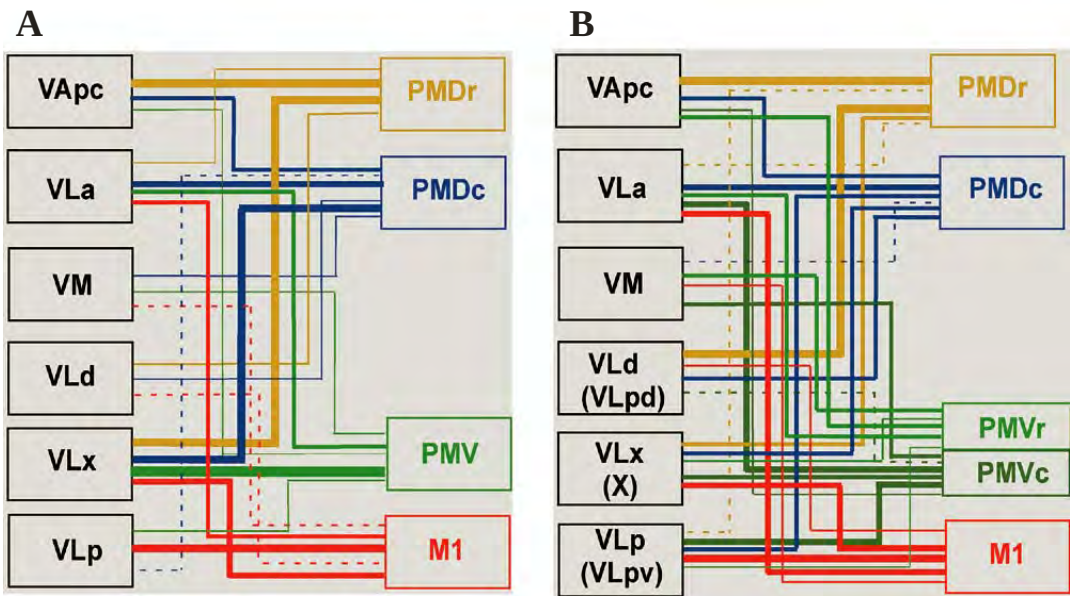


Figure 10: Résumé des projections thalamocorticales des différents noyaux thalamiques sur les aires principales du cortex prémoteur. A: Chez le singe hibou (tiré de Stepniewska et al., 2007); B: Chez le singe macaque (tiré de Morel et al., 2005 dans Stepniewska et al., 2007).

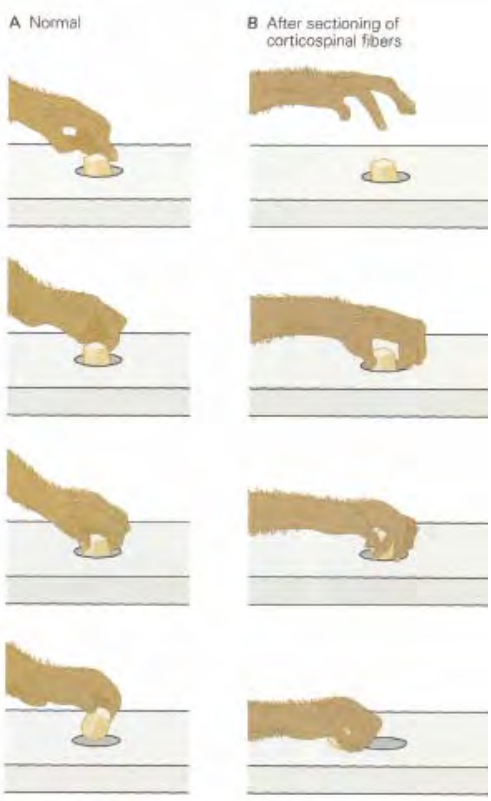
1.7 Lésion du cortex moteur

Les études réalisées chez le primate non-humain ont montré que trois types de lesions pouvaient affecter la voie corticospinale: 1) des lésions du cortex moteur (Rouiller et al., 1998; Passingham et al., 1983; Nudo et al., 1996; Nudo, 1999; Liu and Rouiller, 1999), 2) des lésions effectuées au niveau des pyramides (Woolsey et al., 1972; Lawrence and Kuypers, 1968a; Lawrence and Kuypers, 1968b; Lawrence and Hopkins, 1976; Kucera and Wiesendanger, 1982; Hepp-Reymond, 1982) et enfin 3) des lesions de la moelle épinière (Holmes and May, 1909; Aoki and Mori, 1979; Galea and Darian-Smith, 1997a, b; Sasaki et al., 2004; Schmidlin et al, 2004; Schmidlin et al, 2005; Wannier et al., 2005; Freund et al., 2006; Freund et al., 2007; Freund et al., 2009). Du fait que notre étude porte sur la lésion faite au niveau du cortex moteur primaire, nous nous limiterons ici aux conséquences de lésions du cortex moteur.

L'étude des lésions du cortex moteur primaire a montré son rôle déterminant pour les mouvements volontaires fins, distaux. Chez le singe (Lawrence and Kuypers, 1968a) comme chez l'homme (Lough et al., 1984), l'analyse des mouvements de préhension après des lésions de M1 a montré qu'il y'avait une récupération progressive des mouvements assurant la trajectoire de la main jusqu'à la cible (mouvements proximaux), alors que la prise manuelle était restée incomplète. Des lésions expérimentales du système latéral (Lawrence and Kuypers, 1968b) incluant les deux composantes corticospinale et rubrospinale chez le singe

provoquent une incapacité à réaliser des mouvements des différentes parties du corps, c'est-à-dire une incapacité de mobiliser séparément l'articulation de l'épaule, du coude, du poignet ou les doigts de la main. Les mouvements volontaires dans ce cas sont plus lents et moins précis, toutefois l'animal montre une posture normale. Les lésions du faisceau corticospinal seul causent des déficits moteurs du même type que ceux observés après une lésion plus globale du système latéral, mais la récupération fonctionnelle plus ou moins importante intervient progressivement dans les mois qui suivent la lésion. Le seul déficit permanent qui subsiste est une faiblesse musculaire des fléchisseurs distaux et une incapacité à mobiliser les doigts indépendamment les uns des autres (Fig. 11). Une lésion secondaire du faisceau rubrospinal chez le singe supprime tout effet de récupération fonctionnelle, suggérant que le faisceau cortico-rubrospinal est à même de compenser progressivement les déficits moteurs consécutifs à la destruction du faisceau corticospinal. Chez le singe, des lésions bilatérales de SMA induisent des troubles de la coordination bimanuelle, ainsi qu'une maladresse des mains (Brinkmann, 1984). Des lésions bilatérales de PM perturbent la sélection du mouvement approprié au contexte (Passingham, 1985): lorsque l'animal doit tirer de l'information du contexte environnemental pour attraper ou manipuler un objet, la tache est très perturbée. En revanche, lorsque l'objet en lui-même est source d'information, la tache est normalement exécutée. Les lésions de PM induisent essentiellement des troubles de l'organisation dynamique du mouvement : chaque geste est correctement exécuté s'il est pris isolément; par contre les mouvements élémentaires ne sont pas intégrés dans une conduite gestuelle harmonieuse. Après de telles lésions, le degré de récupération de la dextérité manuelle est directement lié à la taille de la lésion, dans le cas des lésions affectant principalement la voie corticospinale, une récupération plus ou moins importante des fonctions motrices prend place. Un grand nombre de facteur pourrait jouer un rôle dans la récupération observée. La récupération apparaît graduellement durant les mois qui suivent la lésion, mais cette amélioration n'est que partielle. Il y a des évidences que la récupération de fonctions observées après une lésion corticale motrice est en grande partie attribuable à la plasticité adaptative dans le cortex moteur épargné par la lésion (Chollet et al., 1991; Liepert et al., 2000). Suite à une lésion de M1, autant chez l'homme que chez le singe, il a été montré que PM, épargné par la lésion, joue un rôle important dans la récupération fonctionnelle incomplète (Seitz et al., 1998; Liu et Rouiller, 1999). Ce n'est pas surprenant qu'après une lésion du cortex, la structure et la fonction des régions sensorielles et motrices soient radicalement altérées. Une récupération motrice limitée peut arriver spontanément après une

lésion du cortex moteur, toutefois il est intéressant de déterminer les mécanismes neuronaux à la base d'une telle récupération.



<u>Figure 11</u>: Effet direct de la lésion de la voie corticospinale (lésion du cortex moteur primaire) chez le macaque. A: Singe non lésé: le singe est capable d'exécuter la pince de précision (opposition du pouce et de l'index). B: Après lésion unilatérale de la région de la main de M1, le singe saisit les pellets en tentant d'utiliser la main entière, une stratégie inopérante lorsqu'il s'agit de manipuler des objets de petite taille (Lawrence and Kuypers 1968b).

1.8 Mécanisme de récupération fonctionnelle

Plasticité cérébrale

Longtemps, les scientifiques ont cru que le cerveau, une fois mature, se caractérisait par la stabilité de ses connexions, jugées immuables. Depuis une trentaine d'années, cette vision de la structure et du fonctionnement cérébral a volé en éclats. Grâce à la plasticité cérébrale, le cerveau modifie l'organisation de ses réseaux de neurones en fonction des expériences vécues par l'organisme (voir par exemple Donoghue, 1995). La plasticité cérébrale est la capacité du cerveau à remodeler les branchements entre ses neurones par formation ou disparition de synapses (Hallett, 1999; 2000; 2001). Elle est à la base du processus de mémoire et d'apprentissage, mais intervient également parfois pour compenser

les effets de lésions cérébrales en aménageant de nouveaux réseaux. Ces modifications locales de la structure du cerveau dépendent de l'environnement et lui permettent de s'y adapter. Au cours de la décennie passée, des études de cartographies non invasives nouvellement développées comme la neuroimagerie, l'electroencephalographie (EEG), la magnétœncéphalographie (MEG), ou la stimulation magnétique transcrânienne (TMS) chez les humains, correspondant aux études neurophysiologiques et neuroanatomiques chez les animaux, fournissent des preuves substantielles que le cerveau adulte est capable de plasticité fonctionnelle significative. Les neurones et les réseaux neuronaux peuvent ainsi adapter leurs fonctions. La connaissance des mécanismes cellulaires à la base de la plasticité chez les humains est importante afin de mettre en place de nouvelles approches pour la réhabilitation suite à une lésion corticale.

Après une lésion focale de la zone de représentation d'un doigt du cortex moteur primaire (M1), la représentation endommagée réapparaît dans le territoire cortical adjacent. Les études faites par Jenkins et Merzenich (1987) sur le cortex somatosensoriel sont en accord avec ces résultats. Cependant, utilisant la technique de microstimulation (ICMS), des découvertes quelque peu différentes ont été observées dans le cortex moteur (Nudo et al., 1996a, Nudo et al., 1996b, Nudo and Milliken, 1996). Ces études suggérent que le cortex moteur adjacent à la lésion se réorganise après stimulation électrique. Les études récentes chez le macaque ont démontré que les aires motrices secondaires ont des projections directes sur les motoneurones de la moelle épinière, bien qu'elles soient moins nombreuses et moins efficaces que celles de M1. Ainsi, bien que ces projections puissent contribuer à la récupération, il est peu probable qu'elles puissent se substituer aux projections de M1. Il est clair qu'une lésion corticale provoque des changements neurophysiologique et neuroanatomique spécifiques tant dans le tissu cortical adjacent que éloigné. Une réorganisation des cartes motrices a lieu aussi dans les aires motrices interconnectées avec le territoire lésé (Nudo and Milliken, 1996; Dancause et al., 2006). Par exemple, une observation plusieurs mois après une lésion presque totale dans la région de la main (partie distale) de M1 chez les singes écureuils montre une extension considérable de la région de la main (partie distale) de PMv (Frost et al., 2003). L'une des questions les plus cruciales qui doivent être posées par la recherche fondamentale est s'il y a une période de temps après une lésion corticale durant laquelle le reste du système intact est plus disposé à une intervention rééducatrice, par des traitements pharmacologiques (Lee et al., 2004; Adkins and Jones, 2005; Barbay et al., 2006), une stimulation électrique (Adkins and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003), le réapprentissage ou une combinaison de divers traitements. Des études

récentes sur des modèles animaux ont commencé à confirmer que l'entraînement comportemental (par exemple physiothérapie) après une lésion focale augmente la récupération de la fonction motrice perdue et favorise des changements neuroanatomiques dans l'hémisphère contralésionnel dès les premières semaines après la lésion (Murata et al., 2008).

En résumé, une lésion du cortex moteur aboutit à une perturbation forte des réseaux coordonnés et leurs propriétés émergentes sous jacentes, aboutissant à la perte du contrôle moteur fin, et l'emploi de stratégies de mouvements compensatoires. Il apparaît maintenant qu'une telle perturbation du réseau cortical moteur déclenche un recrutement des réseaux inter- et intra-hémisphériques (Dancause et al., 2007; Dancause and Nudo, 2011).

Plasticité liée au développement du système corticospinal

De nombreuses investigations de la neuroplasticité ont été faites au cours des 10 dernières années et la découverte de la capacité de développement remarquable du cerveau à se former par une activité et un apport environnemental. Chez l'enfant, le cortex moteur et/ou la voie corticospinale est un site fréquemment affecté par des lésions du cerveau; la période pré- ou immédiatement péri-natale est la période durant laquelle elles se produisent le plus souvent. Il est maintenant de plus en plus reconnu que le système corticospinal est capable d'une réorganisation substantielle après des lésions et de telle réorganisation est de nature à être à la base d'une récupération spontanée partielle des fonctions (Terashima, 1995; Rouiller et al., 1998; Raineteau and Schwab, 2001). Dans le système nerveux mature, la plasticité synaptique dans les voies préexistantes, et la formation de nouveaux circuits à travers le bourgeonnement des collatérales des fibres lésées ou non lésées sont les principales composantes de ce processus de récupération (Raineteau and Schwab, 2001). Par rapport au système nerveux mature, durant le développement du système nerveux, il est clair qu'il y a un beaucoup plus grand potentiel pour la plasticité, qui pourrait impliquer la plasticité non seulement de l'aire motrice du cortex cérébral ipsilésionnel, mais aussi le cortex contralésionnel, la formation de la voie corticospinale et le développement des réseaux de la la moelle épinière (Benecke et al., 1991; Carr et al., 1993; Cao et al., 1994; Lewine et al., 1994; Maegaki et al., 1995; Terashima, 1995; Muller et al., 1997, 1998; Nirkko et al., 1997; Graveline et al., 1998; Holloway et al., 1999; Wieser et al., 1999; Balbi et al., 2000; Chu and Jones, 2000; Eyre et al., 2000, 2001; Thickbroom et al., 2001). Des données fonctionnelles et anatomiques ont démontré que la plasticité spontanée peut être potentialisée par l'activité (Nudo et al., 1996), également par des manipulations expérimentales spécifiques.

Chez les mammifères (non-primate), des lésions substantielles du cortex sensorimoteur ou de la voie corticospinale au début de la vie post-natale mènent à une hypertrophie du cortex moteur intact et de sa projection corticospinale (Hicks and D'Amato, 1970; Huttenlocher and Raichelson, 1989; Rouiller et al., 1991; Jansen and Low, 1996; Uematsu et al., 1996). Ces changements sont associés avec la maintenance accrue de la projection corticospinale de l'hémisphère intact. Les cellules d'origines des axones ipsilatéraux sont largement distribuées et distinct des cellules d'origines de la projection croisée ou corticospinale (Huttenlocher and Raichelson, 1989; Reinoso and Castro, 1989; Stanfield, 1992; Jansen and Low, 1996). Rouiller et ses collaborateurs (1998) ont réalisé des lésions focales dans le cortex moteur de singes nouveaux nés et ont démontré par la suite une réorganisation somatotopique substantielle du cortex moteur environnant dans le même hémisphère que la lésion. De même, des études d'imagerie par résonance magnétique (IRM) sur des sujets avec une lésion unilatérale précoce du cerveau ont démontré une augmentation de la taille de la projection corticospinale de l'hémisphère intact accompagné par un changement des fonctions sensorimotrices corticales de l'hémisphère intact (Cao et al., 1994; Lewine et al., 1994; Maegaki et al., 1995; Muller et al., 1997, 1998; Nirkko et al., 1997; Graveline et al., 1998; Holloway et al., 1999). Pendant que certaines études indiquent que l'augmentation des projections corticospinales ipsilatérales résultent du cortex moteur primaire de l'hémisphère intact (Sabatini et al., 1994; Nirkko et al., 1997), la découverte la plus commune est la projection qui résulte du cortex moteur non primaire et des aires d'association multimodaux de l'hémisphère intact (Pascual-Leone et al., 1992; Lewine et al., 1994). En résumé, il est probable que la réorganisation est influencée par le developpement cortical général et la spécification au moment de la lésion, la disponibilité du cortex juxtalésionnel suffisant et approprié, l'absence ou la présence de projections spinales passagères (Martin et al., 1999, Martin and Lee, 1999; Eyre et al., 2001b).

Contribution des voies ipsilatérales

Une autre hypothèse avancée par plusieurs chercheurs implique l'activation des voies corticospinales ipsilatérales. Les voies corticospinales étant croisées à 80-90%, il en reste donc 10-20% qui ne sont pas croisées et qui ont une projection ipsilatérale. Suite à une lésion du cortex moteur primaire, l'hémisphère lésé n'est plus en mesure d'assurer la motricité du côté opposé du corps (côté parétique) et l'inhibition transcallosale de l'hémisphère non-lésé est altérée. Des études utilisant l'imagerie par résonance magnétique fonctionnelle (IRMf) ont démontré une activation bilatérale (cortex moteur lésé et non-lésé) lors d'un mouvement de la

main parétique alors que seulement le cortex moteur non-lésé est activé lors d'un mouvement de la main non-parétique, ce dernier patron d'activation étant similaire à celui enregistré chez les personnes saines (Marshall et al., 2000; Foltys et al., 2003; Jaillard et al., 2005). Il est donc suggéré que l'hémisphère non-lésé prendrait la relève du contrôle moteur via les voies ipsilatérales (hémisphère non-lésé vers les muscles parétiques). Par contre, ces études utilisant la technique de l'IRMf ne permettent pas de déterminer si cette activation ipsilatérale reflète des processus inhibiteurs ou facilitateurs dans le contrôle des muscles parétiques. Les voies contralatérales et ipsilatérales sont donc souvent investiguées à l'aide de TMS lors d'expériences visant à évaluer la réorganisation du contrôle moteur suivant une lésion cérébrale, puisque les TMS permettent cette discrimination entre processus inhibiteurs et facilitateurs et testent le lien fonctionnel entre l'activation du cortex moteur (par exemple) et l'activité musculaire.

1.9 Traitement des lésions cérébrales

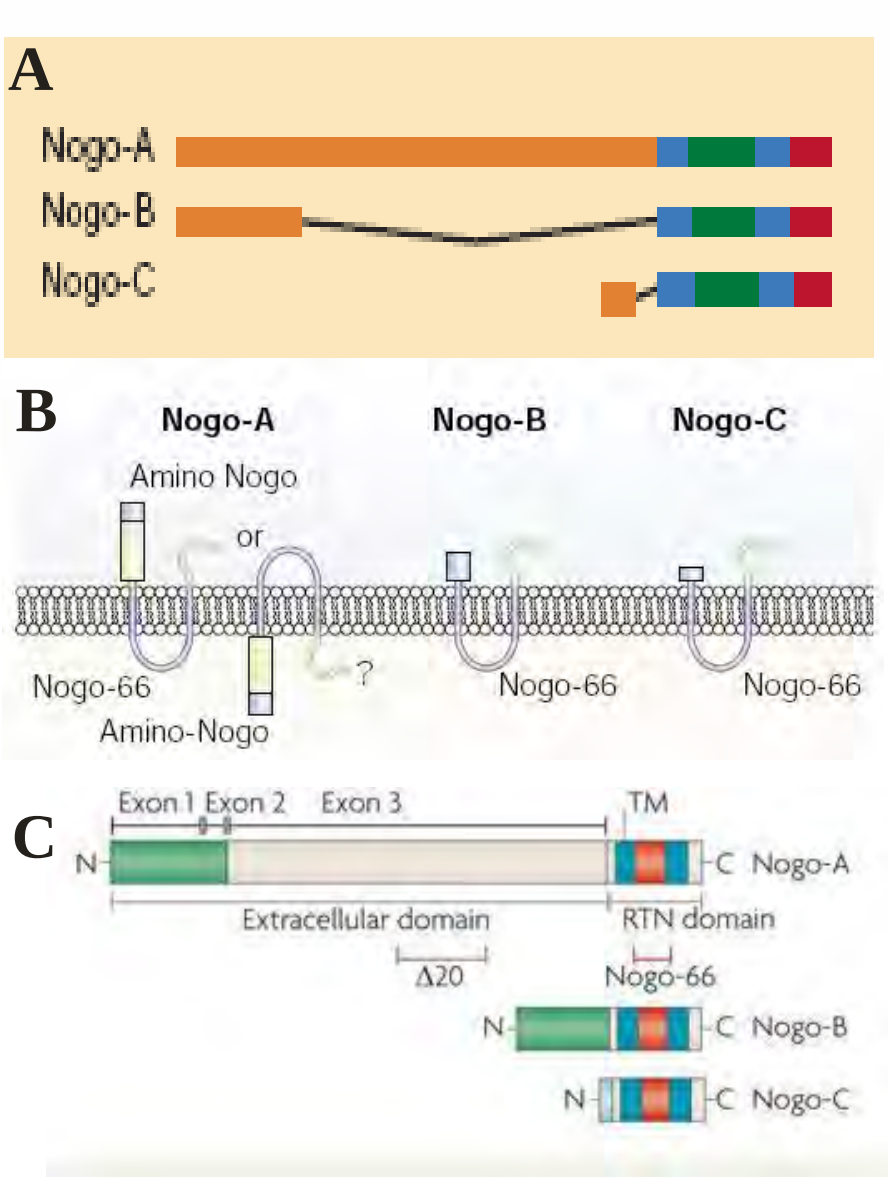
Chez les mammifères adultes, les fibres nerveuses du système nerveux central, localisées dans le cerveau et la moelle épinière, sont normalement incapables de se régénérer après une lésion. Le système nerveux périphérique des mammifères comme le système nerveux central des vertébrés inférieurs sont capables, à la suite d'une axotomie (interruption nerveuse), de pousser et de se reconnecter avec les organes cibles, en d'autres termes il présente des capacités de récupération fonctionnelle considérables si les lésions sont modérées. Une récupération partielle peut également être observée dans le système nerveux central des mammifères lorsque le traumatisme survient en bas âge (Rouiller et al., 1991). Les fibres nerveuses sont ainsi capables de former des connexions fonctionnelles avec leurs tissus cibles, c'est-à-dire la capacité intrinsèque de se régénérer à condition d'être placées dans un environnement approprié. On a récemment constaté que certaines molécules peuvent promouvoir la repousse des fibres nerveuses, alors que d'autres l'inhibent au contraire. Les gaines de myéline, qui entourent les fibres nerveuses et permettent la transmission des impulsions électriques d'une cellule nerveuse à l'autre, produisent certaines substances qui peuvent empêcher la repousse des axones. Ces molécules qui sont capables d'empêcher la croissance des axones in vitro ont été découvertes dans le SNC intact (Caroni et Schwab, 1988, Schwab, 1990) et sur le site de lésions (Fawcett and Asher, 1999, Pasterkamp et al., 2001). Parmi ces molécules, Nogo a été identifié comme une des composantes majeures de la myéline capable d'inhiber la croissance axonale dans le système nerveux central lésé chez l'adulte. Plusieurs autres inhibiteurs myéliniques ont été identifiés, comme les protéines MAG

(myelin associated glycoprotein) ou OMgp (oligodendrocyte myelin glycoprotein). La compréhension des mécanismes d'action moléculaire de ces molécules en général, et de Nogo en particulier, génère aujourd'hui des espoirs quant au développement de nouvelles thérapies.

La protéine Nogo

Caroni et Schwab ont élaboré en 1988 un anticorps, IN-1, qui neutralise les effets inhibiteurs des oligodendrocytes et de la myéline sur la croissance axonale *in vitro* et *in vivo*. Par la suite, l'équipe du Professeur Martin Schwab à Zurich a induit, chez le rat, une repousse des fibres nerveuses de la moelle épinière préalablement lésées, en utilisant un anticorps spécifique («anti-Nogo») capable de neutraliser la protéine Nogo. Après lésion du SNC, cette neutralisation permet la croissance de fibres axotomisées sur une distance de 1-2 cm et augmente le bourgeonnement axonal (Caroni et Schwab, 1988b; Bandtlow et al., 1990; Schnell et Schwab, 1990; Bregman et al., 1995; Thallmair et al., 1998; Z'Graggen et al., 1998). L'identité de la protéine reconnue par cet anticorps est restée non élucidée jusqu'en janvier 2000 où trois groupes (Chen et al., 2000; GrandPre et al., 2000; Prinjha et al., 2000) ont cloné le gène en utilisant les séquences peptidiques publiées en 1998 par le groupe de Schwab qu'ils identifient sous le nom de Nogo.

Nogo est une protéine de la famille des réticulons localisées dans le réticulum endoplasmique, et elle est aussi connue comme réticulon 4 (RTN4). La plupart des protéines RTN connues ont une séquence amino (N) terminal relativement courte qui est semblable à celui de Nogo-B et Nogo-C. La famille des RTNs/Nogo est très vielle dans l'évolution et apparaît chez tous les eucaryotes incluant les plantes et les moisissures (Oertle et al., 2003a). Trois isoformes sont produits dans le système nerveux par le gène Nogo (Fig. 12): Nogo-A (le plus grand), Nogo-B et Nogo-C (les plus petits). La séquence du résidu extracellulaire 66 (Nogo-66) est commune à ces trois isoformes. Ces trois protéines possèdent le même domaine carboxy terminal ; Nogo-A et Nogo-B partagent le même domaine amino terminal. Seul Nogo-A contient toutes les séquences peptidiques purifiées à partir des protéines inhibitrices. Même si la majorité de la protéine se situe dans le réticulum endoplasmique, le groupe de Schwab et celui de Strittmater ont montré qu'au moins une fraction de Nogo-A est présente à la membrane plasmique. Nogo-A est exprimé dans les oligodendrocytes et la myéline du SNC et est reconnu par les anticorps bloquant l'effet inhibiteur de la myéline sur la croissance axonale (Huber et al., 2002).



<u>Figure 12</u>: Structure des trois isoformes majeures de la protéine Nogo (Nogo-A, Nogo-B, et Nogo-C). A) Illustration de Goldberg and Barres, 2000a; B) Illustration de Filbin, 2003, C) Illustration de Schwab, 2010 avec les différents domaines fonctionnels.

Toutes ces expériences montrent que Nogo-A remplit toutes les fonctions attendues des inhibiteurs de la régénération axonale présents dans la myéline. La limitation de la régénération axonale est principalement liée à un environnement non permissif et non aux propriétés intrinsèques des axones. A ce jour, Nogo-A est considéré comme le plus puissant inhibiteur de croissance axonale. Son effet a été démontré à la fois à partir d'expériences in vitro et in vivo (Fig. 13). Son action fait intervenir un récepteur (NgR) présent à la surface des neurones, qui est un récepteur commun à ces trois inhibiteurs myéliniques et des voies de signalisation qui restent encore incomplètement élucidées.

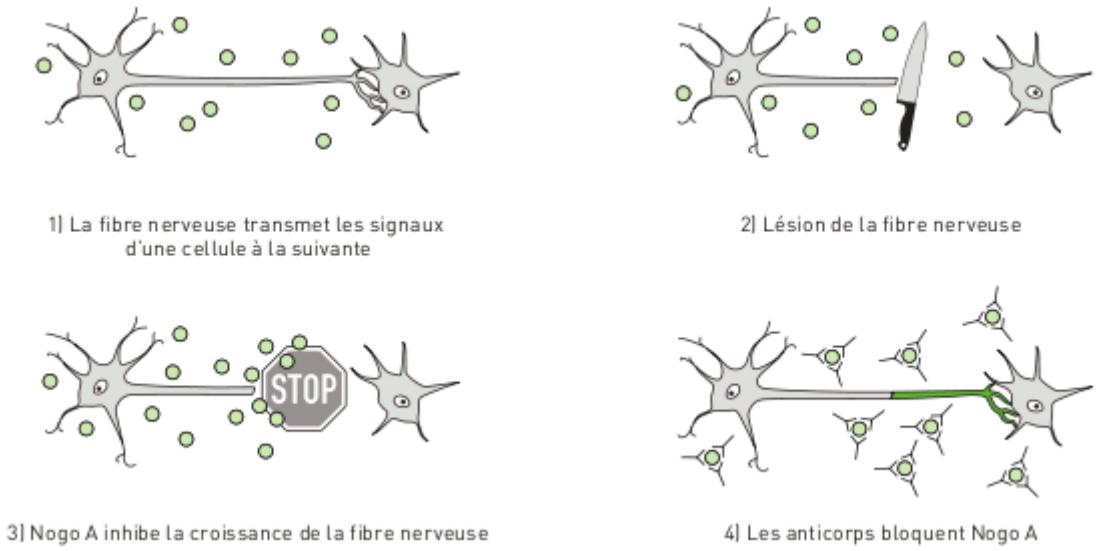
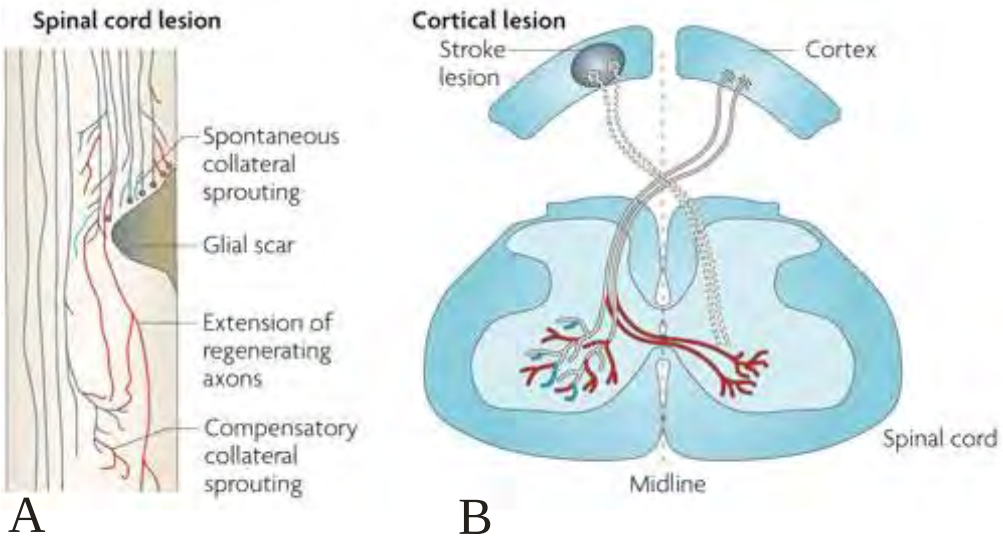


Figure 13: Mode d'action des anticorps dirigés contre Nogo-A. Essais menés chez les primates. http://www.gensuisse.ch/focus/transg/gd0903_f.html

Fonctions de Nogo dans le traitement des lésions

La neutralisation de Nogo ou de la fonction du récepteur de Nogo par des anticorps, des fragments de récepteurs solubles, des peptides antagonistes sont tous des traitements qui permettent une amélioration régénérative du bourgeonnement et une croissance des fibres nerveuses lésées après une lésion de la moelle épinière ou du cerveau. En plus, ces traitements induisent une régénération collatérale compensatoire (Fig. 14) de fibres intactes dans le SNC des adultes après lésion (Schwab, 2010). Ces processus aboutissent à une plasticité accrue et à un certain degré de régénération, dépendant de la taille et du type de la lésion induisant ainsi une réorganisation des circuits du SNC dans la moelle épinière, le cervelet et le cortex. Transitoirement, le bourgeonnement régénératif de courte distance et le degré limité de la réorganisation plastique surviennent spontanément dans plusieurs voies après une lésion. Un plus grand nombre de fibres croîssent et une récupération fonctionnelle observée après une inactivation de Nogo-A s'explique par la suppression de ces effets inhibiteurs de croissance.



<u>Figure 14</u>: A: régénération spontannée mais transitoire (bleu), améliorée par l'inactivation de Nogo-A (rouge) dans le cas d'une lésion de la moelle épinière, B: l'inactivation de Nogo-A induit une croissance des fibres nerveuses à travers la ligne médiane (rouge), phénomène associé à un haut dégré de récupération fonctionnelle dans le cadre d'une lésion corticale (Tirée de Schwab, 2010).

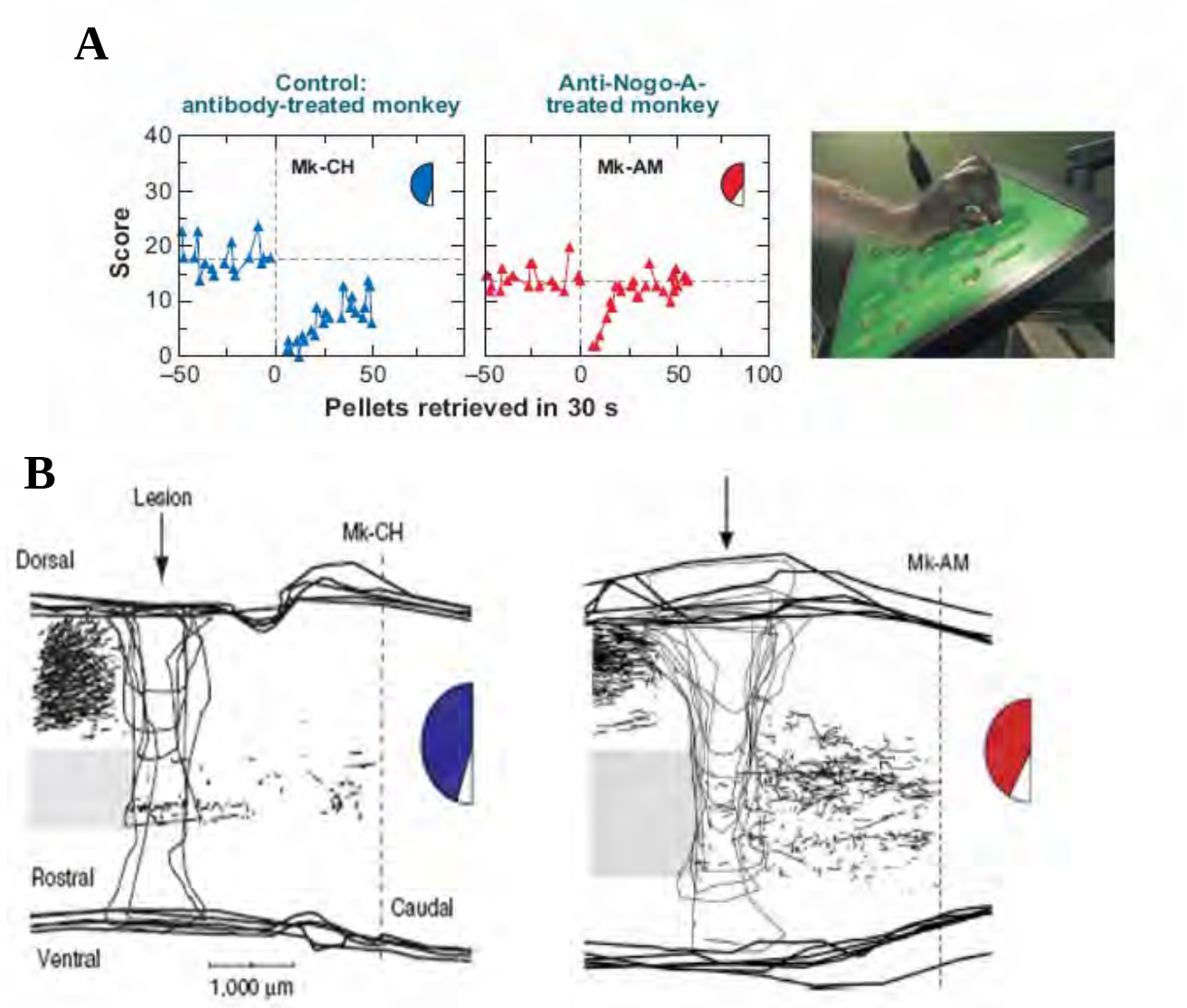
Des études importantes ont été réalisées dans le but de mieux comprendre les fonctions ou le rôle que joue Nogo dans l'inhibition de la croissance axonale. De nos jours, les fonctions de Nogo-B et Nogo-C sont largement inconnus, seul Nogo-A montre une bonne capacité inhibitrice de la croissance axonale dans le SNC des mammifères adultes (Oertle et al., 2003b). L'anticorps bloquant la fonction de Nogo-A a été appliqué dans différents modèles de lésions spinales ou cérébrales, en particulier chez les rats adultes et les primates non-humains, dans le but d'améliorer la régénération des fibres lésées. L'anticorps neutralisant le plus fréquement utilisé contre Nogo a été l'anticorps monoclonal (mAb) IN-1, qui est dirigé contre Nogo-A. Toutefois, d'autres anticorps, qui sont également dirigés contre Nogo-A et ciblant d'autres parties de la protéine, ont été développés dans le cadre de l'étude de la récupération suite à une lésion de la moelle épinière (Fig. 15).

- **mAb IN – 1 (whole length IgM; rFab)** (Caroni and Schwab, 1988; Chen et al., 2000).
  Raised against a myelin fraction enriched in Nogo-A that is able to bind Nogo-A and neutralize its growth inhibitory properties.
- **mAb 7B12 (rFab)** (Oertle et al., 2003; Liebscher et al., 2005).
  Has an epitop towards the C-terminus of the Nogo-A specific region; Binds to the outer surface of living cultured oligodendrocytes.
- **mAb 11C7 (rFab)** (Oertle et al 2003).
  Directed against a 18 amino acid peptide (aa 623-640) close to the active site of the rat Nogo-A specific region. Binds to the outer surface of living cultured oligodendrocytes.
- **mAb hNogo – A (rFab)** is a humanized monoclonal antibody raised against the Nogo-A specific region of the human Nogo-A sequence. Recognizes human and primate Nogo-A specifically on Western blots.

Figure 15: Différents types d'anticorps dirigés contre Nogo-A

L'interruption chez le rat de la voie principale corticospinale dorsale, ainsi que les composantes dorsolaterales mineures, mènent à un faible mais détectable bourgeonnement regeneratif spontané des axones lésés. La présence de IN-1 mAb montre par des études anatomiques une augmentation de la régénération et/ou du bourgeonnement des axones et environ 5-20% des fibres marquées croissent autour du site de la lésion et plus bas au niveau de la moelle épinière, sur une distance de 10 mm au-delà de la lésion (Schnell and Schwab, 1990; Brosamle et al., 2000). Les lésions spinales chez les rats adultes et les singes macaques adultes suivies de l'administration de l'anticorps dirigé contre Nogo-A (anti-Nogo-A) montrent une augmentation de la régénération des fibres axotomisées avec une amélioration de la récupération fonctionnelle (Liebscher et al., 2005; Buchli and Schwab, 2005; Freund et al., 2006, 2007, 2009). Il a été aussi démontré que, suivant une lésion effectuée au niveau de la moelle cervicale, les singes macaques traités avec anti-Nogo-A montraient une augmentation du bourgeonnement des axones corticospinaux rostralement et autour de la lésion pour certaines fibres et également caudalement dans les segments spinaux (Freund et al., 2006, 2007) (Fig. 16A et B). Cependant, il a été montré chez le rat que les effets sur la régénération des fibres lésées et sur la récupération fonctionnelle étaient réduits si le traitement était administré avec un délai de plus d'une semaine après la lésion (Von Meyenburg et al., 1998). Des expériences sur des souris transgéniques knockout (KO) pour Nogo-A, Nogo-A/-B et Nogo-A/-B/-C ont été l'objet d'études récentes. Après lésion de la moelle épinière, les souris KO spécifique à Nogo-A ont montré une augmentation modérée, mais détectable, de la regénération des fibres et de leurs allongements (Simonen et al., 2003). Les mêmes observations ont été quantitativement faites chez les souris KO spécifique à Nogo-

A/-B (Kim et al., 2003). La convergence des résultats anatomiques des voies diverses de l'inactivation de Nogo-A suggère que cette molécule joue un rôle crucial dans l'inhibition du processus de réparation axonal spontané dans la moelle épinière lésée.



<u>Figure 16</u>: Effet de la lésion de la voie corticospinale sur la dextérité manuelle (A) chez le macaque évalué par la tâche du tableau de Brinkman (Nombre de pellets récupérés en 30s) et sur la régénérescence axonale (B). Bleu: singe contrôle non-traité à l'anticorps anti-Nogo-A, Rouge: singe traité avec l'anticorps anti-Nogo-A (Tiré de Freund et al., 2006).

1.10 But du présent travail de thèse

Le présent travail fait suite aux travaux antérieurs effectués dans le laboratoire du Professeur Rouiller portant sur les mécanismes de récupération des fonctions motrices suite à une lésion unilatérale du cortex moteur primaire chez les primates non-humains. Une étude

pilote a été conduite sur 2 singes (Liu et Rouiller, 1999), elle a permis de décrire le déficit induit par une lésion de M1, ainsi que le décours temporel et l'étendue de la récupération fonctionnelle spontanée. Le présent travail s'inscrit dans une seconde phase visant à augmenter le nombre d'animaux lésés et de tester la pertinence d'un traitement anti-Nogo-A, ou encore de l'implantation de cellules progénitrices adultes. Des premiers résultats de ces travaux de longue haleine ont été récemment publiés et font partie de la présente thèse en annexe des résultats (Kaeser et al., 2010, 2011). L'originalité de notre travail repose sur une combinaison d'approches électrophysiologiques et anatomiques, qui visent à déterminer d'une part la réorganisation des connexions dans les différentes aires corticales du cortex moteur et, d'autre part, l'influence de traitements (par exemple anti-Nogo-A) sur cette réorganisation.

De facon spécifique, la présente thèse a pour but de traiter cinq questions essentielles dans la compréhension des mécanismes selon lesquels le SNC se réorganise, après lésion du cortex moteur primaire avec ou sans traitement à l'aide de l'anticorps anti-Nogo-A. Ces questions essentielles seront présentées sous forme de 5 chapitres distincts. Le **chapitre I** est une étude comportementale basée sur les effets à court terme d'une lésion unilatérale du cortex moteur primaire (M1) sur la dextérité manuelle du coté ipsilésionel, ce chapitre est l'objet d'une publication (Bashir et al., 2011). Le **chapitre II** est consacré à une étude électrophysiologique par la méthode de stimulation intracorticale (ICMS) des conséquences de la lésion de M1 sur la réorganisation des cartes somatotopiques corticales. Le **chapitre III** tente de déterminer pharmacologiquement, par une inactivation transitoire (injection de muscimol) dans trois aires importantes du cortex (M1, PMv, PMd), si la réorganisation dans les aires corticales adjacentes à la lésion est différente entre les groupes d'animaux traités et ceux non-traités avec l'anticorps anti-Nogo-A. Afin d'établir une éventuelle corrélation avec la réorganisation des connexions, les **chapitres IV et V** consistent en des études anatomiques de traçage à deux niveaux importants des afférences au cortex cérébral. Le chapitre IV concerne la réorganisation des connexions callosales provenant de l'hémisphère contralésionel intact et le chapitre V traite des connexions thalamocorticales, cela dans le contexte d'une comparaison préliminaire entre les singes non-traités et les singes traités avec l'anticorps anti-Nogo-A.

2 Matériels et méthodes

Cette partie du manuscrit de thèse a pour but de donner un bref aperçu des différentes approches méthodologiques (comportementales, électrophysiologiques et anatomiques) utilisées au cours de cette thèse. Des informations détaillées sur chacune des procédures expérimentales seront données dans les différents chapitres. Tous les travaux ont été réalisés au département de Médecine de l'Université de Fribourg, suivant les recommandations du guide de soins et d'utilisation des animaux de laboratoire (ISBN 0-309-05377-3 ; 1996) et approuvées par les autorités vétérinaires cantonales et fédérales.

2.1 Etudes comportementales

Les expériences ont été effectuées sur les singes de l'espèce *Macaca fascicularis*. Au total onze jeunes macaques adultes des deux sexes, d'âge compris entre 3 et 5 ans et pesant entre 3 et 6 kg au début des tests comportementaux, ont été utilisés pour les expériences, et ont été soumis à une lésion unilatérale de la région de la main du cortex moteur primaire. Ces animaux lésés ont été subdivisés en deux sous-groupes: un sous-groupe non traité à l'anticorps anti-Nogo-A et un autre ayant reçu le traitement à l'anticorps anti-Nogo-A. Pendant toute la durée des travaux comportementaux et électrophysiologiques, les animaux ont été placés sous la responsabilité du même expérimentateur pour chaque singe. Pour s'assurer d'une bonne condition physique des animaux, le poids a été mésuré régulièrement avant chaque session quotidienne comportementale ou électrophysiologique. Tous les animaux étaient logés dans notre animalerie dans des chambres de $12m^3$, contenant 2-4 animaux de même sexe libres de mouvements et pouvant interagir entre eux[1]. Avant chaque expérience, les animaux sont installés dans une chaise de primate en plexiglas (Fig. 17) depuis leur chambre dans l'animalerie. Celle-ci est adaptée à leur taille, avec une ouverture ajustable sur le dessus pour la tête et deux petites portes coulissantes indépendantes sur le devant pour chacun des bras. Le primate est installé sur des barres de fer horizontales adaptées à sa taille, permettant à l'animal d'être dans une position confortable. Durant toute notre étude, une chaise pour primate a été affectée à chaque singe.

[1] Dés septembre 2010, une nouvelle législation impose une chambre de 45 m^3 pour détenir 2-5 macaques.

<u>Figure 17</u>: Chaise de primate en plexiglas utilisée lors des tests comportementaux. Le singe utilise alternativement une des deux mains.

Au début, les animaux étaient entrainés à effectuer de manière alternative avec chacune des mains la tâche unimanuelle sur un tableau appélé «Brinkman modifié» (Fig. 18) permettant d'évaluer la dextérité manuelle des primates (Rouiller et al., 1998; Liu et Rouiller, 1999; Schmidlin et al., 2011). Ce tableau est construit à partir d'une planche rectangulaire de Perspex® vert (10 cm x 20 cm) contenant 50 puits de forme ovale, dont 25 puits horizontaux et 25 puits verticaux. Les dimensions des puits étaient de 15 mm de long, 8 mm de large et 6 mm de profondeur. Avant le début chaque expérience, des granulés à l'arôme de banane mesurant environ 4 mm de diamètre sont insérés dans chacun des 50 puits. La récupération des granulés exige du primate une bonne exécution des mouvements fins des doigts consistant en une opposition entre le pouce et l'index, correspondant ainsi à ce qu'on appelle «pince de précision» (Lemon, 2008; Freund et al., 2009). Les primates étaient entrainés à effectuer la tache de la dextérité manuelle sur le Brinkman modifié quatre à cinq jours par semaine en utilisant séparement l'une des deux mains pendant plusieurs mois jusqu'à atteindre un plateau qui indique un niveau stable de performance. La durée journalière de chaque session comportemantale était d'une heure maximum. La performance de chaque main est analysée en comptant le nombre de granulés récupéré en 30 secondes dans les deux types de puits respectivement.

Des tests comportementaux additionnels ont été effectués par les primates afin d'évaluer d'autres aspects de la dextérité manuelle (Schmidlin et al., 2011), parmi ces tests on

peut citer le Brinkman rotatif, la boite de Brinkman et la tâche du tiroir (Fig. 18). Ces différentes tâches peuvent être visualisées par des séquences vidéo sur le site web http://www.unifr.ch/neuro/rouiller/research/motorcontcadre.php

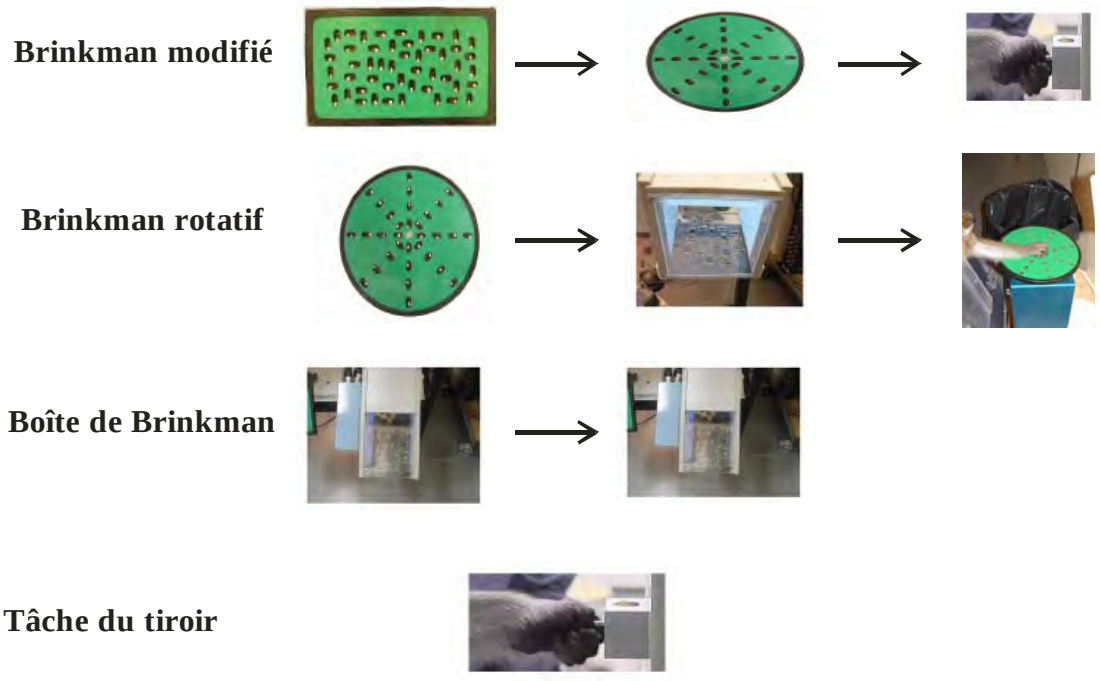


Figure 18: Différents tests comportementaux utilisés pour l'évaluation de la dextérité manuelle chez les primates non-humains.

2.2 Procédures chirurgicales

Les animaux sont soumis à une craniotomie afin d'exposer le cortex moteur primaire. Tout d'abord les animaux sont endormis avec de la ketamine (Ketalar; Parke-Davis, 5 mg/kg, IM), puis de l'atropine a été injectée (0,05 mg/kg, IM) afin de réduire les sécrétions bronchiales qui pourraient obstruer les voies respiratoires. Avant l'opération, les animaux sont traités avec un analgesique qui est le carprofen (Rymadil, 4 mg/kg, SC). Par la suite, ils sont anesthésiés avec une perfusion intraveineuse de 1% propofol (Fresenius) mélangé avec une solution de glucose à 5% (1 volume de propofol et 2 volumes de solution de glucose); la ketamine était ajoutée à la perfusion (65mg/100mL). Afin de prévenir d'éventuels oedèmes, une injection intramusculaire (IM) de déxaméthasone (Dexacortin 0,1mg/kg) a été faite. La quantité moyenne de mélange injecté de propofol/glucose pour obtenir une anesthésie stable était 0.1 ml/min/kg. Toute l'opération chirurgicale était effectuée sous des conditions stériles. Le niveau de l'anesthésie a été ajusté en fonction des paramètres suivants qui étaient surveillés pendant la chirurgie entière : la fréquence cardiaque, la saturation de l'oxygène du sang artériel, la fréquence respiratoire, le CO2 expiré, et la température centrale de l'organisme

mesurée dans le canal anal. Une chambre d'enregistrement en acier inoxidable (22 x 17 x 15 mm) a été fixée sur le crâne, au dessus de l'hémisphère gauche de chaque primate, notamment au niveau du cortex moteur primaire permettant ainsi de faire des sessions de stimulation intracorticale dans le but de déterminer la région de la main du cortex moteur primaire et, en même temps, les autres aires comme le cortex prémoteur. Après l'opération, les primates recevaient un analgésique (carprofen sous forme de comprimé de Rymadil dans de la banane) chaque jour et un antibiotique (Albipen; injection sous-cutanée) tous les deux jours pendant 1 à 2 semaines afin d'empêcher la douleur postopératoire et de prévenir une infection.

2.3 Stimulation intracorticale, lésion et traitement

Les micro-stimulations intracorticales (ICMS) ont été effectuées deux fois, premièrement avant la lésion afin de déterminer la région de la main du cortex moteur primaire pouvant permettre de nous guider dans la lésion et, deuxièmement, plusieurs mois après la lésion lorsque le macaque a atteint (récupéré) un niveau stable de performance manuelle dans les taches motrices (Rouiller et al., 1998; Liu and Rouiller, 1999; Schmidlin et al., 2004). Grâce à une chambre chroniquement implantée, une microélectrode de tungstène (0.1 - 1 MΩ impedance, FHC Inc, USA.) a été utilisée pour stimuler le cortex moteur par des pénétrations distantes de 1 mm (Schmidlin et al., 2004, 2005). Le long de chaque pénétration d'électrode, l'ICMS était appliqué au-dessous de la surface de la dure mère à intervalles de 1 mm sur une distance variable (≤ 10-12 mm). L'effet de l'ICMS était évalué par inspection visuelle et/ou palpation du corps où le mouvement était observable. Le courant minimal (seuil ICMS) produisant le mouvement était déterminé à chaque site de stimulation, puis une carte ICMS était finallement représenté sous la forme d'une carte dépliée (Park et al., 2001, 2004; Kaeser et al., 2010). Après l'établissement de cette carte, une lésion unilatérale a été faite sur des sites selectionnés au niveau de la région de la main du cortex moteur primaire par infusion d'acide iboténique (10µg/µl dans une solution saline de phosphate tamponné) à l'aide d'une microséringue Hamilton, telle que décrite précédemment (Liu and Rouiller, 1999; Kaeser et al., 2010). Le nombre de sites d'injection et le volume d'acide iboténique injecté dans la région de la main de M1 étaient basés sur l'étendue de la région de la main établie pour chaque macaque. Quelques minutes après la lésion, certains macaques ont été traités par un anticorps anti-Nogo-A durant 4 semaines à l'aide de 2 pompes osmotiques placée sous la peau et dont le cathéter était placé, pour la première pompe, à quelques millimètres de la lésion corticale. La deuxième pompe a délivré l'anticorps anti-Nogo-A dans la moelle cervicale, comme décrit précédemment (Freund et al., 2006, 2007, 2009). Le but de la première pompe

était de délivrer l'anticorps anti-Nogo-A dans le cortex cérébral pour favoriser la plasticité de la région à l'origine de la projection corticospinale. La deuxième pompe, au niveau cervical, servait à tenter d'augmenter le bourgeonnement axonal dans la région cible de la projection corticospinale.

2.4 Inactivation transitoire, injection de traceur et histologie

Plusieurs mois après la lésion lorsque l'animal a atteint un plateau comportemental post-lésionnel, une lésion réversible, basée sur la carte ICMS post-lésion, a été faite par infusion de muscimol (un agoniste de GABA) (Sigma, 1 µg dans 1 µl de solution saline 0.9%), dans trois aires différentes du cortex: M1, PMd et PMv. Le volume de muscimol injecté, le nombre de sites d'injection et la position des sites ont été choisis de telle sorte à couvrir le plus possible tout le territoire de l'aire du cortex visée. Ces expériences d'inactivation réversible post-lésionnelle du cortex moteur avaient pour but de déterminer si une aire corticale donnée contribue à la récupération fonctionnelle et, si oui, dans quelle mesure.

L'infusion de muscimol dans les différentes aires a été faite à intervalle d'une semaine pour chaque aire du cortex testée. Immédiatement avant et sitôt après infusion de muscimol, les macaques étaient amenés à effectuer la tâche unimanuelle de dextérité manuelle sur le Brinkmann modifié.

Lorsque toutes les trois aires du cortex moteur ont été inactivées (expériences du muscimol) et 3 semaines avant le sacrifice de l'animal (perfusion), le traceur neuroanatomique Biotine Dextran Amine (BDA, MW 10'000, 10% dans une solution saline de phosphate-tamponné, Molecular Probes, Eugene, OR) était injectée dans le cortex prémoteur de l'hémisphère lésé en utilisant une microséringue Hamilton comme décrit précédemment (Rouiller et al., 1994 a,b, 1996, 1999, 2003; Liu et al., 2002; Tanné-Gariépy et al., 2002; Boussaoud et al., 2005; Morel et al., 2005; Cappe et al., 2007, 2009). Après une période de survie de 3 semaines permettant au traceur (BDA) d'atteindre l'hémisphère intact (opposé), le thalamus et autres structures sous-corticales, les macaques ont été sacrifiés en injectant une dose léthale de sodium pentobarbital (90mg/kg de poid, i.p) et perfusés de manière transcardiaque par une solution saline de 0.9% (500ml), puis fixés par une solution de paraformaldehyde à 4 % dans 0.1M de phosphate tamponné (pH=7.6) et successivement avec des solutions tampons avec des concentrations croissantes de sucrose (10, 20, 30 %). Le cerveau est par la suite placé dans une solution de sucrose à 30 % durant 3-5 jours. Des coupes frontales du cerveau (50µm d'épaisseur) ont été réalisées par la méthode de

congélation en 5 séries. Les 3 premières séries ont été traitées histologiquement pour visualiser la BDA, SMI-32 et Nissl, tandis que les 2 séries restantes ont été conservées comme réserve.

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3 Résultats

3.1 Chapitre 1:

Effets à court terme d'une lésion unilatérale du cortex moteur primaire (M1) sur la dextérité de la main ipsilésionnelle chez les singes macaques adultes.

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Les quatre premiers auteurs ont contribué également à l'étude

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ORIGINAL ARTICLE

Short-term effects of unilateral lesion of the primary motor cortex (M1) on ipsilesional hand dexterity in adult macaque monkeys

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Abstract Although the arrangement of the corticospinal projection in primates is consistent with a more prominent role of the ipsilateral motor cortex on proximal muscles, rather than on distal muscles involved in manual dexterity, the role played by the primary motor cortex on the control of manual dexterity for the ipsilateral hand remains a matter a debate, either in the normal function or after a lesion. We, therefore, tested the impact of permanent unilateral motor cortex lesion on the manual dexterity of the ipsilateral hand in 11 macaque monkeys, within a time window of 60 days post-lesion. For comparison, unilateral reversible pharmacological inactivation of the motor cortex was produced in an additional monkey. Manual dexterity was assessed quantitatively based on three motor parameters derived from two reach and grasp manual tasks. In contrast to the expected dramatic, complete deficit of manual dexterity of the contralesional hand that persists for several weeks, the impact on the manual dexterity of the ipsilesional hand was generally moderate (but statistically significant) and, when present, lasted less than 20 days. Out of the 11 monkeys, only 3 showed a deficit of the ipsilesional hand for 2 of the 3 motor parameters, and 4 animals had a deficit for only one motor parameter. Four monkeys did not show any deficit. The reversible inactivation experiment yielded results consistent with the permanent lesion data. In conclusion, the primary motor cortex exerts a modest role on ipsilateral manual dexterity, most likely in the form of indirect hand postural control.

Keywords Permanent lesion · Reversible lesion · Primate

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Introduction

In primates, the corticospinal projection system plays a major role in the control of skilled movements performed with the contralateral hand (see Lemon 2008, for review), consistent with the notion that most corticospinal axons addressing motoneurons that control distal forelimb muscles decussate (about 85–98% of all corticospinal axons; Rouiller et al. 1996; Lacroix et al. 2004; Rosenzweig et al. 2009; Yoshino-Saito et al. 2010). In contrast, the small contingent of uncrossed corticospinal axons (about 2–15%) terminates mainly in zones of the cervical cord comprised of the motoneurons of proximal and axial muscles. As a consequence, it is generally assumed that the motor cortex limits its control on the ipsilateral forelimb to movements executed by proximal muscles (see Ganguly et al. 2009; Bradnam et al. 2010, for recent functional data). Nevertheless, a possible contribution of the motor cortex to the control of ipsilateral skilled hand movements remains a matter of debate, as an activation of the motor cortex associated with skilled movements performed with the ipsilateral hand was found in normal human subjects (e.g. Kim et al. 1993; Sadato et al. 1996; Catalan et al. 1998; Kawashima et al. 1998; Cramer et al. 1999; Ehrsson et al. 2000; Porro et al. 2000; Hummel et al. 2003; Rau et al. 2003; Verstynen et al. 2005). The activity in the ipsilateral

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motor cortex was related to the complexity of the task (e.g. Catalan et al. 1998; Hummel et al. 2003; Verstynen et al. 2005), and compared to the site of activation in the contralateral hemisphere, it was shifted ventrally, laterally and anteriorly (Cramer et al. 1999; Verstynen et al. 2005).

In response to unilateral transcranial magnetic stimulation (TMS) of the motor cortex, motor-evoked potentials (MEPs) can be recorded from ipsilateral distal muscles (Wassermann et al. 1991; Alagona et al. 2001; Chen et al. 2003; Ziemann et al. 1999), although pre-contraction of the target muscles was required. In other studies, however, no such ipsilateral MEP activity was observed in hand muscles (Carr et al. 1994; Netz et al. 1997; Bawa et al. 2004). Ipsilateral MEPs are related to the degree of handedness (Bernard et al. 2011). When TMS was used to generate a transient inactivation of the motor cortex while the subject was performing hand motor tasks with the ipsilateral hand, deficits were observed in a sequential finger task (Chen et al. 1997), and in grip-lift and in step-tracking tasks (Davare et al. 2007). This effect may be mediated by indirect corticoreticulospinal fibers (Chen et al. 1997) or through the opposite motor cortex via transcallosal fibers, although the hand representations of the two motor cortices are less strongly connected via the corpus callosum than other body territories (Jenny 1979; Rouiller et al. 1994). Finally, a study by Foltys et al. (2001) demonstrated that a perturbation of the ipsilateral motor cortex with TMS affects the reaction time to generate simple unimanual or bimanual movements.

The possible role of the ipsilateral motor cortex in the control of hand movements is indirectly supported by motor deficits of the ipsilesional hand observed after unilateral stroke (Hermsdörfer and Goldenberg 2002; Hermsdörfer et al. 2003; Sunderland et al. 1999; Sunderland 2000; Yarosh et al. 2004; Wetter et al. 2005; Nowak et al. 2007; Chestnut and Haaland 2008; Noskin et al. 2008). A clear limitation of lesional data in human subjects is the highly variable extent of the lesion as well as its location, rarely if ever restricted to the primary motor cortex. Furthermore, depending on the time separating the lesion from the observation, often several weeks, months or years post-lesion, a re-organization of the motor system may have significantly modified or adapted the performance of the ipsilesional hand, as compared to the pre-lesion performance (e.g. Kaeser et al. 2010). Finally, the lesion studies in patients are based on comparison with a group of normal subjects, a procedure inherently confounded by large inter-individual variability.

Given the above-outlined limitations, the necessity of using a non-human primate model becomes evident, especially considering the fact that manual dexterity is considered to be a prerogative of primates (see Lemon 2008, for review), and monkeys such as macaques exhibit considerable manual dexterity. In macaques, using the technique of intracortical microstimulation (ICMS), in a restricted sub-region of the primary motor cortex (M1), located at the limit between the standard hand and face representations of the contralateral body side, ICMS elicited EMG responses in distal muscles not only on the contralateral forelimb as expected, but also in distal muscles on the ipsilateral side (Aizawa et al. 1990). Moreover, in various tasks executed with one or the other hand, several studies demonstrated the presence of neuronal activity in M1 correlated with movements of the ipsilateral hand (Matsunami and Hamada 1981; Aizawa et al. 1990; Chen et al. 1991; Donchin et al. 1998, 2002; Kermadi et al. 1998, 2000; Kazennikov et al. 1999; Cisek et al. 2003). However, it cannot be completely ruled out that such ipsilaterally related neuronal activity may be, at least in part, due to parallel activation of proximal muscles, or that it may correspond to an inhibition of movements with the opposite hand in unimanual tasks (for instance to prevent mirror movements). Due to these limitations of interpretation, the goal of the present study was to use the non-human primate model to assess the role of M1 in the control of the ipsilateral hand using an experimental lesional approach. The advantages of this model are multifold: (1) each trained animal can be used as its own control (by comparing pre-lesion vs. post-lesion performance); (2) observations can be conducted at very early time points post-lesion; and (3) the lesion can be mainly restricted to M1. In a previous study that employed small lesions of the hand area of M1, some paralyses of the ipsilateral hand were cursorily mentioned (Glees and Cole 1950; Cole and Glees 1952). The present study aims at extending these initial data using: (1) a larger population of monkeys; (2) a more quantitative assessment of manual performance; and (3) lesions whose extent and location are guided electrophysiologically by ICMS. Furthermore, the effect of M1 lesion was tested on the ipsilateral hand following both permanent and reversible lesions.

Methods

The present data have been derived from a comprehensive long-term experiment, conducted on 11 adult macaque monkeys (*Macaca fascicularis*) subjected to a unilateral permanent lesion of M1 (see Table 1; Fig. 1a) and 1 additional monkey subjected to pharmacological reversible inactivation of M1 unilaterally. 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.

In our animal facility, monkeys were housed in rooms of 12 m^3, in which usually 2–4 monkeys were free to move

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-BI	Mk-VA	Mk-SL	Mk-MO	Mk-AV	Mk-JO	Mk-JA
Treatment	None	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	5.5	3.5	3.5	4
Weight at time of lesion	3.8	3.6	2.8	3.2	5	4.9	4.6	5.6	4.3	3.4	4.3
Volume of ibotenic acid injected (µl)	40	40	13	18	29.7	15.5	18	20	15	15	38[a]
No. of ICMS sites injected with ibotenic acid	21	21	13	12	29	11	12	20	10	10	38
Total volume of lesion (mm^3), gray matter (motor cortex + post-central gyrus)	112.8	63.01	48.7	14	20.13	20	78.2	41.8	33.2	33.6	22.2
Volume of lesion in post-central gyrus (mm^3)	10.1	0	7.6	0	0	5.8	1.8	0	0	3.8	2.5
Lesion spread subcortically to the white matter (mm^3)	86.5	28.9	0	0	0	0	130.6	0	69.8	23.6	38.4

Mk-LA (involved only in the transient inactivation of M1 with infusion of muscimol) was 5 years old and weighted 2.6 kg. A total volume of 15 µl of muscimol was infused in M1, at 10 sites, previously identified based on ICMS (see text)

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

and to interact with each other.[1] Before daily behavioral testing in the morning, the animal caretaker moved the monkeys to temporary cages for subsequent transfer to a primate chair, in which the monkeys were transported to the behavioral laboratory. The monkeys had free access to water and were not food deprived. The rewards (pellets) obtained during the behavioral tests were the first daily access to food. After completion of the behavioral tests, the monkeys received additional food (fruits, cereals). The body weight of the animals was monitored on each working day. In case the body weight dropped by 10% or more, the experiment was interrupted until the monkey regained the lost weight (this criterion for interruption was not met during the course of the present experiments).

Treatments

The 11 monkeys subjected to unilateral permanent lesion of the motor cortex were included in two pilot studies aimed at assessing the possible effects of two different treatments: (a) anti-Nogo-A antibody treatment; (b) cell therapy with injection of autologous adult progenitor cells collected from the prefrontal cortex (see Brunet et al. 2005; Kaeser et al. 2011). The anti-Nogo-A antibody treatment has been found to significantly enhance functional recovery and sprouting of corticospinal axons after cervical cord injury in rats (Gonzenbach and Schwab 2008, for review) and primates (Freund et al. 2006, 2007, 2009). The same strategy was tested here for a sub-group of three monkeys (Mk-VA, Mk-SL, Mk-MO) and compared with a subgroup of five monkeys also subjected to a unilateral lesion of the motor cortex but without any treatment (Mk-CE, Mk-JU, Mk-GE, Mk-RO and Mk-BI; see Table 1). As outlined in Table 1, three monkeys were included in the pilot cell therapy project, two monkeys (Mk-JO and Mk-JA) received an implantation of adult progenitor cells in the vicinity of the cortical lesion, whereas one monkey (Mk-AV) was a sham control (infusion of vehicle only). The therapeutic effect of the two treatments on the contralesional hand is reported elsewhere (e.g. Kaeser et al. 2011). The possible impact of the treatments on the ipsilesional hand will be addressed in "Discussion".

Behavioral analysis

Monkeys were trained to perform two variations of a manual skill task consisting of grasping small food pellets using a precision grip, namely the opposition of the thumb and index finger. Food pellets were made of dried banana or glucose powder that is compressed in a round shape of about 4 mm in diameter. Dried raisins were occasionally given to increase the motivation of the animals, e.g. to

[1] A new Swiss regulation introduced in September 2010 now requires that a volume of at least 45 m^3 be given to a group of up to five macaque monkeys.

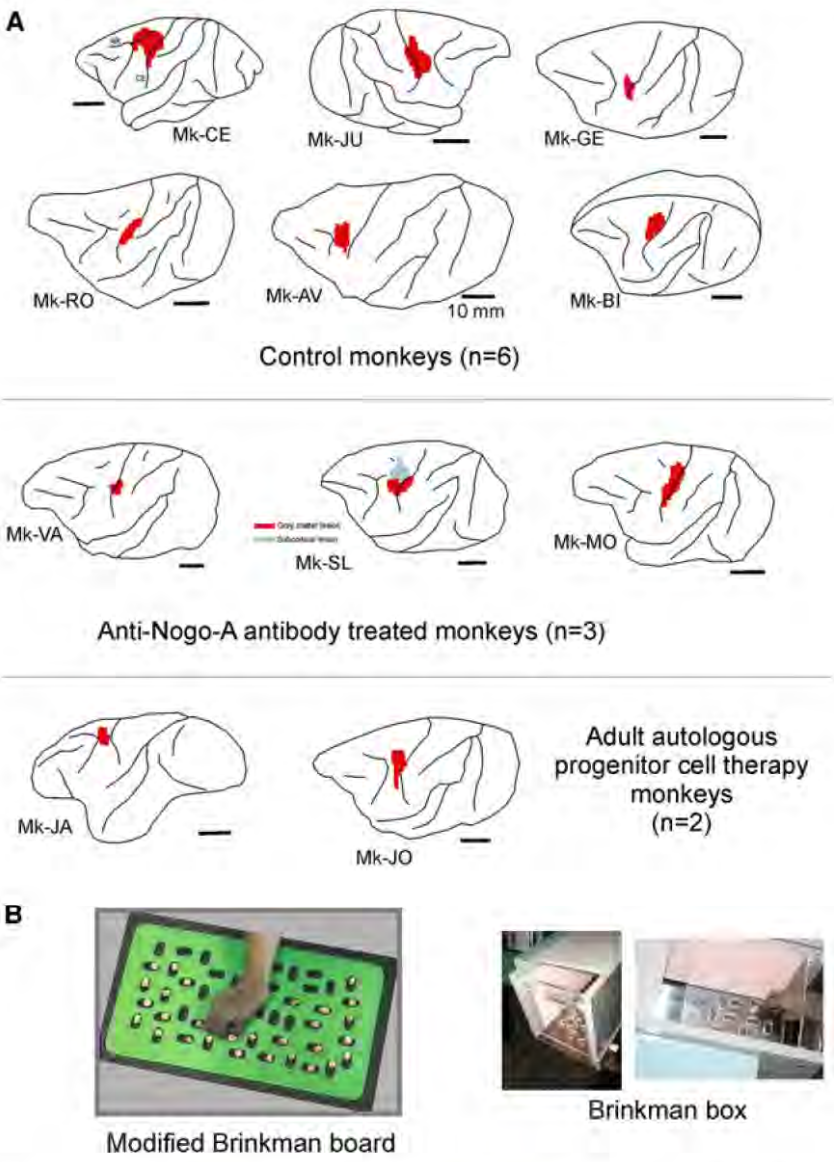

Fig. 1 **a** Location and extent of the permanent unilateral lesion of the M1 hand representation as seen on corresponding lateral views of the brain for 11 monkeys included in the present study (see Table 1). The lesion territory is represented in *red*, as derived from the lesioned zone of cerebral cortex (gray matter) visible on consecutive frontal histological sections. Spread of the lesion to the subcortical white matter below the gray matter is not represented here, except in monkey Mk-SL in which a subcortical white matter territory was lesioned (*gray spot*), in a region located more medially than the *red* territory. The motor cortex lesion was performed in all monkeys on the left hemisphere, except in Mk-JU in which the lesion was in the right hemisphere. Six monkeys (*top panel*) were control animals for two pilot treatment studies: three monkeys were treated with anti-Nogo-A antibody (*middle panel*) whereas two monkeys were subjected to an autologous cell therapy (see "Methods" and Table 1). **b** View of the Modified Brinkman Board (*left*) and the Brinkman box (*right*)

perform a particularly crucial step during training. A daily behavioral session typically lasted 60 min. An initial training period was necessary until the monkeys reached a stable pre-lesion performance, which was then quantitatively determined during a time window ranging from 30 to 128 days before the lesion (Table 1), depending on the specific experimental protocol for each monkey. Post-lesion, the behavioral tasks were pursued during several months (see Kaeser et al. 2010, for long-term effects). In the present report, as the goal was to investigate the short-term effects, the post-lesional behavioral data for the ipsilesional hand were limited to a time window ranging from the day of the lesion up to 60 days post-lesion.

The first manual skill test corresponds to our "Modified Brinkman Board" task (Fig. 1b, left panel), previously described in detail (e.g. Rouiller et al. 1998; Liu and Rouiller 1999; Schmidlin et al. 2004; Freund et al. 2006, 2009; Kaeser et al. 2010, 2011): briefly, the tests were performed on a perspex board (10 cm × 20 cm) containing 50 randomly distributed slots, each filled with a food pellet at the beginning of the test. Twenty-five slots were oriented horizontally and 25 slots vertically. The dimension of the slots was 15 mm long, 8 mm wide and 6 mm deep. This manual prehension task was executed daily, alternately with one or the other hand, 2–5 times per week for several months before and after the cortical lesion. The performance of each hand was videotaped. In the present study, two parameters were assessed: (1) The retrieval score corresponds to the number of pellets successfully retrieved from the slots and brought to the mouth during 30 s, established separately for the vertical and the horizontal slots; (2) The contact time, defined as the time of contact (in seconds) between the fingers and the pellet, calculated for the first five vertical slots and the first five

horizontal slots targeted by the monkey in a given daily session (Kaeser et al. 2010).

In parallel, monkeys were trained to perform the "Brinkman box" task (Fig. 1b, right panel), in which a box containing a perspex board of 20 wells was used (10 vertical slots and 10 horizontal slots). The monkey had visual control on its grasping movements within the box and therefore the test is generally comparable to the above Modified Brinkman Board task, except that the number and spatial distribution of slots is different and the hand is constrained to a restricted space, offering fewer degrees of freedom to reach each slot. The "Brinkman box" task was quantified by counting the total time in seconds needed by the monkey to empty the 20 wells. The two manual skill tasks (Modified Brinkman Board and Brinkman box) can be seen on the following web page: http://www.unifr.ch/neuro/rouiller/motorcontcadre.htm.

Surgical procedures

After the monkey reached a stable pre-lesion performance level, the monkeys were implanted unilaterally with a chronic, stainless steel or tecapeek chamber giving access to M1, but leaving the dura mater in place (see Schmidlin et al. 2004, for detail). The monkeys were first sedated with i.m. injection of ketamine (Ketalar, 5 mg/kg) and pre-medicated with the analgesic carprofen (Rymadil, 4 mg/kg, s.c.) to reduce pain after surgery, as previously described (Schmidlin et al. 2005; Wannier et al. 2005; Freund et al. 2006, 2007, 2009). The surgical intervention itself was conducted under aseptic conditions, and profound anesthesia was 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 (ampicillin 10%, 30 mg/kg, s.c.) and analgesics (pills of Rymadil mixed with food) for several days. Implanted chambers were fixed to the skull with titanium screws and orthopedic cement (Palacos). The inside of the chronic chamber was cleaned daily with Betadine and an antibiotic ointment was spread on the dura mater surface to reduce the risk of infection.

Mapping M1 with intracortical microstimulation (ICMS)

The surgical goal was to restrict the unilateral lesion mainly to the hand representation in M1. To do so, ICMS sessions were performed to map M1: a tungsten microelectrode (0.1–1 MΩ impedance, FHC Inc, USA) was used to micro-stimulate M1, along penetrations at 1 mm from each other, as previously described in detail (Schmidlin et al. 2004, 2005; Kaeser et al. 2010). Along each electrode track, ICMS was applied below the surface of the dura at intervals of 1 mm, along a trajectory of up to 10 mm (in the rostral bank of the central sulcus). The effects of ICMS were assessed by visual inspection of the body part (articulation) at which a movement was elicited and at which minimal current (ICMS threshold) it produced the effect. The ICMS map was finally represented in the form of an unfolded map of M1, as previously reported (Park et al. 2001, 2004; Kaeser et al. 2010) and provided the basis with which to guide injections of ibotenic acid in order to produce a lesion of M1 centered on the hand area.

Lesion of M1 hand representation with ibotenic acid (permanent lesion)

The cortical lesion was targeted to the hand representation in M1 on one hemisphere. Ibotenic acid (10 μg/μl in phosphate buffer) was infused using a Hamilton microsyringe at selected ICMS sites of the hand area in M1 unilaterally, as previously reported in detail (Liu and Rouiller 1999). The number of ICMS sites injected and the total volume of ibotenic acid infused in M1 are indicated for each monkey in Table 1. Several minutes after the ibotenic acid infusion, the contralateral hand exhibited a dramatic paralysis (see Liu and Rouiller 1999; Kaeser et al. 2010, 2011).

Reversible inactivation of M1 (transient lesion)

In one additional monkey (Mk-LA), a pharmacological reversible inactivation of M1 was induced by infusion of muscimol, as previously reported (Kermadi et al. 1997; Schmidlin et al. 2004). Mk-LA was initially included in the present study of permanent lesion, but it turned out that the injection of ibotenic acid in the left M1 failed and, as assessed histologically, did not produce an identifiable permanent lesion in M1 as in the other monkeys. In SMI-32 material from Mk-LA, there was only a very small territory (about 3 mm^3) in which SMI-32 positive neurons in layer V appeared somewhat less densely packed (contrasting with the SMI-32 positive neurons which completely disappeared in the lesion territory in the other 11 monkeys; see Kaeser et al. 2010). In accordance with such a small anatomical disruption, the contralesional hand showed only a small deficit in manual dexterity for a couple of days, and the animal's behavioral performance returned to pre-lesion levels after just 5 days. Thus, for several months following the ineffective ibotenic acid injection, the manual dexterity of both hands was stable and corresponded to the pre-lesion performance. Mk-LA had been implanted with chronic stainless steel chambers on both hemispheres. The intact

right M1 hand area was thus used for two reversible inactivation sessions, in which the GABA agonist muscimol was infused at ICMS sites within the M1 hand representation area. The Modified Brinkman Board task was performed by Mk-LA before and after the muscimol infusion, offering the possibility to assess the immediate (15 min after infusion) effect on the ipsilesional (right) hand.

Data analysis

Within the time window of pre- and post-lesion behavioral analysis for each monkey, the daily retrieval score and contact time were plotted as a function of time (days). The pre-lesion period was used to establish the manual performance of reference, the average retrieval score and its standard deviation (SD). On the plots, the average pre-lesion retrieval score was represented by a thick horizontal line, together with a dashed line positioned at mean retrieval score minus 2SDs. A decrease in the post-lesion retrieval score below the 2SDs was considered as a statistically significant deficit (see Fig. 2). For contact time, a deficit would be signaled by an increase. On the plots, a deficit was considered as statistically significant when contact time was longer than the average pre-lesion contact time plus 2SDs (see Fig. 3). Similarly, for the Brinkman box, a deficit was present when the total time post-lesion exceeded the pre-lesion average total time + 2SDs (see Fig. 4).

At the end of the experiments, the animals were killed 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 during 3–5 days. Sections (50 μm thick) of the brain were cut in the frontal plane and collected in five series. One series of sections was Nissl stained with cresyl violet, whereas a second series of sections was processed to visualize the marker SMI-32, as previously described (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 M1. Finally, the lesion was positioned on a lateral view of the lesioned hemisphere (Fig. 1a). Using an ad hoc function of the Neurolucida software (based on the Cavalieri method), the volume of the cortical lesion (in mm^3) was extrapolated from the reconstructions of the lesion on consecutive histological sections of the brain (see Table 1; Kaeser et al. 2010).

Results

Unilateral lesion of the motor cortex

Unilateral lesion of the motor cortex was produced by infusion of ibotenic acid at multiple sites defined by ICMS (see Kaeser et al. 2010). The goal was to permanently inactivate the hand area in M1. The extent and location of the lesion is shown in Fig. 1a on a lateral view of the lesioned hemisphere for the 11 monkeys included in the present study. The lesion extent was variable from one monkey to the next, but on surface views of the brain generally corresponded to a zone of 4–5 mm, thus matching the size of the hand area. In some monkeys, in one dimension or the other, the lesion extended further, for the largest lesions reaching an extent of up to 10 mm. In one monkey (Mk-SL), there was a large zone of subcortical lesion (in the white matter) spreading more medially than the gray matter lesion (Fig. 1a). In some of the other monkeys, subcortical damage was also observed (see Table 1), but it was located below the gray matter injury (and therefore it did not appear on the brain surface views in Fig. 1a).

Effect of permanent unilateral motor cortex (M1) lesion on manual dexterity

Modified Brinkman Board test: retrieval score

The possible effects of unilateral motor cortex (M1) lesion on the ipsilesional hand was assessed during a short-term time window ranging from 0 to 60 days post-lesion, by comparing the pre-lesion and post-lesion manual performance of each animal in the Modified Brinkman Board task. A first parameter analyzed here is the retrieval score, defined as the total number of pellets retrieved in 30 s (considering both the vertical and horizontal slots). As illustrated in Fig. 2 for four representative monkeys, the pre-lesion manual performance was generally stable as indicated by the distribution of data points around the average "score" (thick horizontal line). The variability pre-lesion across daily sessions is represented by the two dashed horizontal lines placed at average retrieval score + 2 standard deviations (SDs) and at average retrieval score − 2SDs, respectively.

A first subgroup of 4 monkeys (Mk-JA, Mk-VA, Mk-BI and Mk-MO) out of 11 showed a small and transient deficit of manual performance of the ipsilesional hand immediately after the lesion of M1 (Table 2). In two monkeys (Mk-JA and Mk-VA), shortly after the lesion (day 0; vertical dashed line), there were a few sessions in which the retrieval score dropped below the dashed line corresponding to the average pre-lesion retrieval score

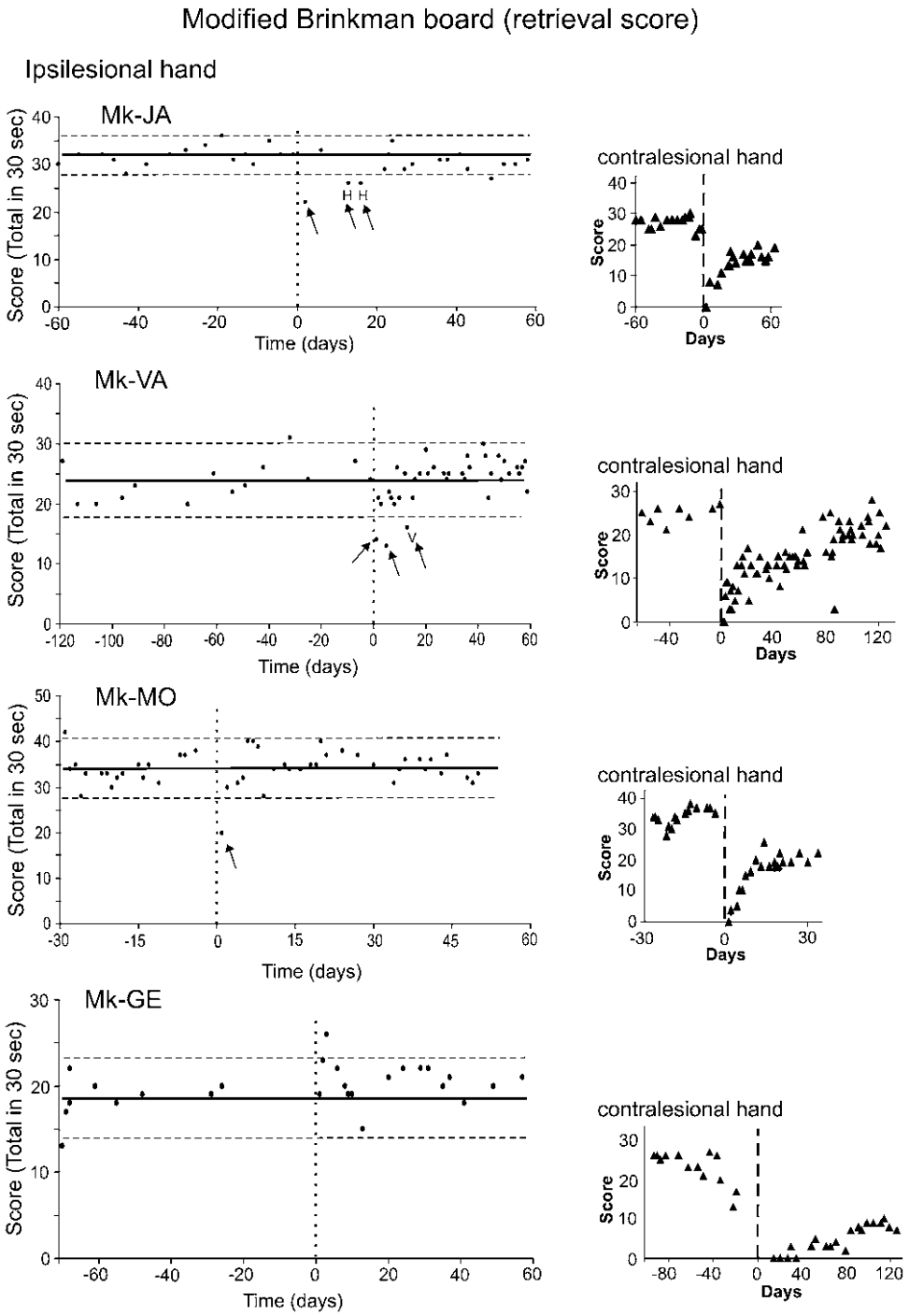

Fig. 2 Behavioral data (manual dexterity) obtained from four representative monkeys (Mk-JA, Mk-VA, Mk-MO and Mk-GE) for the Modified Brinkman Board task. The manual dexterity of the ipsilesional hand is given by the retrieval score, corresponding to the total number of pellets retrieved in 30 s, as a function of time (days). The day of the M1 lesion is at time zero (*vertical dashed line*). The retrieval scores for the vertical and horizontal slots were cumulated. The *thick horizontal line* is the average retrieval score, computed from the pre-lesion daily sessions only. The *horizontal dashed lines* are for the average retrieval score plus 2SDs and minus 2SDs. For comparison, the retrieval scores (*triangles*) are given on the *right* for the contralesional hand for the same monkeys. The period at plateau pre-lesion were of variable duration, depending on the date set for the lesion across monkeys. Similarly, on the *right*, the post-lesion period shown on the graphs was set depending on the variable time course of recovery across monkeys

minus 2SDs, considered as the confidence limit for a statistically significant loss of performance for the ipsilesional hand (Fig. 2). Although significant, the deficit remained modest and transitory, as compared to the more devastating and prolonged effect observed in the same monkeys for the contralesional hand (small graphs on the right with retrieval score indicated by triangles in Fig. 2). In these two monkeys, the letters "H" and "V" represent sessions in which the deficit was significant only due to the horizontal slots (H) or the vertical slots (V), respectively. In the other sessions where retrieval score was lower than the average minus 2SDs, the deficit was significant for both slot orientations. A deficit comparable to those of Mk-JA and Mk-VA was observed for the ipsilesional hand of Mk-BI (not shown). In the fourth monkey of this subgroup (Mk-MO; Fig. 2), the deficit was even more transient, as it was limited to a single daily session shortly after the lesion.

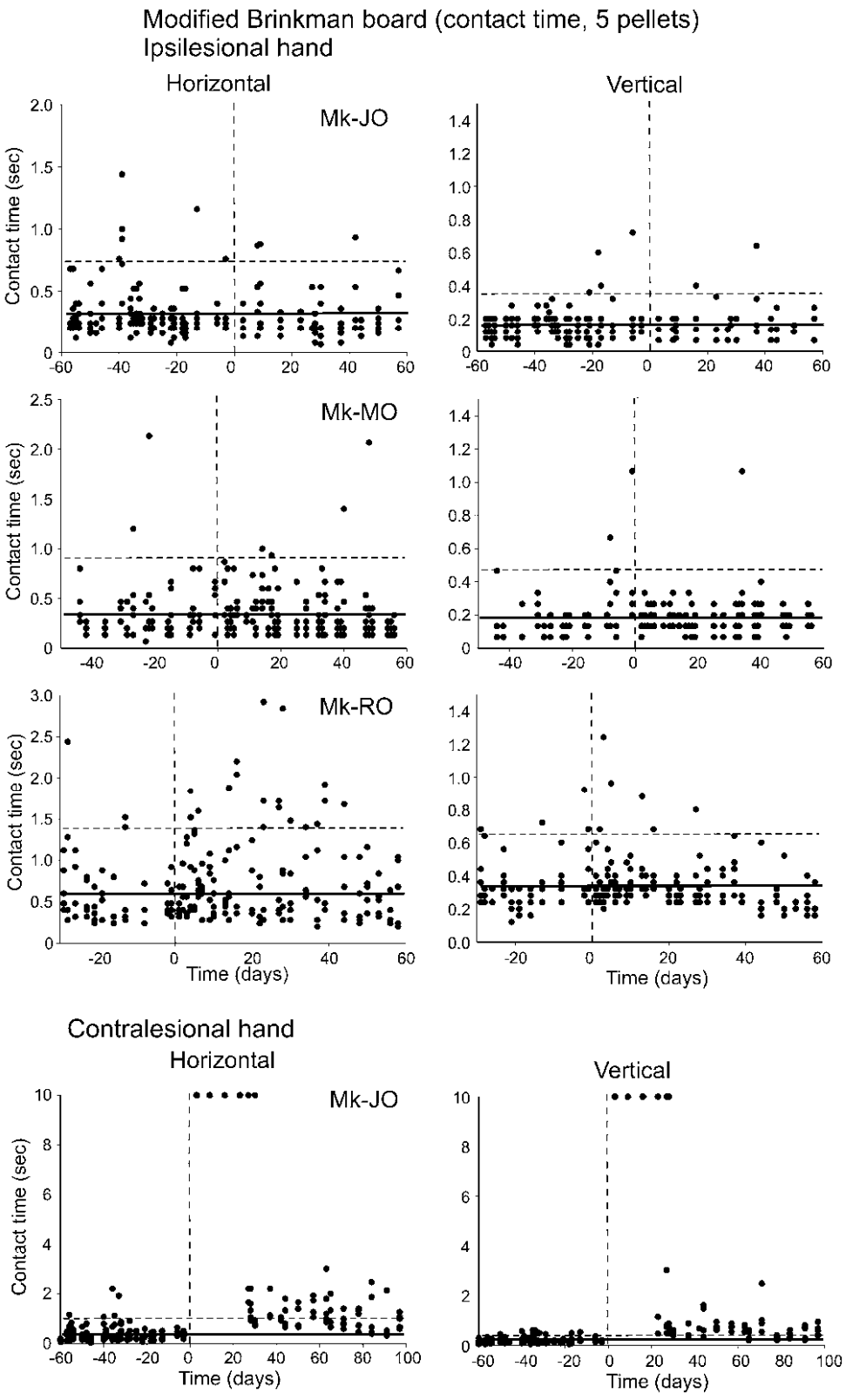

Fig. 3 Behavioral data (manual dexterity) obtained from three representative monkeys (Mk-JO, Mk-MO and Mk-RO) for the Modified Brinkman Board task. The manual dexterity of the ipsilesional hand is given by the contact time, corresponding to the time of contact (in seconds) between the fingers and the first five pellets in vertical slots (*left column*) and the first five pellet in horizontal slots (*right column*) targeted by the monkey. Along the abscissa, the day of the M1 lesion is at time zero (*vertical dashed line*). The *thick horizontal line* is the average contact time, computed from the pre-lesion daily sessions only. The *horizontal dashed line* is for the average contact time plus 2SDs. For comparison, the contact time is given in the *bottom panel* for the contralesional hand in Mk-JO (note the different scale in the ordinate). The contact time values saturated at 10 s indicate that monkey Mk-JO was totally unable to perform the grasping of the pellets on the corresponding daily sessions following the lesion, reflecting the dramatic deficit observed for the contralesional hand

In the other subgroup comprising 7 lesioned monkeys out of 11, as illustrated for Mk-GE (bottom graph in Fig. 2), no significant drop in manual performance was observed for the ipsilesional hand (Table 2), in spite of a massive and long-term deficit in the contralesional hand. Based on the retrieval score data for the Modified

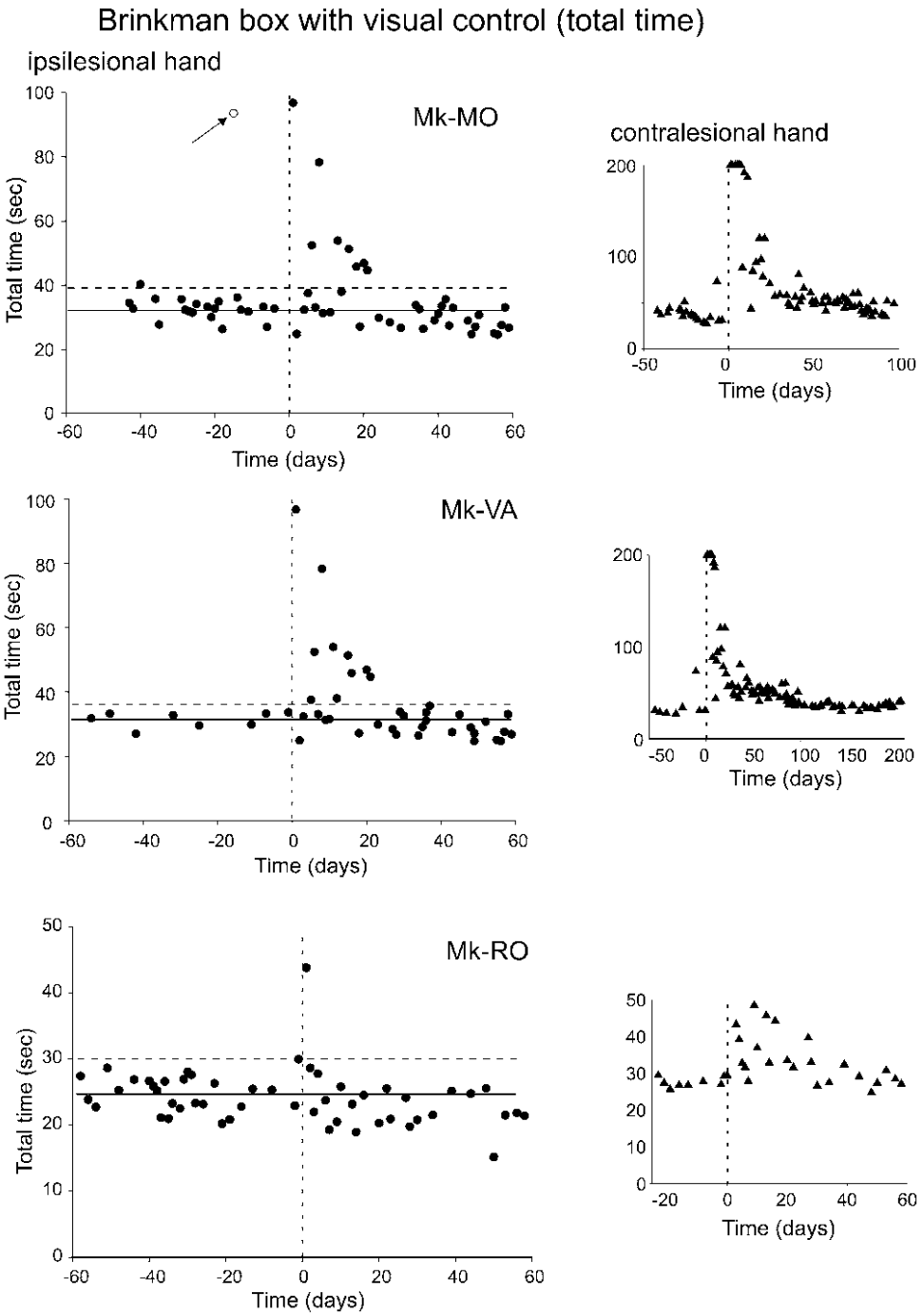

Fig. 4 Behavioral data (manual dexterity) obtained from three representative monkeys (Mk-MO, Mk-VA and Mk-RO) for the Brinkman box task, performed with visual feedback. The manual dexterity of the ipsilesional hand is given by the total time needed by the monkey to empty the 20 wells. Along the abscissa, the day of the M1 lesion is at time zero (*vertical dashed line*). The *thick horizontal line* is the average total time, computed from the pre-lesion daily sessions only. The *horizontal dashed line* is for the average total time plus 2SDs. For comparison, the total time is given in the small graphs on the *right* for the contralesional hand in each monkey (note the different scale in the ordinate). On the *right*, the post-lesion period shown on the graphs was set depending on the variable time course of recovery across monkeys. In the *top graph* (Mk-MO), the *arrow* points to a daily session in which the monkey took a lot of time to empty the box (probably due to poor motivation on that day), thus representing an outlier data point

Brinkman Board task, only four monkeys thus exhibited a modest and transient deficit for the ipsilesional hand shortly after the lesion, whereas the majority of monkeys ($n = 7$) did not show any significant impairment.

Modified Brinkman Board test: contact time

To focus more specifically on the grasping phase of the pellet, reflecting specifically manual dexterity (and thus omitting other components of the task, such as reaching, transporting to the mouth, etc.; see Freund et al. 2009), the parameter contact time measures the time in seconds of contact between the fingers and the pellet within the slot. The shorter the contact time, the more dexterous the monkey during the corresponding trial. The contact time was determined for the first five vertical pellets and the first five horizontal pellets targeted by the monkey on each daily session. Contact time data were thus represented separately

Table 2 Summary of the effects of M1 lesion on the ipsilesional hand manual performance

	Mk-CE	Mk-JU	Mk-GE	Mk-RO	Mk-AV	Mk-BI	Mk-VA	Mk-SL	Mk-MO	Mk-JA	Mk-JO	Mk-LA[a]
Retrieval score	N	N	N	N	N	Y	Y	N	Y	Y	N	Y
Contact time	N	N	Y	Y	N	N	N	Y	N	N	N	Y
Total time	–	–	N	N	N	N	Y	Y	Y	N	N	–
No. of inj. close to proximal sites[b]	5	9	3	6	–	9	2	3	10	12	–	–
Average distance to proximal sites (mm)[c]	1.5	2.6	1.3	1.4	–	1.1	1.3	1.4	1.8	0.5	–	–

Effect of unilateral lesion of M1 hand representation on the manual performance with the ipsilesional hand as assessed with three parameters: retrieval score in the Modified Brinkman Board task, the contact time in the Modified Brinkman Board task and the total time in the Brinkman box task. Y ("YES") means that the lesion had an effect, whereas N ("NO") is for an absence of effect, as observed for each monkey. Note that the parameter total time was not measured in the two monkeys, Mk-CE and Mk-JU, as the Brinkman box task was not introduced yet when these monkeys were under study. The Brinkman box task was not tested in the reversible inactivation sessions with muscimol in Mk-LA. In all monkeys, except Mk-LA, the lesion was permanent (infusion of ibotenic acid)

[a] In Mk-LA, a transient lesion was produced by infusion of muscimol in two separate sessions (see Fig. 5)

[b] The row gives for each monkey the number of sites where ibotenic acid was infused that were close (≤2 mm) to one or several ICMS sites eliciting proximal muscles activation (shoulder or elbow), as illustrated in Fig. 6 for the monkeys Mk-BI and Mk-SL. These data are plotted in Fig. 7a. As the ICMS maps were incomplete in Mk-AV and Mk-JO, this parameter could not be determined

[c] For each site where ibotenic acid was infused and located close (≤2 mm) to a proximal ICMS site (elbow or shoulder), the actual distance between the infusion site and the close proximal ICMS sites were measured and averaged (expressed in mm). These data are plotted in Fig. 7b. As the ICMS maps were incomplete in Mk-AV and Mk-JO, this parameter could not be determined

for the horizontal and vertical slots (Fig. 3, left and right columns, respectively). During the pre-lesion period, it happened that a few contact times were above the significant level given by the average contact time + 2SDs (Fig. 3). In two representative monkeys (Mk-JO and Mk-MO), the post-lesion contact times showed a comparable distribution as pre-lesion, indicative of an absence of effect of the lesion on the grasping ability for the ipsilesional hand. Out of 11 monkeys, 8 monkeys exhibited an absence of effect on the contact time parameter (Mk-JA, Mk-JO, Mk-MO, Mk-BI, Mk-JU, Mk-AV, Mk-CE and Mk-VA; see Table 2). In contrast, there was a transient increase of contact time post-lesion for the ipsilesional hand in three monkeys (Mk-SL, Mk-GE and Mk-RO; see Table 2), as illustrated in Fig. 3 for Mk-RO, exhibiting several contact times above the pre-lesion average contact time + 2SDs. In these three monkeys, the effect of the lesion was present for both slot orientations, though it was somewhat more pronounced in the horizontal slots for Mk-RO (Fig. 3) and Mk-SL, whereas for Mk-GE the deficit was more pronounced for the vertical slots. For comparison, the effect of the M1 lesion on the contralesional hand is shown for Mk-JO in the bottom panel of Fig. 3, with a persistent increase in contact time, lasting more than 100 days post-lesion, reflecting a massive loss of manual dexterity.

Brinkman box test: total time

As explained in "Methods", the manual performance was also tested while the monkeys grasped pellets within a restricted space, corresponding to the Brinkman box (Fig. 1b, right panel). The total time to empty the 20 slots was measured and illustrated for three typical monkeys in Fig. 4. The pre-lesion performance is represented by the average value (horizontal solid line) and a limit for a statistically significant deviation given by the average pre-lesion total time + 2SDs (horizontal dashed line). The monkeys Mk-MO and Mk-VA both exhibited a significant increase in total time for the ipsilesional hand for just over 20 days post-lesion. This increase in total time is, however, clearly less prominent and more transient than the one observed for the contralesional hand (plots on the right in Fig. 4). A deficit for the ipsilesional hand was also present in Mk-SL (not shown). In contrast, Mk-RO (Fig. 4, bottom) does not show an increase in total time for the ipsilesional hand in the Brinkman box, except the very first post-lesion daily session. The contralesional hand of Mk-RO showed a clear and long lasting increase of total time. As for Mk-RO, no deficit was observed in the Brinkman box task for five other monkeys (Table 2).

Effect of transient (reversible) unilateral motor cortex lesion (M1) on manual dexterity

The above data were derived from the assessment of manual dexterity in the ipsilesional hand after permanent unilateral lesion of M1 induced by infusion of ibotenic acid. Although it was possible, with our experimental protocol, to still observe the impact on manual performance already few days after lesion, one cannot exclude a kind of rapid re-arrangement of the system of control of ipsilateral manual dexterity, thus masking a possible role

actually played by the motor cortex on the ipsilateral hand in the normal state. To circumvent this limitation, one may use the approach of pharmacological reversible inactivation, by infusing muscimol unilaterally in the hand representation of M1, as performed in Mk-LA in two separate sessions. On the same daily session, the manual performance was first assessed in the Modified Brinkman Board task immediately before infusion of muscimol (see Table 1). Then, a few minutes after infusion of muscimol, the manual skill task was repeated. As expected, confirming the efficacy of the inactivation, the retrieval score for the hand opposite to the inactivated M1 dropped to zero, indicative of a complete loss of the ability to grasp pellets (not shown; see however Kermadi et al. 1997). In contrast, the hand ipsilateral to the transiently inactivated M1 was still able to grasp pellets, although the retrieval score dropped to some extent as compared to the pre-infusion score. Across two distinct reversible inactivation sessions (Fig. 5a for Mk-LA), the retrieval score for the hand ipsilateral to the reversibly inactivated M1 decreased within a range of 30–73% for the vertical slots and of 25–33% for the horizontal slots (Fig. 5a; Table 2). Consistent with this drop in retrieval score, the contact time increased after the infusion of muscimol as compared to prior to the infusion (Fig. 5b; Table 2). The increase in contact time was more prominent for the grasping of the pellets from the horizontal slots than for the vertical slots (Fig. 5b), reaching an increase of about 100% for the horizontal slots (i.e. the time needed for successful grasping doubled).

Are the effects on manual dexterity with the ipsilesional hand (partly) mediated indirectly via proximal muscles?

The transient deficits observed above for the manual dexterity with the ipsilesional hand after unilateral motor cortex lesion may be due to a dysfunction of cortical territories giving rise to the uncrossed corticospinal projection, involved mainly in the control of proximal muscles (e.g. Lemon 2008). Indeed, if some of the injections of ibotenic acid, aimed at the hand representation, spread to more proximal territories (elbow, shoulder), a dysfunction in the reaching phase as well as in the control of forelimb posture may indirectly impact on the manual dexterity. To address this issue, on the ICMS maps, the sites of ibotenic acid infusion close (less than 2 mm) to proximal muscles territories were identified (purple x symbols in Fig. 6) and the corresponding real distances (to proximal ICMS sites) in the original 3D coordinates system were calculated for each of these sites of injection (Fig. 7; Table 2).

The number of sites of infusion of ibotenic acid close to proximal territories was plotted in Fig. 7a for the individual monkeys as a function of the effects observed on the behavioral tasks described above (no effect = 0; deficit observed for one manual dexterity task = 1; deficit observed for two manual dexterity tasks = 2). There is no correlation between the number of sites infused close to proximal territories (elbow, shoulder) and the number of tasks for which an ipsilesional deficit of manual dexterity was observed. When plotting the average distance between the infused sites and the closely located proximal territories

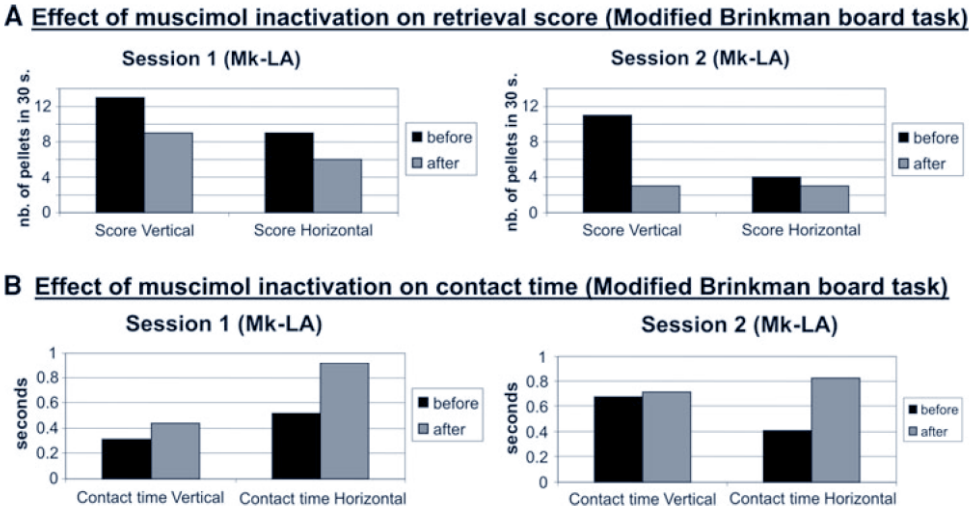

Fig. 5 Immediate effect of reversible inactivation of the M1 hand representation unilaterally in Mk-LA, obtained by infusion of muscimol, on the dexterity of the ipsilateral hand. Two distinct sessions are illustrated (sessions 1 and 2). **a** The plots show the effect of muscimol infusion on the retrieval score in the Modified Brinkman Board task. **b** The plots show the effect of muscimol infusion on the contact time in the Modified Brinkman Board task. **a**, **b** The *black bars* are the pre-infusion data (before inactivation), whereas the *gray bars* are for the post-infusion data (after inactivation). Retrieval scores and contact times are given separately for the vertical and horizontal slots

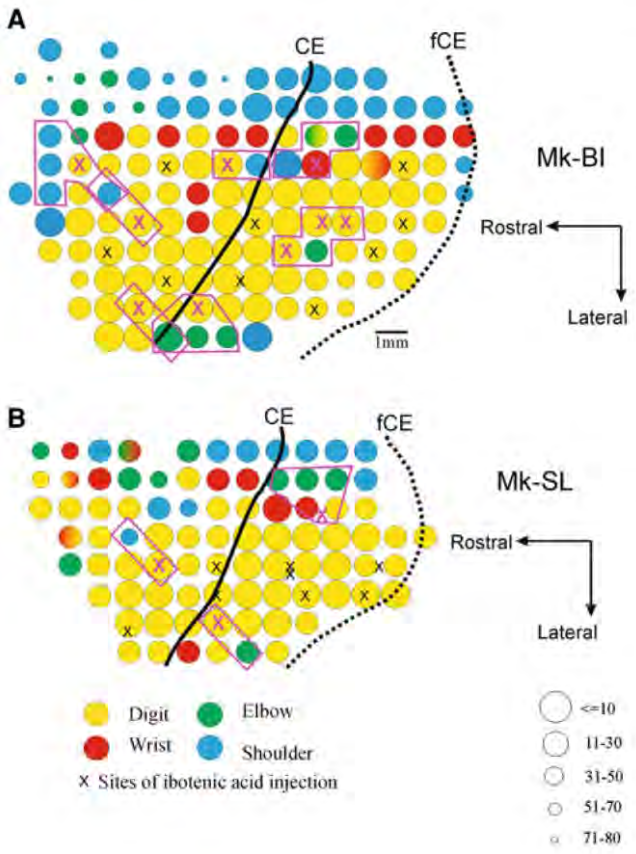

Fig. 6 Representative unfolded ICMS maps (in the left hemisphere) of the forelimb before lesion, derived from two animals, Mk-BI (**a**) and Mk-SL (**b**), with positions of ICMS sites selected for infusion of ibotenic acid (*x symbols*) to produce the lesion of the hand area. The method to unfold the rostral bank of the central sulcus has been described previously (Kaeser et al. 2010). As several electrode tracks running within the same rostrocaudal plane along the rostral bank of the central sulcus are projected on the same line segment, some ICMS sites and/or sites of ibotenic acid infusion may be superimposed. On these surface maps, a few ICMS sites eliciting contralateral finger movements (*yellow circles*), where ibotenic acid was infused, appear to be located close (less than 2 mm) to ICMS sites corresponding to representation of proximal muscles (elbow and shoulder, *green* and *blue circles*, respectively). Such sites of infusion of ibotenic acid are depicted by the *purple x symbols*. The real distance between such sites of infusion of ibotenic acid and the proximal ICMS sites (identified on the maps by *purple polygons*) was calculated from the original 3D coordinates system. These distance data are presented in Fig. 7 and Table 2. *CE* central sulcus, *fCE* fundus of the central sulcus. As coded on the *bottom right corner*, the size of the circles represents the ICMS threshold at which the just noticeable movement was observed. The body territory (digit, wrist, elbow or shoulder) activated by the ICMS is given by the color code (*bottom left*). For clarity, at the periphery of the forelimb representation, sites eliciting movements of other territories (e.g. face) or unresponsive were not represented

with respect to the number of manual dexterity tasks for which a deficit was observed (Fig. 7b), there is no systematic relationship either. The data in Fig. 7 thus suggest that the deficits of manual dexterity with the ipsilesional hand do not result from a systematic spread of ibotenic acid to proximal territories in the monkeys exhibiting more deficit than the other animals.

Discussion

Interpretation of the behavioral data

To the best of our knowledge, the originality of the present study lies in a systematic and quantitative assessment of the manual performance deficit for the ipsilesional hand resulting from unilateral lesion of M1 in non-human primates targeting the hand representation. Indeed, most previous studies on motor cortex lesion in adult monkeys were focused on the contralesional hand (e.g. Passingham et al. 1983; Friel and Nudo 1998; Liu and Rouiller 1999; Frost et al. 2003; Plautz et al. 2003; Pizzimenti et al. 2007; Eisner-Janowicz et al. 2008; Murata et al. 2008; Darling et al. 2009).

Based on 3 parameters characterizing manual performance in 11 macaque monkeys (Table 2), the present study provides evidence that a unilateral permanent lesion of the hand representation in M1 does not induce a systematic and long lasting deficit of motor control for the ipsilesional hand, in contrast to the dramatic deficit observed for the contralesional hand. This conclusion (Figs. 2, 3, 4; Table 2) is in line with the general notion that the corticospinal (CS) projection responsible for fine manual control is largely crossed (about 90% of CS axons decussate) and terminates on distal motoneurons, whereas the uncrossed CS projection exerts its control mostly on proximal and axial muscles (e.g. Brinkman and Kuypers 1973). For each of the three parameters we assessed, there was, however, a minority of monkeys exhibiting a modest (but statistically significant) deficit of manual performance for the ipsilesional hand, limited to a few days immediately following the lesion. How serious are these deficits? For the retrieval score data, significant deficits were found in 4 monkeys (out of 11), limited however to 1–3 daily sessions immediately after the lesion. The extent of the deficit (i.e. the decrease in retrieval score) ranged from 30 to 50%, with the exception of 80% in one monkey on one single daily session. Considering the contact time data, the deficits were also moderate (though significant) but, again, in few monkeys (3 out of 11). Out of the five contact times recorded for each slot orientation in each daily session, most often only a single measurement exceeded the upper confidence limit derived from the pre-lesion period (Fig. 3). In the three monkeys showing a modest deficit, in very rare daily sessions two recorded contact times within the same daily session exceeded the confidence limit (Fig. 3: Mk-RO). However, such cases were limited to one to two daily sessions. The contact time data are thus

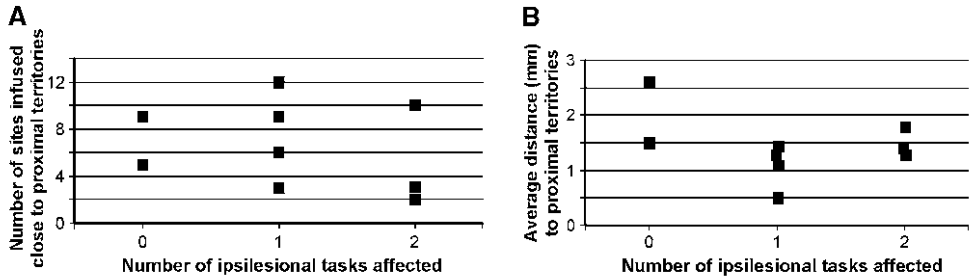

Fig. 7 To assess whether transient deficits for manual dexterity performed with the ipsilesional hand may be due to a spread of ibotenic acid to proximal territories in M1, the number of infusion sites closely located to elbow or shoulder ICMS sites were plotted for individual monkeys as a function of the number of manual dexterity tasks for which a deficit was observed (**a** none, one or two; see Table 2). Similarly, in **b**, the average distance from ibotenic acid infusion sites to the closely located proximal ICMS sites was plotted for individual monkeys as a function of the number of manual dexterity tasks for which a deficit was observed. Mk-AV and Mk-JO were not considered as their ICMS map was incomplete (see Table 2)

indicative of a very modest effect of the motor cortex lesion on the ipsilesional hand's manual dexterity per se (specific precision grip ability). As the majority of contact times remained normal in each daily session, it can be concluded that there is no crucial ipsilateral control of the motor cortex on manual dexterity. The lesion of the motor cortex thus appeared to affect slightly more the retrieval score than the contact time. In other words, the effect of the lesion on the ipsilesional hand was more on components of the task distinct from the manual dexterity itself.

Following this deduction, the effect of the lesion on the ipsilesional hand as assessed with the total time to empty the Brinkman box was more prominent, when present, as observed in three monkeys (Mk-MO, Mk-VA and Mk-SL; see Fig. 4). Indeed, in these three monkeys, the effect lasted at least 20 days and was present in about 50% of daily sessions during this post-lesion time window. This observation is consistent with the notion that the Brinkman box task requires a more precise control of the forelimb posture, as the space to access the slots is more restricted than in the Modified Brinkman Board task. As a consequence, more proximal muscles (in part under the control of the uncrossed CS projection) contribute to performance in the Brinkman box task, which are likely to be more affected by an ipsilateral lesion of the motor cortex than distal muscles specialized for manual dexterity per se. Nevertheless, it remains that the majority of monkeys (8 out of 11) did not exhibit a deficit in the Brinkman box task (Table 2), again supporting the notion that the effect of the lesion on the ipsilesional hand is modest, at least for the behavioral tasks considered in the present study. This conclusion is supported by the observation that the deficits seen for the three parameters (Table 2) are not present systematically in the same three to four monkeys. No monkey showed a deficit of the ipsilesional hand for all three parameters, whereas only three monkeys (Mk-SL, Mk-MO and Mk-VA) showed a deficit for two of the three parameters. Four monkeys exhibited a deficit for only one parameter (Mk-GE, Mk-RO, Mk-BI and Mk-JA). Finally, four monkeys showed no deficits at all, although two of them were not tested for the Brinkman box task (Table 2). Surprisingly, the three monkeys exhibiting deficits of the ipsilesional hand for two of the three parameters were those subjected to anti-Nogo-A antibody treatment. It cannot be ruled out that the presence of the osmotic pumps delivering the antibody during 4 weeks may have contributed, at least in part, and indirectly, to these slight deficits. Considering the monkeys showing deficits of manual performance with the ipsilesional hand for either one or two parameters, there is no obvious correlation with the size and/or the precise position of the lesion (Fig. 1). Moreover, deficits in the ipsilesional hand's manual performance during the days following the lesion of M1 did not correlate with the presence or absence of enhancement of manual performance of the ipsilesional hand on the long-term, found in the monkeys which recovered best their manual performance for the contralesional hand (Kaeser et al. 2010). Finally, there was no systematic relationship between the deficits observed for the ipsilesional hand in some monkeys for one or two motor parameters (Table 2) and the presence/absence of subcortical lesion in the white matter (Table 1). At the other extreme (no deficit), one animal (Mk-AV) was characterized by a very rostral lesion, located mainly in PM, in line with moderate deficits for the contralesional hand and no deficit for the ipsilesional hand.

The deficits observed in the Brinkman box task (increase in the total time that lasted 20 days) for three monkeys after permanent lesion of the motor cortex (Table 2), and while retrieving the pellets from the horizontal slots for Mk-LA that received a reversible lesion, suggest that the impact of the lesion may be indirect via an effect on the posture of the hand. Indeed, the Brinkman box task requires precision control of the hand posture (wrist muscles) within a restricted space in order to perform the

grasping. In Mk-LA, the predominant effect on the horizontal slots is consistent with the notion that grasping is more difficult from the horizontal slots, requiring an additional postural adjustment of the hand, as compared to the vertical slots (see Freund et al. 2009).

Comparison with previous non-human primate studies

Few studies on motor cortex lesions have investigated the effects on the ipsilesional hand. In a recent study, marmosets trained to catch a food reward in the "Hill-and-Valley staircase" test exhibited a deficit lasting about 1 week post-lesion (Bihel et al. 2010), comparable to the transient deficits observed here in a few monkeys. However, the cortical lesions in the marmosets were clearly larger than those in the present study (2–5 times), especially considering the smaller size of the marmoset's brain. As a consequence, the deficit observed in the marmoset for the ipsilesional hand cannot be interpreted as a specific reduction of manual performance, as the lesion spread to more proximal territories in M1, as well as other cortical and subcortical areas. In a previous study, using a similar behavioral test, it was reported that the ipsilesional hand exhibited some deficit, but it was suggested that this deficit was due more to perceptual neglect of the contralesional hemispace than a true motor deficit (Marshall et al. 2003).

A fine motor test (grasping of food pieces) was performed in macaques subjected to a unilateral middle cerebral artery occlusion (Roitberg et al. 2003). As expected, after lesion affecting the motor cortex, the contralesional hand was completely unable to perform the grasping. In contrast, the task was performed successfully with the ipsilesional hand, though more slowly as compared to pre-lesion. The difference, however, was not statistically significant. This result is consistent with a decrease in the retrieval score observed for the ipsilesional hand in the present study in 4 out of 11 monkeys. Once more, the lesion performed by Roitberg et al. (2003) was less constrained to the hand area than that produced in the present study with the infusion of ibotenic acid at sites identified with ICMS.

Possible confounding factors

The deficit observed for the ipsilateral hand already a few minutes after unilateral infusion of muscimol in M1 in Mk-LA (Fig. 5), and comparable to the deficits observed in some of the monkeys subjected to permanent lesion (Table 1), allows to rule out that the latter deficits are due to a general degradation of the health condition of the animal following the infusion of ibotenic acid in M1. In fact, except the expected flaccid paralysis restricted to the contralesional hand, the monkeys did not exhibit other pathological signs, in line with a permanent lesion restricted to the hand representation of M1.

Out of 11 monkeys (Table 1), 5 animals were subjected to a treatment, anti-Nogo-A antibody ($n = 3$) or cell therapy ($n = 2$). What may be the influence of these treatments on the manual performance of the ipsilesional hand within the first few weeks post-lesion (up to 60 days)? As mentioned above, in the case of the anti-Nogo-A antibody-treated monkeys, an indirect influence exerted by the osmotic pumps cannot be ruled out. However, a direct negative effect of the anti-Nogo-A antibody treatment is unlikely as one would have expected instead a neuroprotective effect (instead we observed a deficit in the ipsilesional hand in the three treated monkeys). Furthermore, the enhancement of functional recovery for the contralesional hand possibly promoted by the anti-Nogo-A antibody treatment occurs later than 60 days (unpublished data). The delay for enhancement resulting from cell therapy is also longer than 60 days (Kaeser et al. 2011).

Implication of the present study

The variability of the effects of the motor cortex lesion on the ipsilesional hand's manual performance across monkeys (Table 2) may be consistent with the presence of a small territory in M1 from which motor commands are issued toward distal muscles (see Aizawa et al. 1990). Due to its small size and possibly its variable position across monkeys, it is plausible that the lesions performed in the present study involved this "ipsilateral" territory in some monkeys and not in others. The presence of few ICMS sites in M1 activating ipsilateral distal muscles was not found in the present study because, in contrast to the study of Aizawa et al. (1990), only overt movements were investigated here, an approach less sensitive than recording EMG activity. Furthermore, at most ICMS sites, we did not systematically apply high current intensity, to prevent cortical damage, and therefore the ICMS may have been sub-threshold to elicit ipsilateral distal movements. Nevertheless, the present data also suggest an indirect effect via a transient perturbation of the postural hand control, involving primarily more proximal (wrist) muscles.

The general conclusion of the present study that a unilateral lesion of the motor cortex has only modest effects on the performance of the ipsilesional hand thus suggests that the neuronal activities observed in M1 in monkeys performing ipsilateral hand movements (Matsunami and Hamada 1981; Aizawa et al. 1990; Chen et al. 1991; Donchin et al. 1998, 2002; Kermadi et al. 1998, 2000; Kazennikov et al. 1999; Cisek et al. 2003) are most likely related to activation of more proximal muscles (for postural adjustments) and/or activities aimed at preventing simultaneous movements of the opposite hand. From the clinical

point of view, if a patient exhibits a clear deficit of the ipsilesional hand after cortical lesion, it is likely to be associated to a lesion that is not limited to M1 but rather includes adjacent cortical territories (e.g. premotor cortical areas) more engaged in the control of both hands than M1.

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Conflict of interest The authors have no conflict of interest in relation to the present study.

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3.2 Chapitre 2:

Long-term motor cortical map changes following unilateral lesion of the hand representation in the motor cortex in macaque monkeys: does it predict the degree of functional recovery of the contralesional hand?◊

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◊At the time of the submission of the present thesis, this chapter corresponds to an article in preparation, not yet submitted for publication. It is thus a preliminary version.

Abstract

The goal of the present study was to investigate, in a population of eight adult macaque monkeys subjected to a restricted chemical lesion of the primary motor cortex (M1) targeted to the hand representation, how the motor maps are modified within as well as at the vicinity of the damaged cortical zone. The motor maps are established based on the technique of intracortical microstimulation (ICMS), performed pre-lesion and repeated post-lesion, after the incomplete functional recovery has reached a plateau. It is hypothesized that, as a result of ibotenic acid infusion in the hand area in M1, the lesioned territory remains non responsive to ICMS post-lesion in spite of an incomplete functional recovery. A further hypothesis is that the extent of functional recovery is related to the degree of motor map re-arrangement in the cortical territories immediately adjacent to the lesion.

The pool of eight monkeys subjected to a unilateral lesion of M1 was subdivided into a subgroup of five (control) animals without treatment to improve the functional recovery, whereas a subgroup of three monkeys received an anti-Nogo-A antibody treatment. A preliminary assessment of the possible benefit of the anti-Nogo-A antibody treatment on functional recovery was made here from the eight monkeys, in parallel to the analysis of the electrophysiological data.

Following permanent chemical lesion of the hand area in M1 in the adult macaque monkey, the lesion territory became largely non micro-excitable several months post-lesion, although some functional recovery took place (spontaneous or promoted by a treatment). A few sites within the lesioned territory remained excitable, but without relationship to the degree of functional recovery. Around the lesion in M1, there was no evidence for a reallocation of proximal territories into distal territories to replace, at least in part, the original hand representation affected by the lesion. As compared to pre-lesion, there was post-lesion an increase of thresholds at which ICMS elicited movements of the forelimb muscle territories, but again without relationship to the degree of functional recovery. In addition, based on behavioral data, the present study provides preliminary evidence for an enhancement of functional recovery promoted by the anti-Nogo-A antibody treatment, as reflected by the retrieval score for the horizontal slots in the modified Brinkman board task, extending to the non-human primates previous data in rodents (Papadopoulos et al., 2002; Emerick et al., 2003; Emerick and Kartje, 2004; Seymour et al., 2005; Tsai et al., 2007, 2011; Cheatwood et al., 2008).

Introduction

A lesion of the motor cortex in the adult results in a hemi-paresis of the corresponding body territory, which is long-lasting, although some spontaneous and incomplete functional recovery may occur during the weeks or months following the cortical damage. The knowledge of the mechanisms underlying this limited spontaneous recovery is crucial in order to develop strategies to enhance functional restitution from cortical damage, using rehabilitative training protocols and/or various treatments (pharmacological, cell therapy, etc). Before safe and efficient clinical application, mechanisms of functional restitution need to be elucidated in detail using animal models. In this context, the model of non-human primate is especially valuable as the general organization of the motor system controlling voluntary movements in monkeys is similar to that of humans (Courtine et al., 2007; Lemon, 2008). In the past, several studies conducted in non-human primates have investigated the consequences (deficits) of a lesion of the motor cortex on the ability to perform voluntary movements, as well as to what extent and how some spontaneous recovery may contribute to the restitution of some motor control (e.g. Ogden and Franz, 1917; Glees and Cole, 1952; Brinkman and Kuypers, 1973; Passingham et al., 1983; Friel and Nudo, 1998; Rouiller et al., 1998; Liu and Rouiller, 1999; Frost et al., 2003; Marshall et al., 2003; Plautz et al., 2003; Roitberg et al., 2003; Dancause et al., 2005,2006; Pizzimenti et al., 2007; Eisner-Janowicz et al., 2008; Murata et al., 2008; Darling et al., 2009; Bihel et al., 2010; McNeal et al., 2010; Kaeser et al., 2010,2011; Bashir et al., 2011; Darling et al., 2011). The incomplete spontaneous recovery taking place after a unilateral lesion affecting the primary motor cortex (M1) is based, at least in part, on a vicarious phenomenon in which (ipsilesional) non-primary motor cortical areas (premotor cortex, supplementary motor area, or even the somatosensory cortex) can take over part of the lost function (Sasaki and Gemba, 1984; Liu and Rouiller, 1999; Frost et al., 2003; Dancause et al., 2005, 2006; Eisner-Janowicz et al., 2008; McNeal et al., 2010). The incomplete recovery of manual dexterity in case the lesion of M1 affects the hand representation may include the emergence of compensatory movement patterns, favoured by rehabilitative training (Friel and Nudo, 1998; Murata et al., 2008). Moreover, peri-infarct electrical stimulation may enhance fuctional recovery from the lesion of M1 (Plautz et al., 2003).

One may hypothesize that the more extensive (or severe) the lesion in M1, the more likely is the involvement of a non-primary motor cortical area in the incomplete functional recovery. If the lesion is however restricted to a part of M1, there is evidence that territories in

M1 preserved by the lesion may be re-organized to take over part of the lost motor function, if post-lesion training/practice takes place (Glees and Cole, 1952; Nudo and Milliken, 1996; Nudo et al., 1996; Rouiller et al., 1998; Plautz et al., 2003). The re-organization of such peri-lesion territory in M1 consists in changes of somatotopic representation, as assessed by intracortical microstimulation (ICMS) by comparing pre-lesion and post-lesion motor maps. The goal of the present study was to investigate, in a relatively large group of eight adult macaque monkeys subjected to a restricted chemical lesion of M1 targeted to the hand representation, how the motor maps are modified within as well as at the vicinity of the damaged zone using ICMS performed pre-lesion and repeated post-lesion, after the incomplete functional recovery has reached a plateau. It is hypothesized that, as a result of ibotenic acid infusion in the hand area in M1, the lesioned territory remains non responsive to ICMS post-lesion, although an incomplete functional recovery takes place. A further hypothesis is that the extent of functional recovery is related to the degree of motor map re-arrangement in the cortical territories immediately adjacent to the lesion. Finally, the pool of eight monkeys subjected to a unilateral lesion of the motor cortex was subdivided into a subgroup of five (control) animals without treatment to improve the functional recovery and a subgroup of three monkeys that received an anti-Nogo-A antibody treatment, known to enhance functional recovery after motor cortex lesion in the rat (Papadopoulos et al., 2002; Emerick et al., 2003; Emerick and Kartje, 2004; Seymour et al., 2005; Tsai et al., 2007, 2011; Cheatwood et al., 2008). A preliminary assessment of the possible benefit of the anti-Nogo-A antibody treatment on functional recovery was made here from the eight monkeys, in parallel to the analysis of the electrophysiological data.

Methods

General survey of the experiments

The data were collected from 8 monkeys (*Macaca fascicularis*, 3.5-6.5kg, 4-6 years old, 1 female and 7 male), all trained to perform 3 different behavioural tasks aimed at assessing manual hand dexterity (Schmidlin et al., 2011): the modified Brinkman board task, the rotative Brinkman board task and the Brinkman box task. Once the monkeys attained a stable behavioural performance for several weeks (thus reaching a pre-lesion plateau), a chronic stainless steel chamber was implanted above the left hemisphere (Table 1), to have access principally to the primary motor cortex (M1) and the dorsal and ventral premotor areas (PMd and PMv). The hand area of M1 was defined based on a first series (pre-lesion) of daily ICMS sessions for a period of about 2 months, during which the behavioural training was pursued,

though to a lesser extent. After completion of the pre-lesion ICMS map, behavioural training was more extensively repeated in order to confirm the pre-lesion plateau of manual dexterity obtained before the ICMS phase. Targeting the ICMS sites where a movement of the hand was elicited at low threshold, ibotenic acid was infused at multiple sites to generate a permanent lesion aimed at the hand representation unilaterally, as previously reported (Kaeser et al., 2010, 2011; Bashir et al., 2011). The behavioural assessment was conducted daily during several months to quantify the deficit resulting from the lesion as well as the time course and extent of recovery during several months post-lesion (data reported elsewhere; see also Kaeser et al., 2010, 2011; Bashir et al., 2011). After reaching a post-lesion plateau of behavioural recovery, stable during a few weeks (see Table 1 and Fig. 8), a second series (post-lesion) of daily ICMS session was performed, with the aim to investigate how the initial hand representation has been modified by both the lesion and the recovery of manual dexterity. Again, the second series of daily ICMS sessions lasted also about 2 months, as the same sites were explored a second time. The aim of the present study was to specifically compare the pre-lesion and the post-lesion ICMS maps.

Surgical procedures and animal care, previously described in detail (Kaeser et al., 2010, 2011; Bashir et al., 2011), were conducted in accordance to the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and were approved by local (Swiss) veterinary authorities.

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-BI	Mk-VA	Mk-SL	Mk-MO
Treatment	None	None	None	None	None	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Anti-Nogo-A antibody
Age at time of lesion (rounded 0.5 year)	4.5	5	5	4	5	5.5	5.5	5.5
Weight at time of lesion	3.8	3.6	2.8	3.2	5	4.9	4.6	5.6
Volume of ibotenic acid injected (□L)	40	40	13	18	29.7	15,5	18	20
Nb. of ICMS sites injected with ibotenic acid	21	21	13	12	29	11	12	20
Time interval (days) between lesion and post-lesion ICMS mapping	315	292	97	80	220	210	132	(97)
Estimates of "unfolded hand area pre-lesion (in mm^2)	**37**	**33**	**25**	**56**	**55**	**40**	**45**	**34**
Total volume of lesion (in mm^3) Gray matter (motor cortex + post-central gyrus)	**112.8**	**63.01**	**48.7**	**14**	**20.13**	**20**	**78.2**	**41.8**
Volume of lesion in post-central gyrus (in mm^3)	10.1	0	7.6	0	0	5.8	1.8	0
Lesion spread sub-cortically to the white matter (in mm^3)	86.5	28.9	0	0	0	0	130.6	0
Degree of functional recovery from M1 lesion (expressed in % of post-lesion **total** score at plateau with respect to pre-lesion **total** score in the modified Brinkman board task: **all** slots)	**42%**	**39%**	**38%**	**98%**	**74%**	**87%**	**73%**	**76%**
Degree of functional recovery from M1 lesion (expressed in % of post-lesion **horizontal** score at plateau with respect to pre-lesion **horizontal** score in the modified Brinkman board task: **horizontal** slots)			**11%**	**90%**	**36%**	**91%**	**77%**	**60%**
Degree of functional recovery from M1 lesion (expressed in % of post-lesion **vertical** score at plateau with respect to pre-lesion **vertical** score in the modified Brinkman board task: **vertical** slots)			**57%**	**100%**	**94%**	**87%**	**77%**	**84%**

Manual dexterity tests (behavioural assessment)

The eight monkeys were trained to perform behavioral tasks aimed at quantifying manual dexterity. The monkeys were trained to perform three different manual tasks, in which they had to retrieve food pellets from wells in different environments: (i) the modified Brinkman board task; (ii) the rotative Brinkman board task; and (iii) the Brinkman box task (see Schmidlin et al., 2011 for a detailed description and for visualization of the 3 manual dexterity tasks). Monkeys were trained before the cortical lesion until they reached a behavioral plateau corresponding to a pre-lesion stable score. Then, the cortical lesion was performed and the behavioral assessment was pursued during the functional recovery phase, as well as after the monkeys reached a post-lesion behavioral plateau, during several weeks/months. The methods of analysis of the behavioral data have been reported in a recent report (Schmidlin et al., 2011).

Surgical procedures

All surgical procedures were conducted under deep anesthesia. Before all implant surgeries, the monkeys were sedated with ketamine (Ketalar®; Parke-Davis, 5 mg/kg i.m.) and atropine was injected to reduce bronchial secretion (0.05 mg/kg, i.m.). Monkeys were then anesthetized deeply with an intravenous perfusion of 1% propofol (Fresenius®) mixed with a 5% glucose saline solution (1 volume propofol and 2 volumes of gluco-saline, delivered at a dose of 0.1 mg/kg/min). To prevent brain oedema, Dexamethasone (Decadon®) was injected i.m. (0.05 ml/kg diluted 1:1 in saline). At that step, a preventive pain killer (Carprofen) was administered i.m. (Rimadyl®; 50mg/ml; dose: 4 mg/kg). To further reduce possible activation of pain receptors during the surgery itself, ketamine was added to the i.v. perfusion solution (0.0625 mg/minute/kg), as previously reported (Freund et al., 2007). The surgery was carried out under continuous monitoring of the following parameters: heart rate, respiration rate, expired CO_2, arterial O_2 saturation and body temperature. All monkeys received, before surgery, a subcutaneous injection of the antibiotic Albipen® (Ampiciline 10%, 30 mg/kg). Immediately after surgery and offset of anesthesia, the monkey was continuously supervised by the experimenters until it was sufficiently awake and started to eat and drink. During the week following the surgery, additional doses of Carprofen were given daily (pills of Rymadil® mixed with food). Antibiotic (ampiciline as above) was administered on alternate days for the first week after surgery. All surgeries were performed in a facility under sterile conditions and approved by the (Swiss) cantonal veterinary office.

Cortical chronic chamber Implant

After reaching a stable behavioral performance (pre-lesion plateau), a square or a rectangular stainless steel chamber (Table 1) was stereotaxically implanted above the forelimb area of M1 on the left hemisphere (except in Mk-JU above the right hemisphere). The chamber were centred 15mm anterior and 15mm lateral and at an angel of 30° with respect to the misdsagittal plane, allowing perpendicular electrode penetrations with respect to the cortical surface. Six to ten titanium screws were used to anchor the chamber to the skull. Moreover, for better stability, two flat wings soldered to the chamber, one rostral and one caudal was also anchored to the skull by titanium screws. The edge of the chronic chamber next to the skull, as well as the titanium screws were covered with dental acrylic or orthopaedic cement (Palacos®). Over the mid-occipital and frontal regions of the skull, two stainless steel cylinders were anchored with 3-4 titanium screws and then cemented as described above for the chronic chamber. These cylinders allowed head restraining during the cleaning of the chronic chamber and most importantly during the ICMS sessions conducted while the monkey was awake. Nevertheless, a partly flexible head restrain attached to the cylinders allowing limited movement of the head. To prevent infections, the chronic chamber was cleaned daily with Betadine and an antibiotic ointment (Morrhulan).

Intracortical microstimulation (ICMS) procedures

During the initial phase of behavioural training, the monkeys were progressively habituated to be passively manipulated, allowing the experimenter to generate passive movement of the forelimb and the hindlimb. The goal of this habituation was to offer the possibility to the experimenter to modify the posture of the limbs during the subsequent ICMS sessions, for better assessment (visual and by palpation) of the overt motor response elicited the electrical microstimulation and searching the threshold. Electrophysiological ICMS procedures were performed twice in each monkey, first before the cortical lesion in order to guide the lesion procedure by mapping the M1 hand representation and, second, several months after the lesion when the monkeys reached a stable post-lesion manual performance (see Rouiller et al. 1998; Liu and Rouiller 1999; Schmidlin et al. 2004).

For ICMS, we used glass- or mylar-insulated platinum-iridium electrodes with typical impedances between 0.1 to 1.0 MΩ (Frederick Haer & Co., Bowdoinham, ME). The monkey sat in the primate chair, as described previously (Liu and Rouiller, 1999). The head was restrained and the electrode advancing system (Narishige group, Japan, Model MO-95) was attached to the chronic chamber. Electrode penetrations were performed systematically in the

precentral and postcentral cortex in a 1 mm grid interval, along the rostro-caudal and medio-lateral axes. The electrode was manually advanced (usually 1 mm step in depth), starting from 2 mm below the dura, on a maximal distance of 10 12 mm for penetrations along the rostral bank of the central sulcus, as recently illustrated (Kaeser et al., 2010).

The ICMS consisted of 12 electric pulses, delivered in a train of 33 ms duration, at a rate of 330 Hz. The effect of ICMS was assessed by visual inspection and/or palpation of muscle contraction at which a movement was elicited. The minimal current (ICMS threshold) producing the motor response was determined at each ICMS site along the electrode penetration. The intensity of stimulation ranged from 80 to 1 microampere. The intensity of 80 microamperes was mostly applied at the beginning of the electrode penetration (close to surface) and it was decreased until threshold. At the next ICMS site (1mm deeper), the initial intensity of stimulation was set slightly above (10 microamperes) the threshold intensity of the previous stimulation site. If this intensity did not elicit any movement, then it was progressively increased until threshold, but not above 80 microamperes (in absence of response at 80 microamperes, the site was considered as non-responsive). On the other hand, if the slightly increased intensity produced an effect, then it was decreased until threshold. During the entire stimulation period at each ICMS site, one experimenter hold the monkey's forelimb and passively moved it to different postures to determine the effect of the ICMS on the different joints (fingers, wrist, elbow, shoulder, etc).

Cortical lesion and anti-Nogo-A antibody pump implant

In each monkey, after completion of the ICMS map and precise delineation of the hand representation in M1, as well as some behavioral retraining, a permanent lesion of M1 was performed unilaterally, targeted to the hand area. The cortical lesion was produced by infusion of ibotenic acid at multiple sites (Table 1), where movements of the fingers were obtained at low threshold in response to ICMS. The precise procedure of ibotenic acid infusion was described earlier (Liu and Rouiller, 1999; Kaeser et al., 2010). In a separate and recent study, the time course of the neurotoxic effect of ibotenic acid infusion in the cerebral cortex of a monkey was assessed based on cerebral blood flow and MRI measurements (Peuser et al., 2011). A volume of 1µl to 1.5µl of ibotenic acid solution (10µg/µl phosphate buffer saline) was injected at each selected ICMS site, via a 10µl Hamilton syringe replacing the electrode at the corresponding penetration site. The total volume of infusion was adapted to the extent of the hand representation in M1 previously determined by ICMS. Ibotenic acid is expected to diffuse approximately 1.5 mm around the center of injection site (Martin et al., 1991). The

distance between adjacent infusion sites thus ranged between 1.5 to 3 mm, in order to cover most of the hand representation. In the majority of monkeys (Table 1), the infusion of ibotenic acid took place when the animal was awake and sat quietly in the primate chair. A few minutes after infusion of ibotenic acid, a progressive motor deficit of the contralateral hand appeared, reflected a flaccid paralysis of the hand.

At that step, the monkeys all subjected to a lesion of M1 were separated into two groups (Table 1). One group of 5 monkeys did not receive any treatment (control monkeys) whereas the second group of three monkeys was treated with an anti-Nogo-A antibody. The treated monkeys were implanted in the neck region (in a subcutaneous pouch) with two osmotic pumps (Alzet®, model 2ML2, 5µl/h), containing the anti-Nogo-A antibody 11C7, under deep anesthesia. One osmotic pump was used to infuse the antibody intrathecally in the spinal cord at cervical level, as previously reported in our studies on spinal cord injury (Freund et al., 2006, 2007, 2009). The second osmotic pump was used to deliver the anti-Nogo-A antibody at cortical level, close to the lesioned territory. The catheter of this second osmotic pump was tunneled under the skin to the head of the monkey. Through a small opening in the skull, the tip of catheter was push under the dura in close proximity to the motor cortex. Once the catheters from both osmotic pumps were in place, the muscles and then the skin were sutured. The osmotic pumps delivered the anti-Nogo-A antibody treatment during 4 weeks and then they were removed under deep anesthesia. The untreated monkeys were not implanted with osmotic pumps.

Processing of ICMS data: Unfolding the motor cortex maps

ICMS data were represented in two different types of cortical maps: a standard (raw) ICMS map (see Liu and Rouiller, 1999; Kaeser et al., 2010: supplemental Fig. 1 panel A) and an unfolded ICMS map (see Park et al., 2001, 2004; Kaeser et al., 2010: supplemental Fig.1). The standard ICMS map consisted in projecting the lowest ICMS threshold obtained along an individual electrode track on the surface of the motor cortex, at the location of electrode penetration. The resulting ICMS map forms a grid with intervals of 1mm x 1mm and allows ICMS positioning on the brain surface. The limitation of this method is that electrode tracks running in the rostral bank of the central sulcus in parallel to the cortical layers is represented only by a single point on the surface, although the electrode may have stimulated layer V neurons at low threshold at several adjacent sites (see Kaeser et al., 2010: supplemental Fig. 1). In contrast, the unfolded map represents all sites where the lowest thresholds were obtained by ICMS, presumably at the location of the pyramidal cells in layer V. As a

consequence, the multiple ICMS sites in layer V encountered along an individual electrode track in the rostral bank of the central sulcus appear in the unfolded ICMS map (see Kaeser et al., 2010: supplemental Fig. 1). In summary, a two-dimensional rendering of cortical layer V in the rostral bank of the central sulcus required flattening and unfolding its curvature. To this aim, all electrode tracks were first grouped according to their medio-lateral coordinate. Within each group, the tracks were then ordered according to their rostro-caudal coordinate. On the basis of electrophysiological data and observations, a parasagittal diagram was constructed to represent the cortex that was explored and stimulated. The white matter was identified by a sharp increase of the ICMS effective intensity or even a loss of effect of ICMS on the forelimb joints. The reconstruction of the position of the precentral sulcus was based on the successive ICMS sites that produced effects at the lowest threshold and the sensory cortex was identified by the absence of forelimb movement or by a significant increase of the ICMS effective intensity, mostly in the fundus of the central sulcus. For each electrode track, sites corresponding to cortical layer V were identified using a combination of electrode depths, and the lowest threshold ICMS intensity site. Electrode penetrations on the convexity of the precentral gyrus traversed cortical layers perpendicularly and, in such cases, it was relatively easy to identify the ICMS site closest to layer V, corresponding to the lowest threshold. For electrode penetrations running along the rostral bank of the central sulcus, roughly parallel to the cortical layers, it was more difficult to identify layer V sites. In such cases, output effects from sites at the same depth from different electrode tracks along the rostro-caudal axis were compared. This analysis yielded a series of reconstructed parasagittal cortical sections oriented along the rostro-caudal axis of the chronic chamber in the plane of the electrode tracks. The first ICMS site of the identified convexity of the rostral bank of the central sulcus will be used as the axis for rotating and unfolding the layer V (see Kaeser et al., 2010: supplemental Fig. 1).

Results

1) Monkeys involved in the present study

The present data were collected from eight monkeys, already included in recent reports from this laboratory, related to the performance of the ipsilesional hand after M1 lesion (Kaeser et al., 2010; Bashir et al., 2011) or representing a preliminary approach to establish a cell therapy based on implantation of autologous adult progenitor cells (Kaeser et al., 2011). In the present study, the eight monkeys subjected to M1 lesion were specifically analyzed to address the issue of motor map changes using ICMS (Table 1). The left M1 was lesioned in

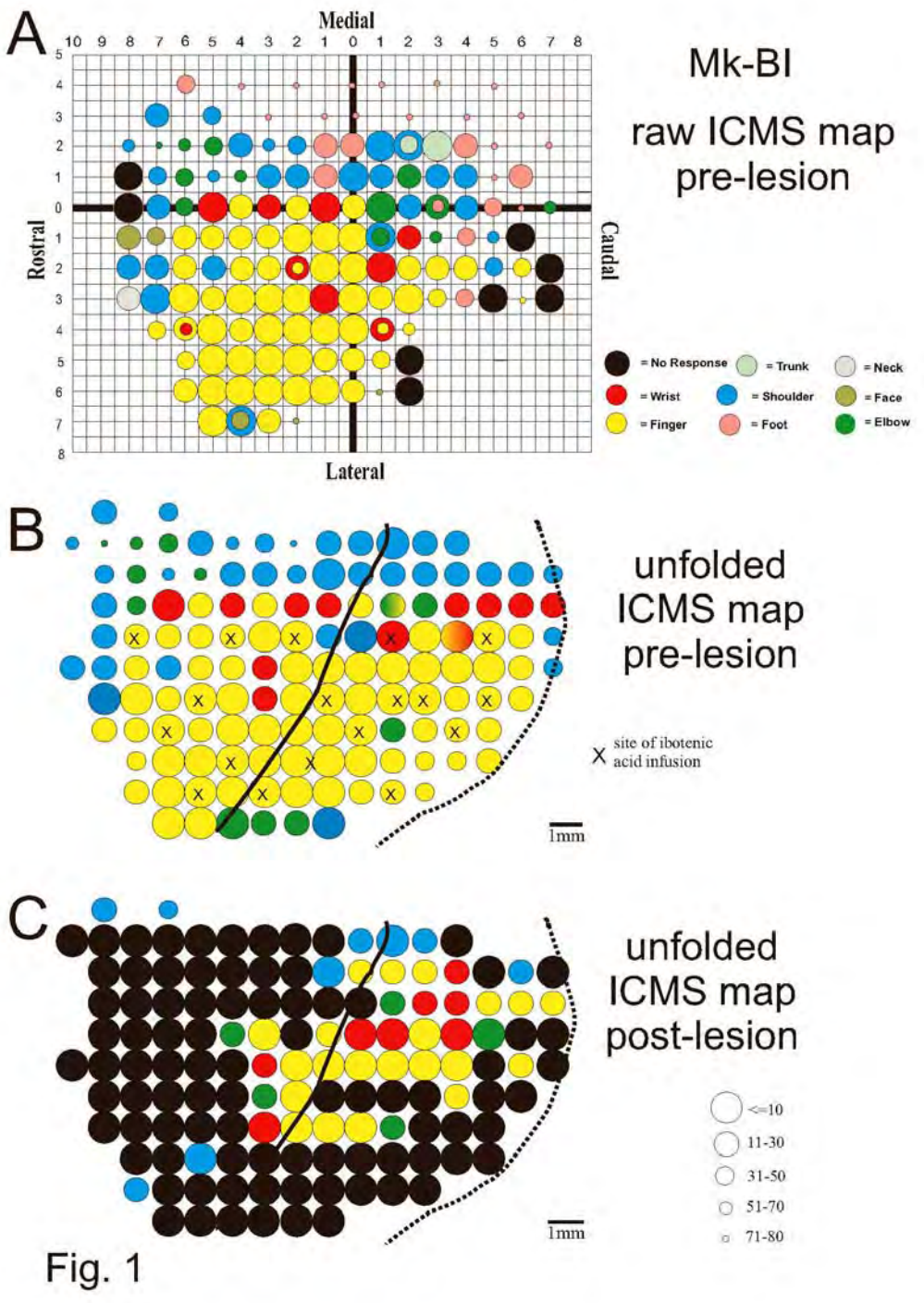
seven monkeys, whereas it was the right M1 that was lesioned in the eighth monkey (MK-JU). In the context of a long-term study aimed at assessing the possible beneficial effect of a treatment to enhance functional recovery from M1 lesion, three of these eight monkeys were treated during 4 weeks with continuous administration of anti-Nogo-A antibody, whereas the other five monkeys, also subjected to M1 lesion, did not receive any treatment, thus corresponding to control animals (Table 1).

2) Pre-lesion motor maps

In all monkeys, before the lesion of M1, ICMS experiments were conducted to delineate the hand representation in M1, defined as the cortical sites where ICMS elicited movements of the digits of the contralateral hand at the lowest threshold. After about 1-2 months of daily ICMS sessions, a raw cortical motor map was obtained (Fig. 1A; in Mk-BI), showing the position on the cortical surface of the electrode penetrations perpendicular to the cortical surface and distant by 1 mm from each others. The hand area on the cortical surface corresponds to the sites of electrode penetrations along which the ICMS effect observed at the lowest threshold was a movement of digits of the contralateral hand (yellow circles in Fig. 1A). As expected, ICMS delivered at more medial and/or rostral electrode penetrations elicited at lowest threshold movement of more proximal body territories (wrist, elbow, shoulder or foot) and/or face muscles (Fig. 1A). To avoid damage of the cerebral cortex as might induce too many electrode penetrations, the ICMS was mostly focused on the hand representation in M1, without extensive mapping neither of the adjacent motor cortical areas (PM, SMA) nor of the other body territories in M1 (e.g. face, trunk, leg, etc).

A first analysis of these raw data aimed at obtaining a more comprehensive representation of the motor map focused on the hand representation by unfolding the central sulcus (mostly the rostral bank of the central sulcus, as explained in the methods: Fig. 1B). In the rostral bank of the central sulcus (mostly where the hand representation is located), the electrode penetrations are roughly parallel to the cortical layers. Consecutive ICMS sites eliciting digit movements at low threshold are encountered along the same electrode penetration (presumably following layer V), thus corresponding to an enlargement of the hand area (yellow circles) in the caudal direction (Fig. 1B in the same Mk-BI), as compared to the raw motor map (Fig. 1A). Similarly unfolded pre-lesion motor maps have been established for the other seven monkeys (left column in Figs. 2 and 3). In the unfolded map pre-lesion, only the ICMS responsive sites were considered. These data show that the extent of the hand area, as delineated by ICMS, is quite variable across monkeys (see Table 1: estimates of “unfolded”

hand area). As expected for M1, most ICMS sites elicit corresponding body movements at low intensity (below 10 micro-amps).



<u>Figure 1</u>: Example of ICMS maps established in Mk-BI, in the initial (raw) format pre-lesion (panel A) and after unfolding the central sulcus, pre-lesion (panel B) and post-lesion (panel C). In panel A, each circle corresponds to the location on the cortical surface of an electrode penetration. Still in panel A, the

movement elicited by ICMS at the lowest threshold along each electrode penetration is represented by the color code shown on the right (e.g. wrist, finger, etc; at a few sites, a circle represented with 2 colors indicate an effect at the same threshold for two body territories). The same ICMS sites as in panel A appear in panel B and C, plus some additional sites along the rostral bank of the central sulcus, as explained in the method. Same color code in panels B and C as in panel A. Applying to the three panels, the set of circles on the bottom right of the figure indicates the threshold (in micro-amps) at which the corresponding body movement was still observable at the lowest intensity. The five graded circle sizes (encountered in the ICMS maps) correspond to different threshold ranges, as indicated on the right of the circles. In each ICMS map, five graded circle sizes have been used to distinguish the excitability of each ICMS site. In panels B and C, the solid oblique line is for the approximate position of the central sulcus as it appears on the surface of the cerebral cortex, whereas the oblique dashed line is for the fundus of the central sulcus.

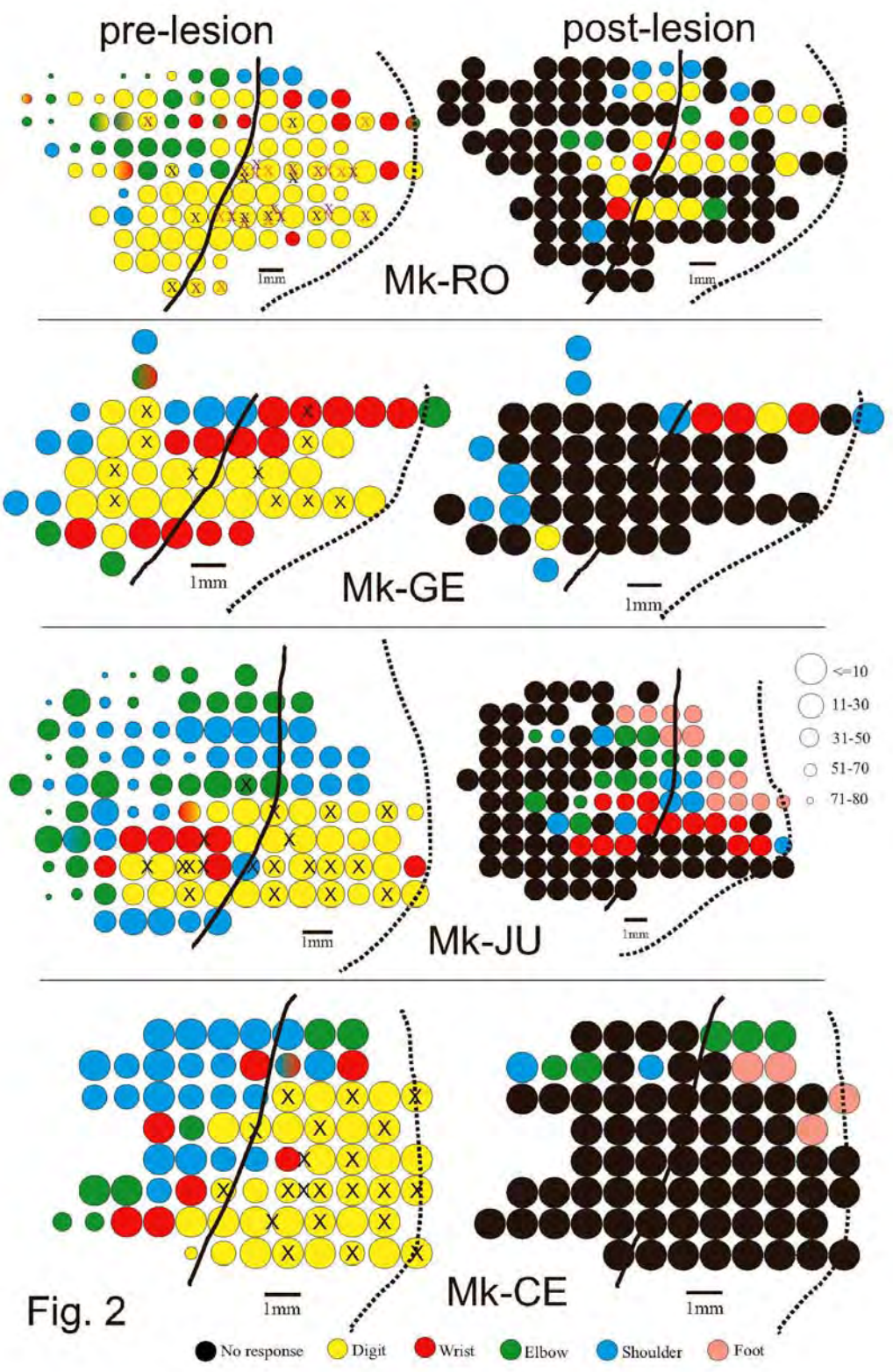


Figure 2: Unfolded ICMS maps established pre-lesion (left column) and post-lesion (right column) in the monkeys Mk-RO, Mk-GE, Mk-JU and Mk-CE. Same conventions as in Figure 1. In Mk-JU, the ICMS map has been reverted horizontally so that it appears as derived from the left hemisphere for better comparison with the other monkeys, although the mapping was conducted originally on the right hemisphere (see Figure 4: Mk-JU).

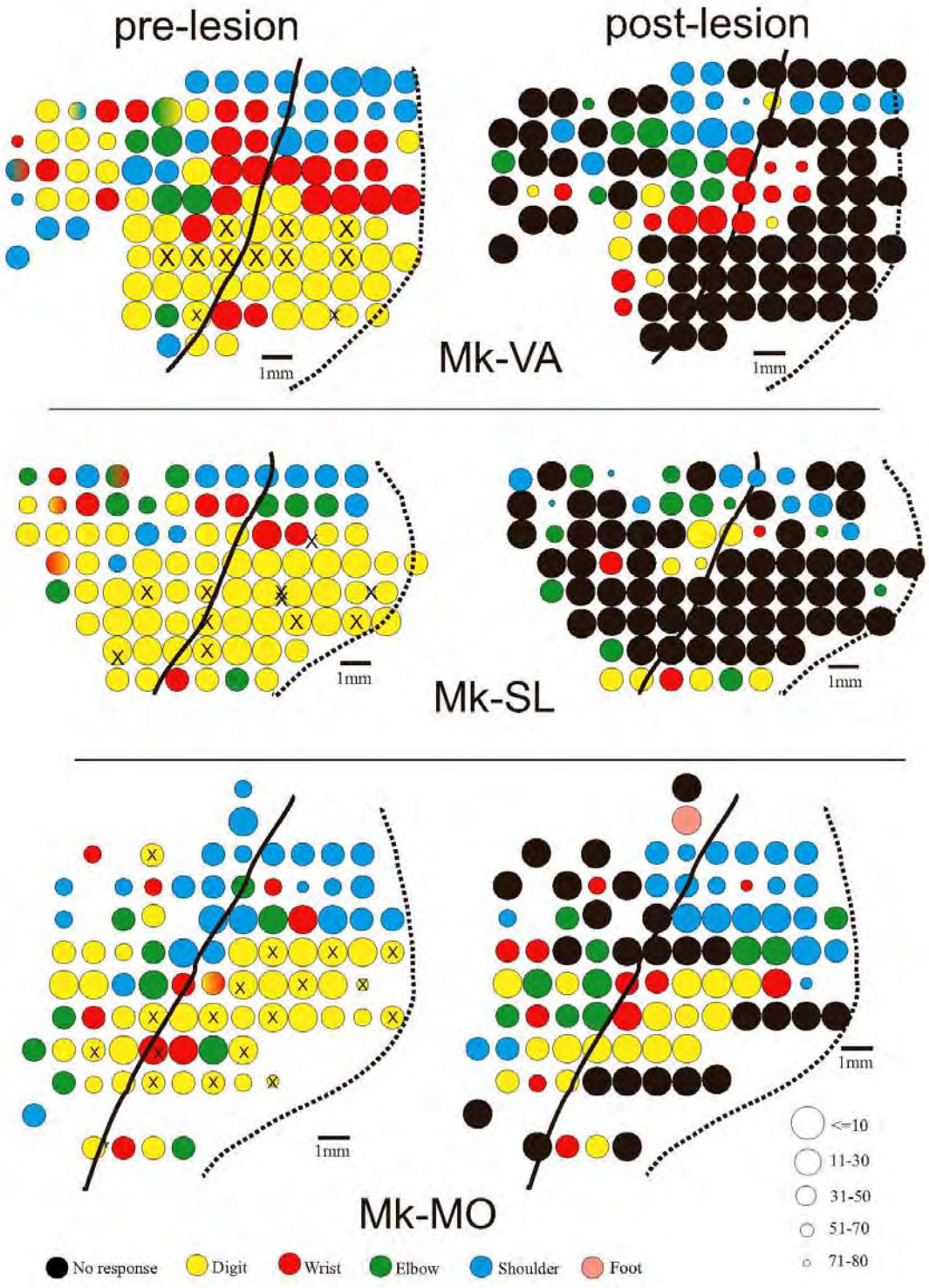


Fig. 3

Figure 3: Unfolded ICMS maps established pre-lesion (left column) and post-lesion (right column) in the monkeys Mk-VA, Mk-SL and Mk-MO. Same conventions as in Figure 1. In contrast to the monkeys illustrated in Figure 1 and 2, these three monkeys were subjected to anti-Nogo-A antibody treatment.

The “x” symbols in the pre-lesion unfolded motor maps represent the ICMS sites selected for infusion of ibotenic acid to generate a permanent chemical lesion of the hand representation in M1. The reconstruction of the lesion, as seen on a lateral view of the cerebral cortex, is represented in Figure 4 for each of the eight monkeys included in the present study. The volume of the lesioned territory has been assessed (Table 1), from histological sections treated for the marker SMI-32, as previously reported (Kaeser et al., 2010; Bashir et al., 2011).

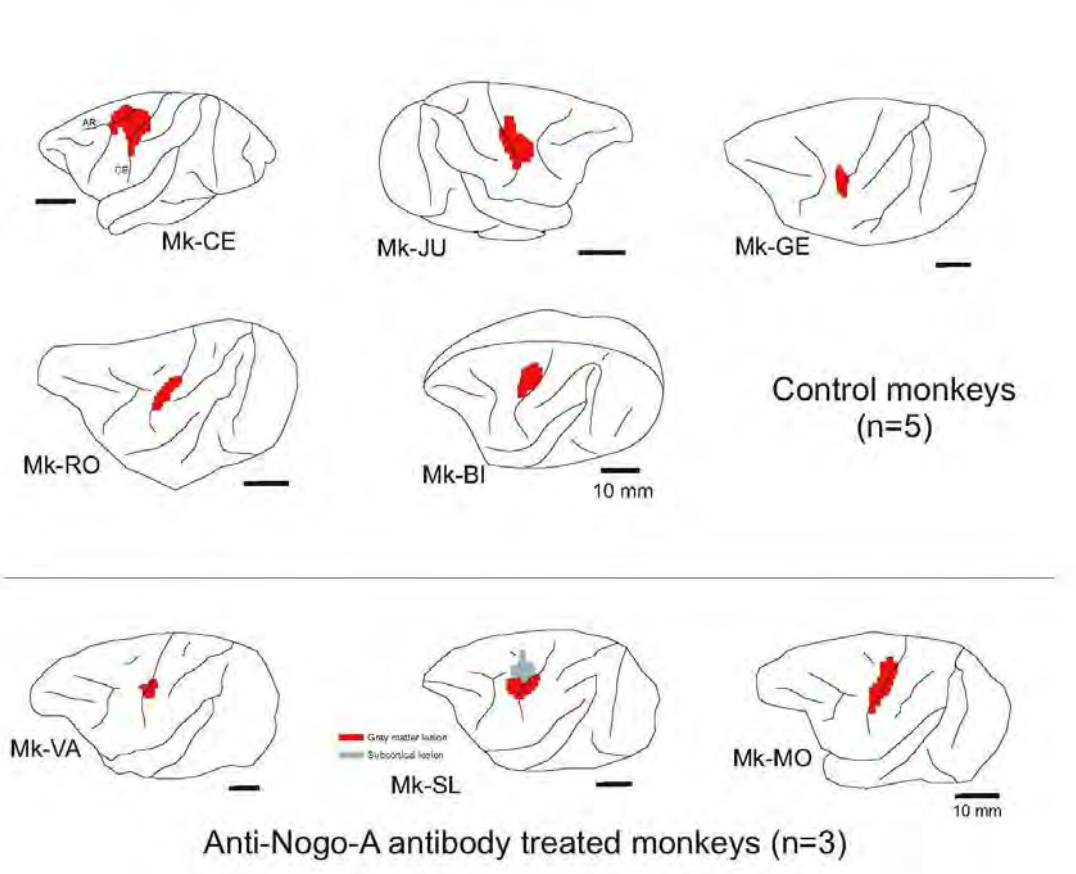


Fig. 4

<u>Figure 4</u>: Lateral views on the surface of the corresponding hemisphere of the lesion extent (red area) in the motor cortex, as seen in transparency of the cortical surface (same data as shown previously for some monkeys in Kaeser et al., 2010, 2011; Bashir et al., 2011). The surface representation of the lesion does not reflect directly the actual volume of the gray matter territory impacted by the infusion of ibotenic acid (see for that Table 1 and Figs. 9 and 10).

Starting a couple of days after the lesion, the behavioral sessions were pursued, as before lesion, to assess manual dexterity. As a result of ibotenic acid infusion in the hand area in M1 unilaterally, the contralateral hand was paralyzed, corresponding to a score of zero in

all manual tests (see Liu and Rouiller, 1999; Kaeser et al., 2010, 2011; Bashir et al., 2011; Schmidlin et al., 2011). During the following weeks, there was a progressive recovery of manual dexterity, reaching a post-lesion plateau of manual performance. In most cases, the extent of functional recovery is incomplete (Table 1). After the post-lesion plateau of manual performance has been established for several weeks or months, a second series of ICMS sessions took place to determine the post-lesion motor map in the cortical area in and around the lesion. The time interval between the lesion of the motor cortex (ibotenic acid infusion) and the onset of the second ICMS mapping is indicated in Table 1 for each monkey.

3) Post-lesion motor maps

Post-lesion, the same ICMS sites as in the unfolded pre-lesion map were re-visited, when the monkeys reached a post-lesion plateau. The unfolded ICMS maps established post-lesion (panel C in Fig. 1 and right columns in Figs. 2 and 3) appear very different from the pre-lesion unfolded ICMS maps, mainly because of non responsive ICMS sites. As expected for a permanent lesion induced by ibotenic acid infusion, a majority of territories became non-responsive to ICMS (black circles in Figs. 1C, 2 and 3), in spite of high intensity stimulation (up to 80 microamps). In one monkey (Mk-CE; Fig. 2), none of the pre-lesion sites where ICMS elicited digit movements was preserved after the lesion: the entire previous hand area was replaced post-lesion by a non micro-excitable territory (except at 2 sites, replaced by an effect on foot muscles). In Mk-JU, in the post-lesion ICMS map, there was a replacement of hand sites either by a non micro-excitable territory or by more proximal body territories, such as wrist, shoulder or even foot (Fig. 2). In all other monkeys (Figs. 1-3), a large proportion of ICMS sites belonging to the hand area pre-lesion became non-responsive post-lesion. However, in these monkeys (Mk-GE, Mk-VA, Mk-SL, Mk-RO, Mk-BI, Mk-MO) to an increasing extent following this order, there were ICMS sites still eliciting digit movements at the lowest threshold along the corresponding electrode track. In parallel, in these monkeys, some other ICMS sites eliciting digit movements pre-lesion were replaced by more proximal muscle territories (Figs. 1-3). Overall, out of eight monkeys, the change of ICMS map post-lesion of the motor cortex was quite variable from one animal to the next. With extremely rare exception (one site in Mk-VA, one site in Mk-GE) none of the ICMS sites located pre-lesion clearly outside the hand area (e.g. proximal territory) was changed post-lesion into an ICMS site eliciting digit movements. On the other hand, when digit ICMS sites were present post-lesion, it was in general a site that was already devoted to the hand area pre-lesion (Figs. 1-3).

4) Comparison of ICMS maps pre-lesion versus post-lesion

The number of sites where ICMS was applied pre-lesion was quite variable from one monkey to the next (variable size of hand representation) and therefore it is not a meaningful parameter. In contrast, as the very same ICMS sites were re-visited post-lesion, comparing this initial number to the number of still responding sites post-lesion provides a valuable assessment of ICMS map changes (Fig. 5). In all monkeys, there was a substantial decrease of responding ICMS sites post-lesion (Fig. 5A), though to a quite variable extent across animals, as better seen when plotting the percent of non-responding ICMS sites post-lesion with respect to the total number of responding sites pre-lesion (Fig. 5B). The percentage of non-responding ICMS sites ranged from 29% to 85%, in line with the relatively high number of black circles in Figures 1-3. As the number of responding sites displayed in Figure 5 are unique observations (one pre-lesion map only and one post-lesion ICMS map only), no statistical comparison can be conducted.

In Figure 6, for each monkey, the bar graphs in black show the pre-lesion percent distribution of ICMS sites with respect to the corresponding body territories activated: as expected, the goal of the ICMS is being to delineate the hand representation, the majority of ICMS sites elicited digit movements at threshold. The gray bar graphs in Figure 6 show the same percent distribution but post-lesion, for the sites which still elicited a motor response. The percent of digit sites decreased dramatically or even totally disappeared (Mk-JU and Mk-CE). Nevertheless, the distribution of activated body territories pre- versus post-lesion, as shown in Figure 6, did not vary significantly, as assessed with the chi-square test ($p>0.05$). For nearly all other territories, there was also a decrease of responding sites, but generally to a lesser extent than for the fingers.

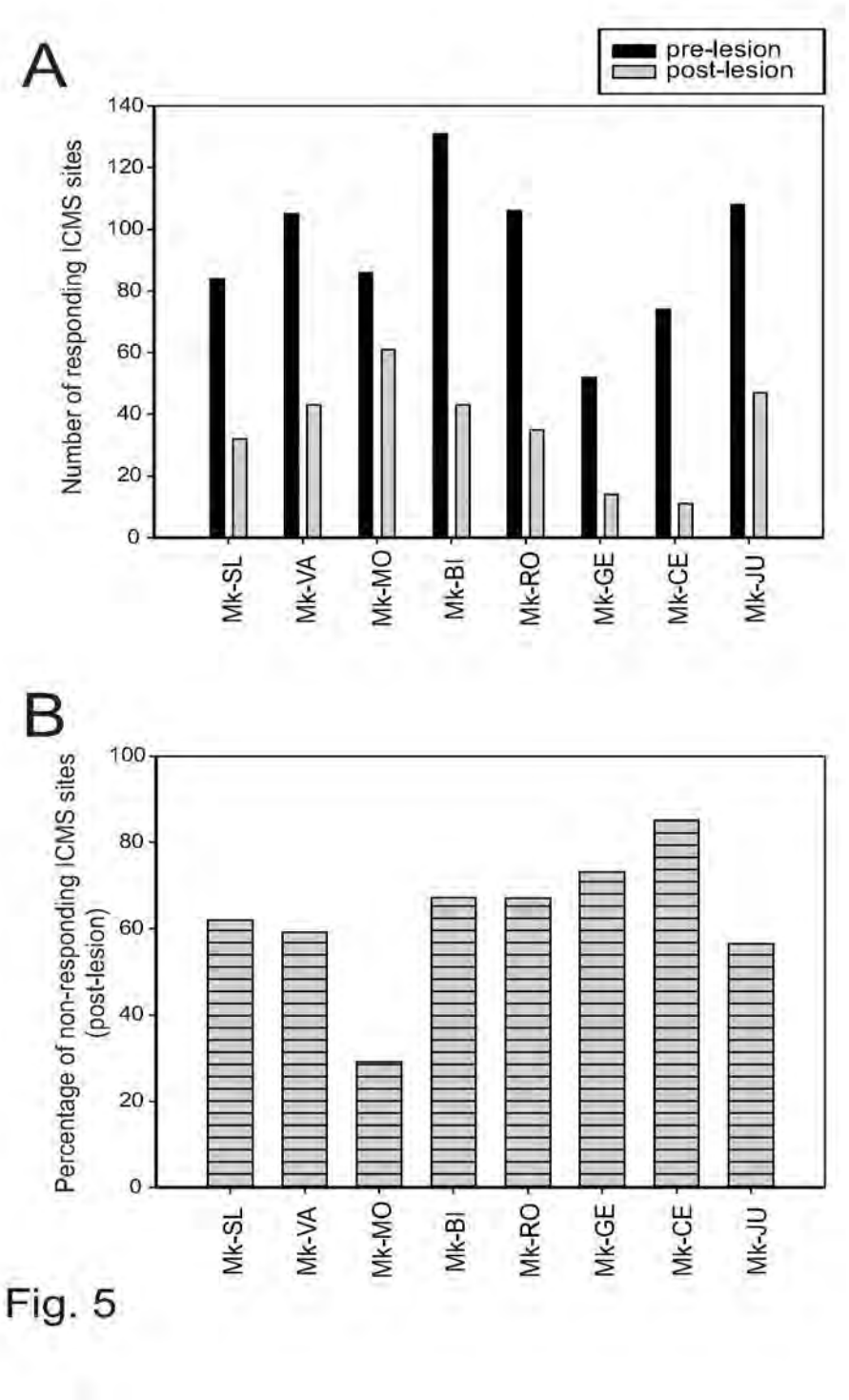


Fig. 5

<u>Figure 5</u> A: Comparison pre- versus post-lesion for each monkey of the number of ICMS responsive sites (effect on joints elicited at an intensity lower than 80 microamps). The three monkeys on the left were subjected to anti-Nogo-A antibody treatment wheres the five monkeys on the right were control.

B: Derived from panel A, the bar graphs show for each monkey the percentage of ICMS sites which became non-responsive as a result of the lesion in the motor cortex. The very same ICMS sites were tested post-lesion as those visited pre-lesion.

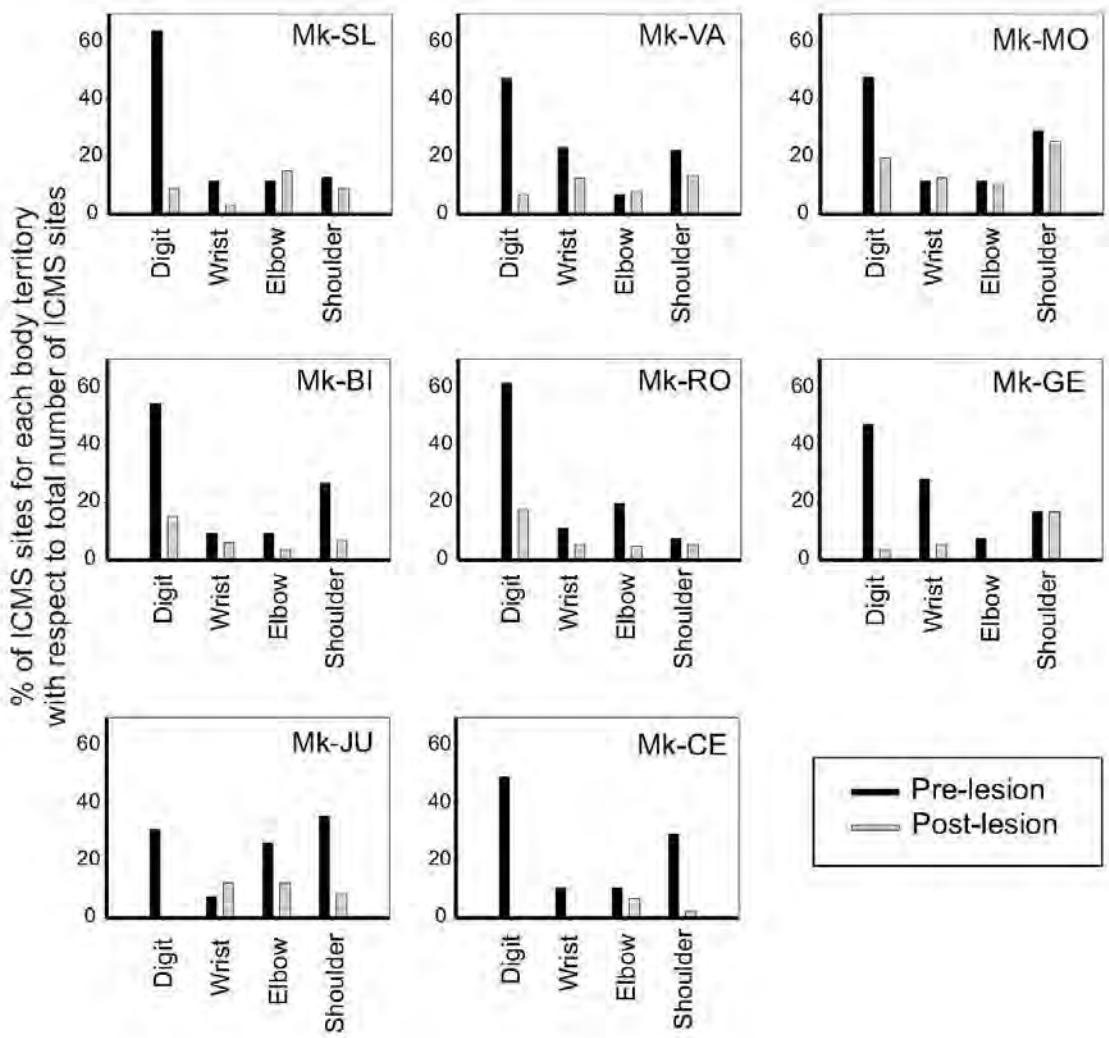


Fig. 6

<u>Figure 6</u>: For each monkey, the histograms show the distribution of the ICMS sites in M1 with respect to the body territory (forelimb), at which the current at threshold elicited a movement before the lesion of M1 (black bars). In each graph, the sum of the four black bars is 100%. The grey bars show the distribution of the same ICMS sites post-lesion, showing that a large proportion of ICMS sites became non-responsive (only the responsive ICMS sites post-lesion have been distributed; their sum is thus clearly smaller).

Due to the motor cortex lesion, one would expect that the ICMS sites still present after the lesion in the ICMS map elicit movements at higher threshold than pre-lesion. This prediction has been generally confirmed in some monkeys (Mk-SL, Mk-VA, Mk-JU and Mk-CE: statistically significant difference pre- versus post-lesion for all or most territories using the Mann and Whitney test), but not in other monkeys (Mk-MO, Mk-BI, Mk-RO and Mk-GE:

statistically non-significant differences for most territories), as illustrated in Figure 7. In only two cases (Mk-BI and Mk-RO), the threshold was lower post-lesion than pre-lesion for a given body territory, respectively the elbow and the shoulder. The extent of the threshold increase was again quite variable from one monkey to the next (Fig. 7).

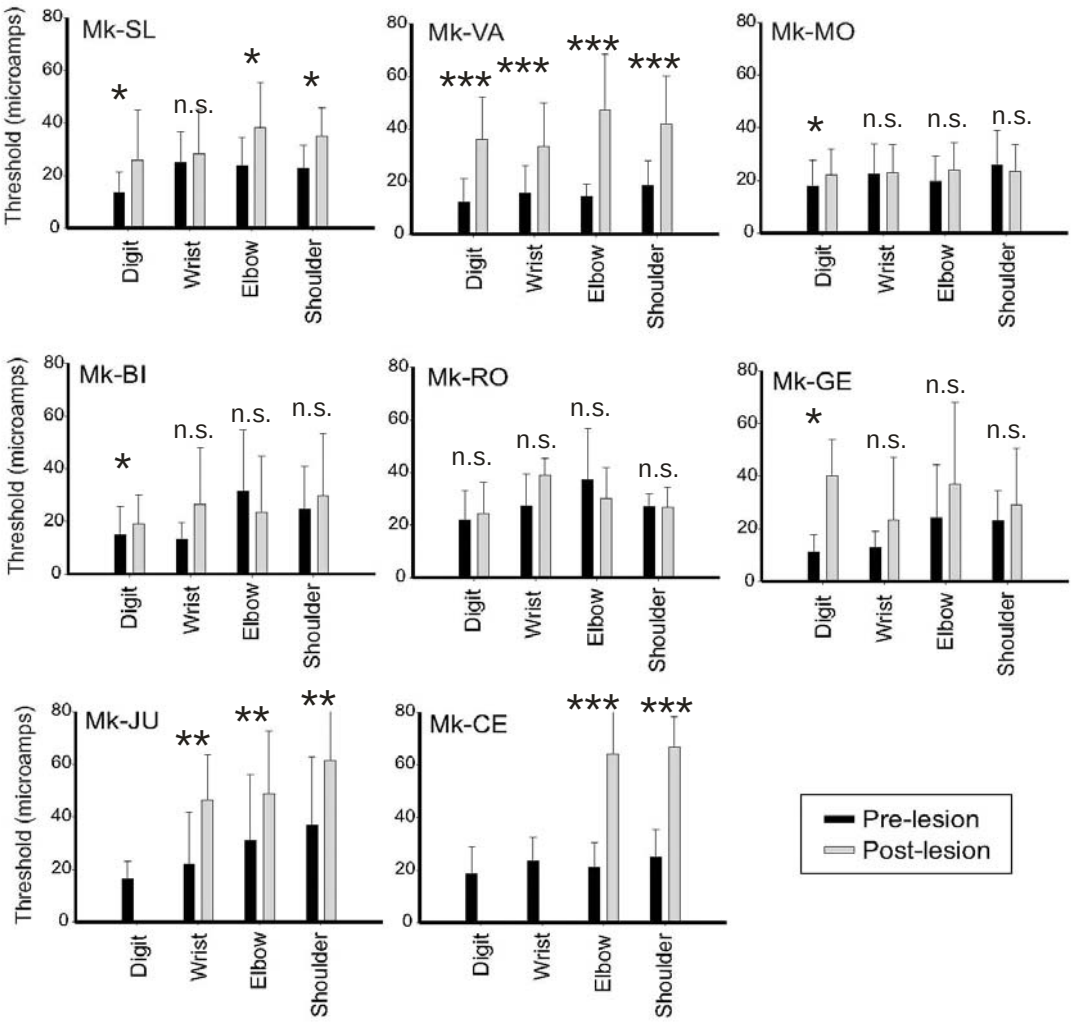


Fig. 7

Figure 7: For each monkey, the histograms show the distribution of the ICMS threshold values at which the effect was observed, pre-lesion (black bars) and post-lesion (grey bars). For each body territory (forelimb), the bars indicate the mean threshold and the standard deviation (SD). Note a general increase of threshold post-lesion as compared to pre-lesion. The number of ICMS sites considered post-lesion is much smaller than pre-lesion, as the majority of ICMS sites became non-responsive post-lesion (see Figs 1-3; 5-6). The distributions of thresholds were compared in each monkey and for each territory (e.g. digit, wrist, etc) pre-lesion versus post-lesion, using the Mann and Whitney test: n.s. is for p>0.05; * is for p<0.05; ** is for p<0.01; * is for p<0.001.**

5) Behavioral data

Based on the modified Brinkman board task, manual dexterity was assessed by the number of pellets (score) retrieved during the first 30 seconds (Schmidlin et al., 2011) on each daily behavioral session. In contrast to our recent report (Kaeser et al., 2011) focused on the total number of pellets retrieved (total score), the score data were analyzed here separately for the vertical slots and the horizontal slots. Plots of the behavioral performance of the three anti-Nogo-A antibody treated monkeys are shown in Figure 8 (panel A). Before the lesion, after an initial training phase (not shown), the monkeys reached a generally stable manual performance, reflected by a pre-lesion plateau of score. Immediately after the lesion, as expected, the manual dexterity of the contralesional hand dropped to zero, before a progressive functional recovery took place during several weeks, to reach a post-lesion plateau. The beginning of the post-lesion plateau (vertical dashed line in panel A of Figure 8), was determined based on criteria as defined in Kaeser et al. (2011): the onset of the plateau was defined as the first individual data point (total score) for which, among the next 3 individual data points, none exhibits a higher score. In two out of the three anti-Nogo-A antibody treated monkeys (Mk-MO and Mk-VA), there was a rebound of score, with a subsequent (significant) increase of the number of pellets retrieved, initiated on the day pointed out by the green arrow (see Kaeser et al. 2011, for criteria to define the onset of the rebound). The same, original plots of score, showing the deficit immediately after the lesion and the subsequent functional recovery in the five control monkeys, have been published in recent reports (Kaeser et al., 2010, 2011; Bashir et al., 2011). These plots (reproduced here in Fig. 8B) show that, in the five control monkeys, there was no such rebound of score post-lesion, as observed in Mk-VA and Mk-MO (panel A in Fig. 8). The ratio of the post-lesion plateau score (median value) to the pre-lesion plateau score (median value) defines the percentage of functional recovery, computed for each monkey and illustrated in the form of box and whisker plots in Figure 9. In the control monkeys (Fig. 9), the degree of functional recovery ranged between 46% and 100% for the vertical slots and between 9% and 90% for the horizontal slots. Taking into account the final second plateau post-lesion in Mk-VA and Mk-MO, the degree of functional recovery among the anti-Nogo-A antibody treated monkeys ranged between 77 and 87% for the vertical slots and between 60 and 91% for the horizontal slots. Such variability within the two subgroups of monkeys is related, at least in a large part, to the size of the cortical lesion. For this reason, the degree of functional recovery in percent was plotted as a function of the volume of the cortical lesion (Fig. 10). As expected, considering all eight monkeys, there was a general trend towards a decrease of functional

recovery as a result of increasing lesion size (see regression lines in Fig. 10). First of all, the control monkey Mk-RO with the smallest lesion exhibited an excellent spontaneous functional recovery. Looking at the vertical slots data (middle panel in Fig. 10), the distribution of functional recovery for the control monkeys was fairly comparable to that obtained for the anti-Nogo-A antibody treated monkeys. In contrast, the functional recovery was distributed in a clearly more separate way when considering the horizontal slots (bottom panel in Fig. 10). As grasping from the horizontal slots is more challenging than from the vertical slots (see Freund et al., 2009; Schmidlin et al., 2011), the deficit in control monkeys is more prominent whereas, in the anti-Nogo-A antibody treated monkeys, the treatment appeared to favor a substantial restitution of this sophisticated manual ability (contribution of precision grip and arm rotation needed for grasping from the horizontal slots). In more detail, the anti-Nogo-A antibody treated monkey Mk-VA recovers the ability to grasp from the horizontal slots a bit more than the control monkey Mk-RO, subjected to a smaller lesion (bottom panel in Fig. 10). The two anti-Nogo-A antibody treated monkeys Mk-MO and Mk-SL recovered clearly better than the other control monkeys (bottom panel in Fig. 10). As expected, the plot based on the total score (sum of vertical and horizontal slots; top panel in Fig. 10) yields an intermediate figure between the two slot orientations taken separately.

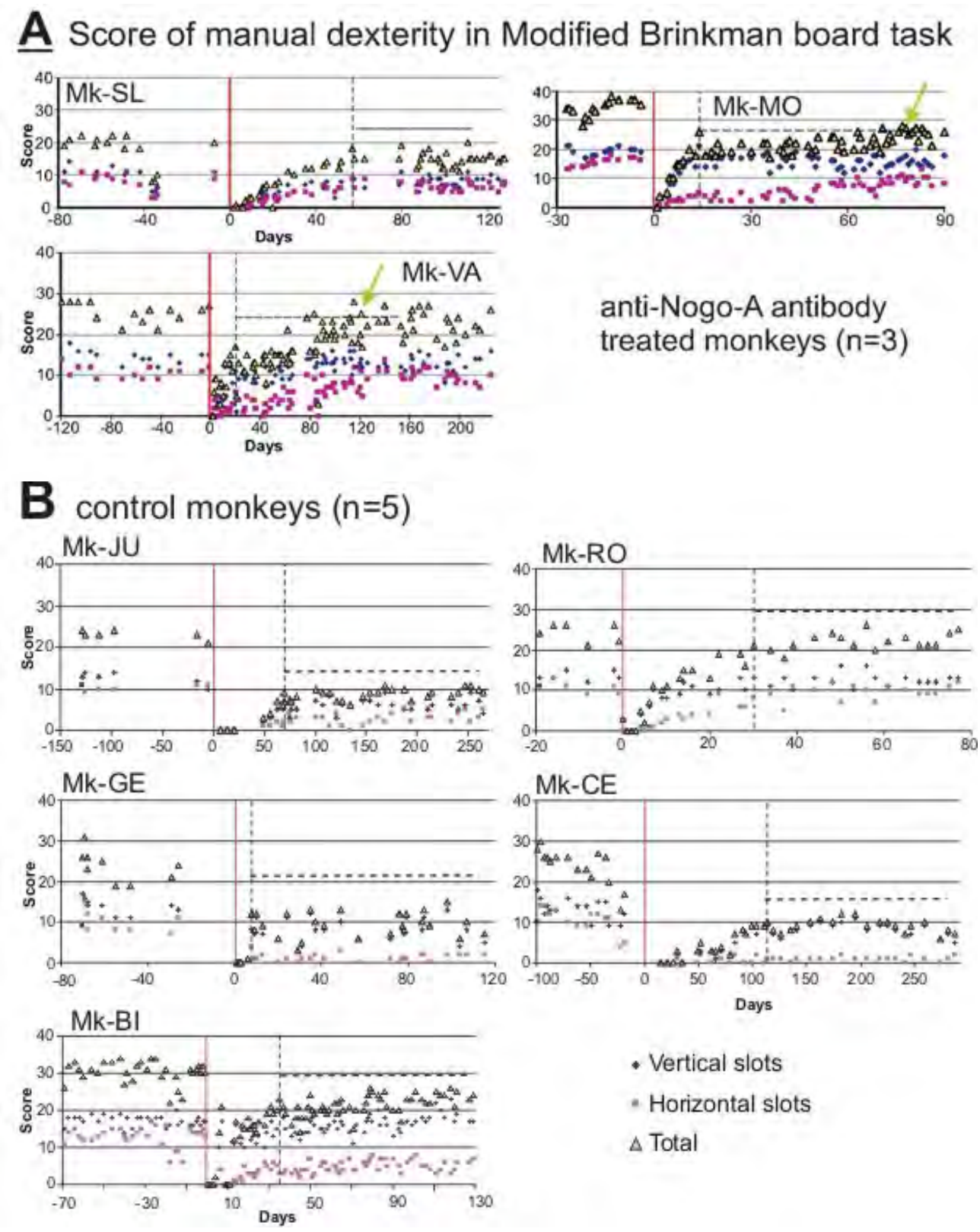
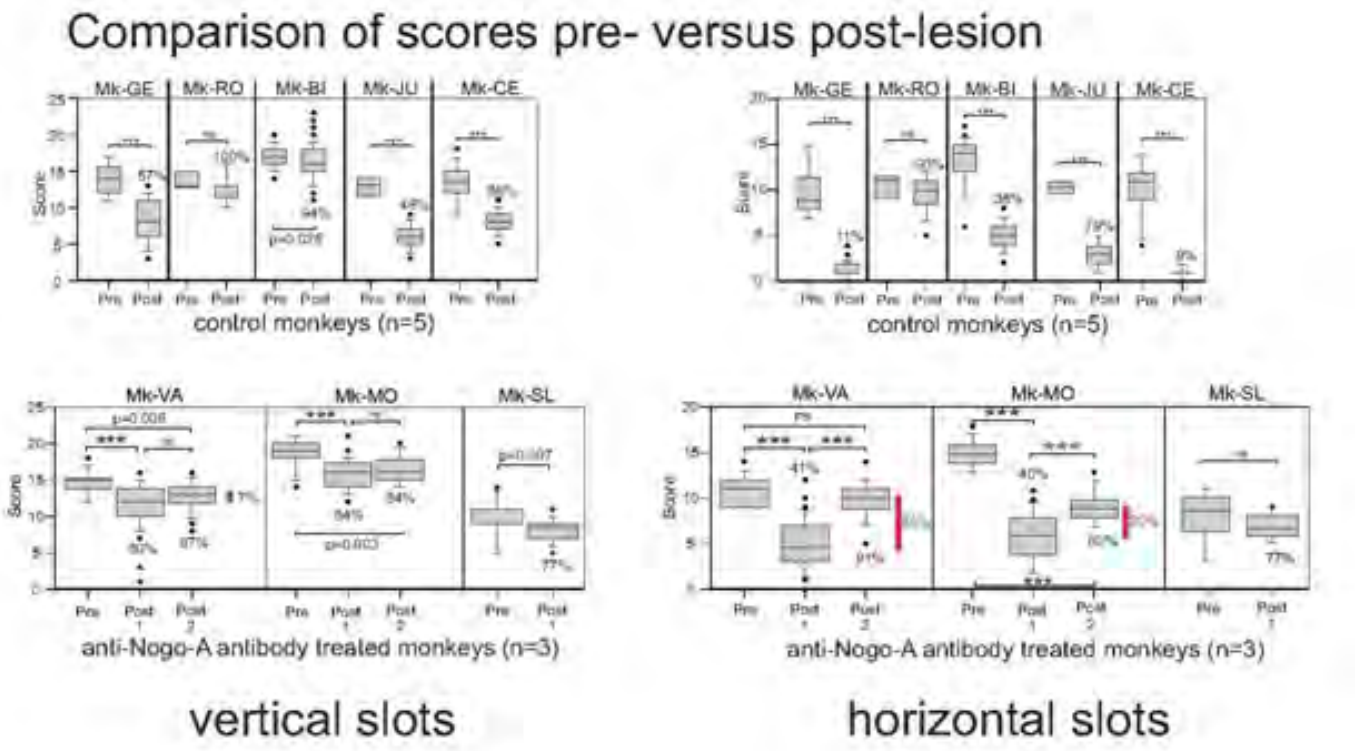


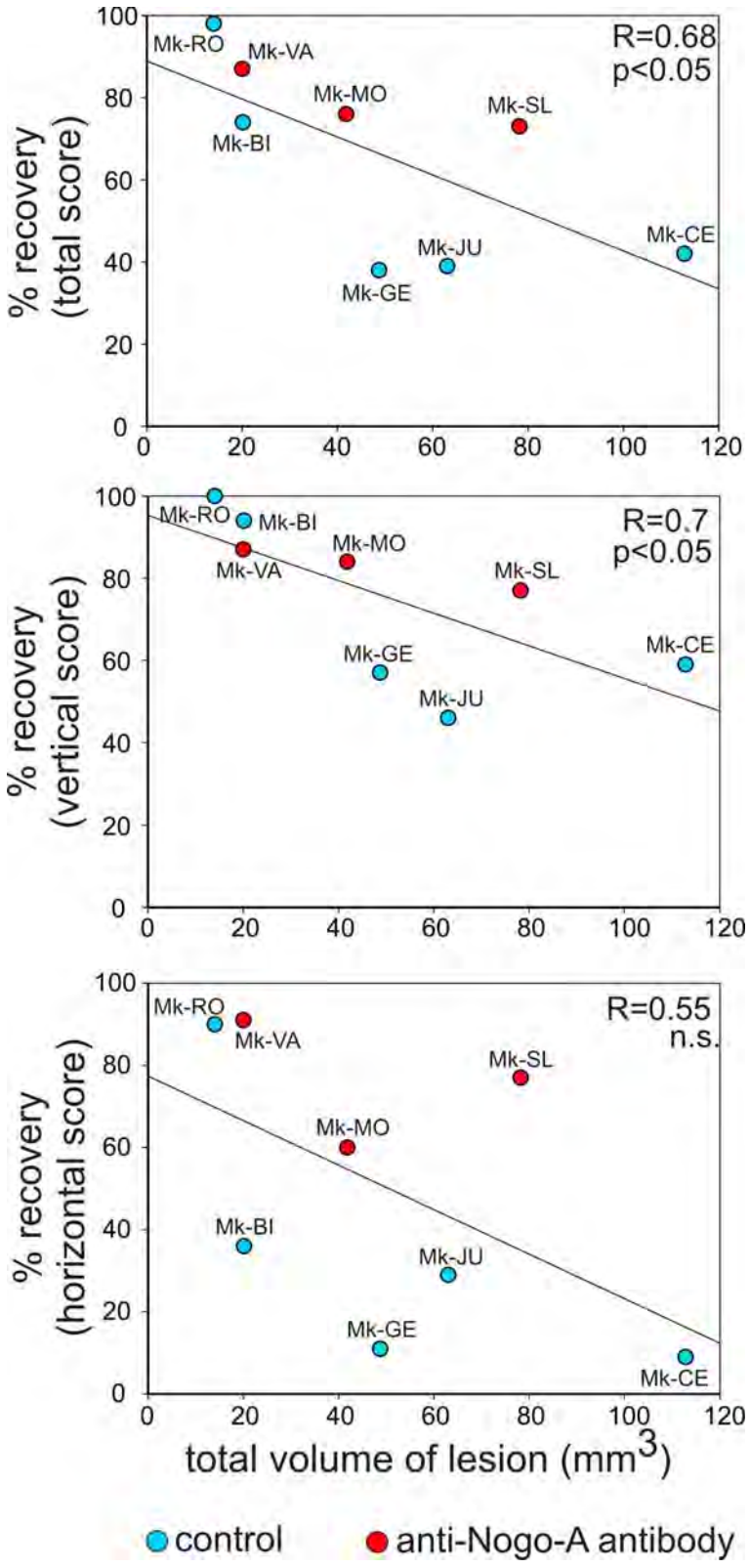
Figure 8 A: Behavioral data for the three monkeys subjected to anti-Nogo-A antibody treatment, showing the manual dexterity performance (score) in the Brinkman board task, as a function of time corresponding to daily sessions pre-lesion (negative days) and post-lesion (positive days). The vertical red line is for the day of M1 lesion. Note the dramatic drop of manual dexterity immediately after the lesion of M1. The score (number of pellets retrieved in 30 seconds) is given for the vertical slots (blue diamonds),

the horizontal slots (red squares), whereas the yellow triangles are for the total score (sum of vertical and horizontal slots). As described earlier (Kaeser et al., 2010), the vertical dashed line points to the onset of a first post-lesion plateau, reflecting most likely the "spontaneous" functional recovery level. The horizontal dashed line shows an upper limit of score given by the median value at first plateau plus twice the difference between the median value and the highest value at plateau. In two monkeys (Mk-VA and Mk-MO), later during the post-lesion the score reached this upper limit (green arrow), interpreted as a second plateau or rebound of functional recovery, possibly reflecting the effect of the anti-Nogo-A antibody treatment. The same plots presented earlier for the five control monkeys (Kaeser et al., 2011) showed that none of the control monkeys exhibited such a rebound of functional recovery)

B: Behavioral data for the five control monkeys, showing the manual dexterity performance (score) in the Brinkman board task, as a function of time corresponding to daily sessions pre-lesion (negative days) and post-lesion (positive days)

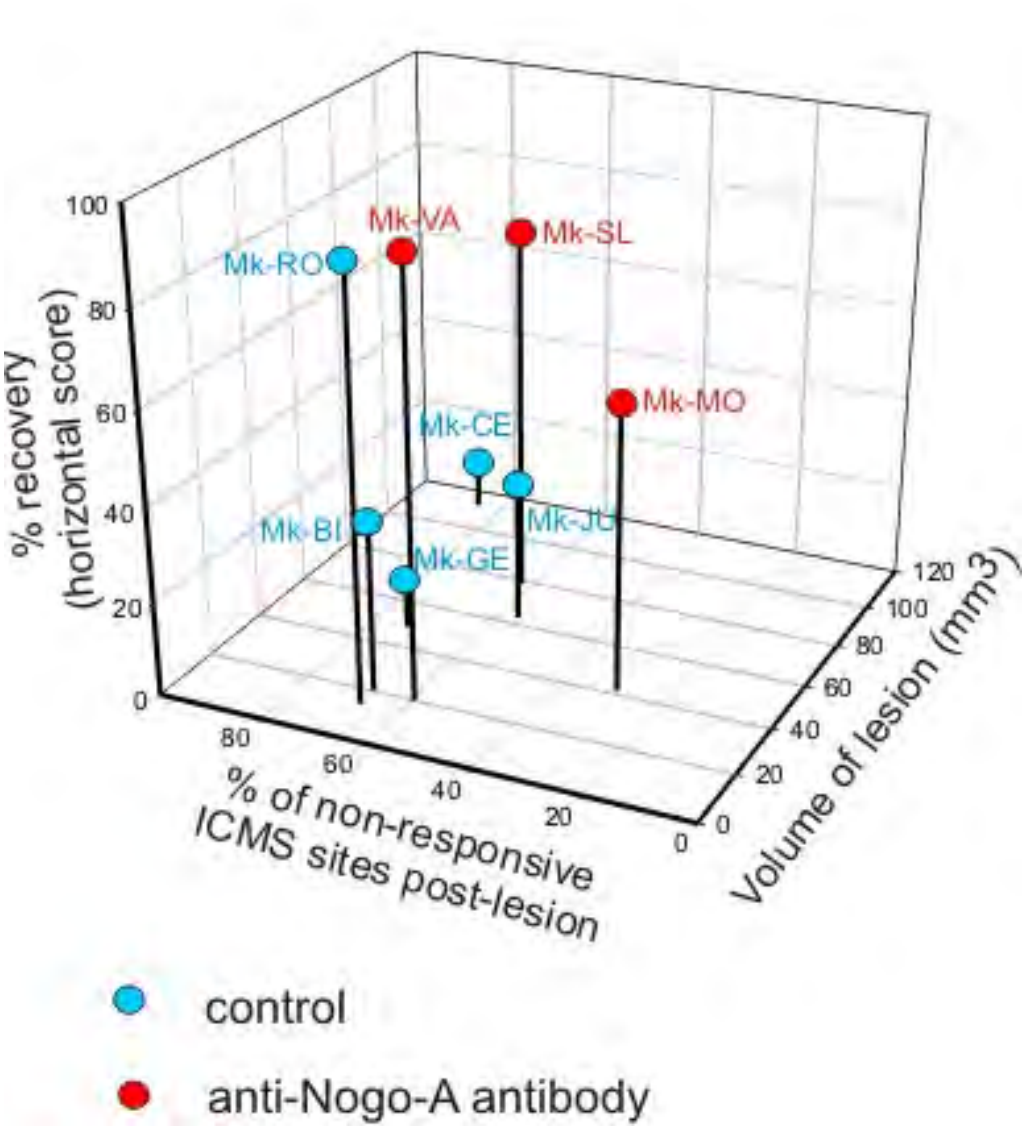


<u>Figure 9</u> : Comparison in the form of box and whiskers plots of pre-lesion ("pre") and post-lesion (post 1) scores in the five control monkeys (top panels) and in the three anti-Nogo-A antibody treated monkeys, separately for the vertical slots (left) and the horizontal slots (right). In the two monkeys with a rebound of functional recovery (second plateau=Post 2), the middle box is for the first post-lesion plateau (Post 1) and the rightmost box is for the second post-lesion plateau (Post 2). The percent values in red are for the enhancement of functional recovery obtained by reaching the second plateau as compared to the first post-lesion plateau. In the statistical comparison between pre-lesion median value and post-lesion median values (first or second plateaux), the three stars is for p<0.001 whereas "n.s." is for not significant (p>0.05).



<u>Figure 10</u>: Scatter plots of functional recovery (in percent) as a function of the volume of the lesion in the motor cortex (in mm^3), for the total score (top panel), the vertical slots (middle panel) and the horizontal slots (bottom panel). Control monkeys are represented by blue symbols whereas red circles are for the anti-Nogo-A antibody treated monkeys. The regression line was calculated for all data points, reflecting the general trend towards a decrease of functional recovery for increasing lesion sizes, as expected.

To address the question whether the functional recovery may also be correlated to the change of motor maps in M1 (in and around the lesioned territory), the three parameters percent of functional recovery for horizontal slots, volume of lesion and the percentage of non-responsive ICMS sites post-lesion were plotted in the form of a 3D plot (Fig. 10). It appears that the two subgroups, differing to some extent with respect to the percentage of functional recovery (except the control monkey Mk-RO with the smallest lesion), superimpose to a large extent as far as the percentage of non-responsive ICMS sites is concerned. In other words, the number of ICMS sites in M1 still eliciting forelimb movements is a poor predictor of the functional recovery for the horizontal slots, which is a more sensitive parameter than the vertical slots.



<u>Figure 11</u>: 3D scatter plot showing the relationship between the degree of functional recovery, the volume of the lesion and the percent of non-responsive of ICMS sites.

Discussion

The results of the present study can be summarized as follows: 1) after permanent chemical lesion of the hand area in M1 in the adult macaque monkey, the lesion territory becomes mostly non micro-excitable several months post-lesion, although some functional recovery takes place (spontaneous or promoted by a treatment); 2) some sites within the lesioned territory remains excitable, but without relationship with the degree of functional recovery; 3) around the lesion in M1, there was no evidence for a reallocation of proximal territories into distal territories to replace, at least in part, the original hand representation affected by the lesion; 4) compared to pre-lesion, there was post-lesion an increase of thresholds at which ICMS elicited movements of the forelimb muscle territories, but again without relationship to the degree of functional recovery; 5) there is preliminary evidence for an enhancement of functional recovery promoted by the anti-Nogo-A antibody treatment, as reflected by the retrieval score for the horizontal slots in the modified Brinkman board task, extending to the non-human primates previous data in rodents (Papadopoulos et al., 2002; Emerick et al., 2003; Emerick and Kartje, 2004; Seymour et al., 2005; Tsai et al., 2007, 2011; Cheatwood et al., 2008).

Although, as expected the lesioned hand territory in M1 became mostly non-responsive to ICMS, there were some sites where ICMS applied post-lesion elicited movements of the contralateral forelimb (Fig. 1-3), though at a higher intensity for some of them (Figs. 2 and 3). Do they correspond to preserved cortical sites, spared by an insufficient spread of ibotenic acid? This is most likely not the case, as the responsive sites post-lesion do not correspond to zones where the spots selected for infusion of ibotenic acid were more distant from each others than in zones which became completely non-responsive (see e.g. Mk-RO and Mk-MO). In other words, a poor of spread of ibotenic acid cannot easily explain the persistence of still micro-excitable sites post-lesion in the cortical area delineated pre-lesion as hand representation. Ibotenic acid infusion does not generate a cavity in the cerebral cortex (Peuser et al., 2011), in contrast to a surgical lesion, suggesting that during the period of recovery post-lesion, some connections (most likely indirect) with the spinal cord may be reconstructed, whose mechanism is unknown. One may speculate that few cortico-cortical connections may have been restored.

In absence of sustained rehabilitative training protocol in the present study, that would complement the short test sessions, there was no dramatic change of motor maps at the periphery of the lesion in the adult monkey (e.g. proximal territory reverted in finger sites), in

line with the previous results of Nudo and Milliken (1996). This situation contrasts with a lesion of M1 performed neonatally in infant monkeys, in which later at adult stage the functional recovery was associated to a re-arrangement of the somatotopic representation, with the emergence of a hand territory at a location normally occupied by proximal territories (Rouiller et al., 1998). There is a contrast also with the data obtained by Nudo et al. (1996), reporting a significant re-arrangement of the motor around the lesion, but this change was triggered by a sustained rehabilitative training protocol, not conducted in the present study. The present data thus confirms the notion that training is necessary to trigger substantial cortical map reorganization, representing most likely a substrate for an enhanced functional recovery (see Dancause and Nudo for a recent review, 2011). The absence in the present study of cortical map reorganization related to the functional recovery is consistent with previous observations on two control monkeys subjected to M1 lesion that the territory immediately adjacent to the lesion is little, if not, involved in the recovery, in contrast to the ipsilesional premotor cortex which plays a significant role (Liu and Rouiller, 1999). As outlined above, a contribution of the perilesional territory in M1 may appear in case of very small lesion of the hand area (Glees and Cole, 1952) or when the lesion is followed by intense rehabilitative therapy (Nudo et al., 1996; Dancause and Nudo, 2011).

Based on the retrieval score for the horizontal slots in the Brinkman board task, the present study provides preliminary evidence for an enhancement of functional recovery promoted by anti-Nogo-A antibody treatment (Fig. 9, bottom panel). Besides the trend for a better functional recovery in anti-Nogo-A antibody treated monkeys, on the qualitative point of view, two (Mk-MO, Mk-VA) of the three anti-Nogo-A antibody treated monkeys exhibited a rebound in the post-lesion plateau. The third treated monkey (Mk-SL) did not show such a rebound, but the functional recovery was surprisingly high after such a large lesion (Fig. 10). The rebound in the post-lesion plateau (Mk-VA and Mk-MO) is similar to the rebound observed in two monkeys which were transplanted with autologous adult progenitor cells (Kaeser et al., 2011). Interestingly, in sharp contrast, out of five control monkeys subjected to M1 lesion but without any treatment (neither anti-Nogo-A antibody nor progenitor cells transplantation) none exhibited a rebound of functional recovery in the post-lesion plateau (Kaeser et al., 2011). Both treatments (in 4 out 5 monkeys) thus provide a secondary enhancement of functional recovery, added to the level presumably reached by spontaneous recovery. As far as the effect of the anti-Nogo-A antibody treatment is concerned, the present preliminary data based on 3 treated monkeys compared to five control monkeys need to be extended to a larger population of monkeys subjected to a lesion of M1.

On the behavioral point of view, the present study emphasizes the pertinence of horizontal slots in the Brinkman board task. It is clearly more challenging than the vertical slots and it thus represents a more sensitive test, better suited to reflect a benefit of a therapy in our animal model, based on a compromise between a large enough lesion to detect a deficit sufficiently prominent to be compensated better after a treatment and a lesion not too large to avoid a poor condition for the monkeys due to an ethically not acceptable handicap. The sequence of movements to retrieve the pellets from the vertical slots is less difficult than for the pellets in the horizontal slots. For the vertical slots, the monkey has to use the precision grip to grasp the pellet, with a posture of the wrist in its natural orientation. For the horizontal slots, the monkey has to perform a half pronation of wrist, followed by the precision grip and a flexion. The behavioral data show that the lesioned monkeys have serious difficulty to execute this flexion. Thereby, the initial retrieval score of the two orientations is different and so is the functional recovery after the lesion of M1.

Following lesion of M1, and in absence of treatment, there is evidence based on reversible inactivation experiments that the “spontaneous” functional recovery depends, at least in part, on the ipsilesional premotor cortex, spared by the injury (Liu and Rouiller, 1999). A possible anatomical support for such a vicarious contribution of PM after M1 lesion is the enhancement of the projection of PM to the post-central gyrus (Dancause et al., 2005). Along the same line, there is also anatomical and functional evidence that, after a lesion including M1 and the lateral PM, the ipsilesional SMA contributes to the incomplete functional recovery (McNeal et al., 2010). In the present study, it is likely that the functional recovery observed in the five control monkeys subjected to M1 lesion results from a substitution to some extent of M1 by non-primary motor cortical areas, especially the ipsilesional PM. Furthermore, the present ICMS data largely exclude a role played by more proximal territories in M1 spared by the lesion, in line with reversible inactivation experiments conducted in two of the five control monkeys (Liu and Rouiller, 1999). The mechanisms of the vicarious contribution of PM and/or SMA may include sprouting of the corticospinal projections originating from these non-primary areas, as demonstrated for SMA after lesion of M1 and the lateral PM (McNeal et al., 2010). Through its projection to cervical and thoracic segments of the spinal cord (Rouiller et al., 1996; Dum and Striock, 1996), SMA is in position to take over some direct influence of M1 on hand motoneurons, though to a limited extent as its influence on distal motoneurons in the normal function is clearly less prominent than corticospinal inputs arising from M1 (Maier et al., 2002; Boudrias et al., 2006,

2010a). For PM, its corticospinal projection exerts effects on distal motoneurons, but also clearly lower than those originating from M1 (Boudrias et al., 2010b), reflecting mainly an indirect linkage with the motoneurons. In particular, PMv was shown to exert an influence mostly via its corticocortical projection to M1 (Cerri et al., 2003; Shimazu et al., 2004; Schmidlin et al., 2008), although some descending influence via intraspinal circuits in the spinal cord cannot be excluded (Sasaki et al., 2004; Takei and Seki, 2010; Alstermark et al., 2011). Contributions of non-primary motor cortical areas to the functional recovery after lesion of M1, irrespective of whether they are direct or indirect, can be interpreted as an unmasking of pre-existent, but mostly silent, projections and/or axonal sprouting forming new connections (see Dancause and Nudo, 2011 for a recent review). It is likely that the anti-Nogo-A antibody treatment may have enhanced some axonal sprouting, especially involving the ipsilesional PM, as observed for the callosal projections from the intact hemisphere terminating in the ipsilesional PM (see chapter 4 of the present thesis). Whether other connections of PM (or of other cortical areas, such as SMA for instance), such as their corticospinal projection or corticorubral projection have also been promoted by the anti-Nogo-A antibody treatment remains an open question at that step. As the anti-Nogo-A antibody was delivered not only around the lesion at cortical level, but also at cervical level, an enhancement of re-organization of intrapsinal circuits may also have promoted functional recovery.

In conclusion, the present study shows that the re-arrangement of motor maps in and around the lesioned territory in M1 is variable across monkeys, but without relationship to the degree of functional recovery of manual dexterity for the contralesional hand. This conclusion holds for experimental conditions in which there was no intensive rehabilitative training during the weeks post-lesion. The situation is likely to be different in case intensive rehabilitative training takes place (Nudo and Milliken, 1996; Nudo et al., 1996). Nevertheless, in the absence of intensive rehabilitative training, there is evidence that some functional restitution of manual dexterity after lesion of the hand representation in M1 in the adult macaque depends to a large extent on the vicarious role played by non primary motor areas (premotor cortex, SMA) (Liu and Rouiller, 1999; Mc Neal et al., 2010). A significant rehabilitative therapy protocol needs to be implemented in order to favor the re-arrangement of the motor map in the immediate vicinity of the lesion (and maybe also enhance the contribution of non-primary motor areas), although care should be taken to adopt the most adequate timing in the interventions. In addition, therapies such neutralization of nerve growth inhibitors (present study) or cell therapy (Kaeser et al., 2011) may further boost the

capacity of functional restitution, although a larger pool of monkeys is needed to better decipher the precise mechanisms of such enhancements.

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3.3 Chapitre 3:

Effects of reversible inactivation of the primary motor cortex (M1) or of the premotor cortex (PM) on the recovered manual dexterity after unilateral lesion of M1 in adult macaque monkeys, in absence or presence of anti-Nogo-A antibody treatment.

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Introduction

Several previous studies have shown that a unilateral lesion of the hand representation in the primary motor cortex (M1) in adult monkeys led to a loss of manual dexterity with the contralesional hand, such as the precision grip defined as the opposition of the thumb and index finger to manipulate small objects (e.g. Denny-Brown and Botterell, 1947; Fulton, 1949; Travis, 1955; Liu and Rouiller, 1999). A similar deficit has been observed in adult macaque monkeys after reversible inactivation of the hand representation in M1 (Matsumura et al., 1991; Kermadi et al., 1997; Rouiller et al., 1997). M1 plays a major role in the control and execution of dexterous hand movements, as demonstrated by lesional studies, either permanent or transient (e.g. Murray et al., 1991; Hoffman and Strick, 1995). M1 is connected to the premotor cortex (PM), especially via a strong reciprocal projection between the ventral PM (PMv) and M1 (Matsumura and Kubota, 1979; Muakkassa and Strick, 1979; Matelli et al., 1986; Dum and Strick, 2002; Dancause et al., 2005). The dorsal PM (PMd) is more involved in the control of movements with more proximal and axial muscles (Humphrey, 1979). A lesion affecting PM results in a strong deficit in the execution of conditional motor tasks (Passingham, 1988), although other studies suggest that PMd plays a more prominent role in such deficits than PMv (Petrides, 1985; Passingham, 1987). In a pilot study conducted on two adult macaques monkeys subjected to a unilateral lesion of the hand representation in M1, it was shown that the incomplete functional recovery of manual dexterity depends on the ipsilesional area PM, based on reversible inactivation experiments (Liu and Rouiller, 1999).

In the present part of the thesis, the goal was to extend these preliminary data of Liu and Rouiller (1999) to additional monkeys subjected to a unilateral lesion of M1, to address the following issues: i) confirm that PM contributes to the functional recovery of manual dexterity after lesion of the ipsilesional M1; ii) investigate whether the lesioned territory in M1 and its surrounding zone plays also a role in the functional recovery of manual dexterity; ii) what is the respective contribution of PMd versus PMv to the functional recovery of manual dexterity after lesion of the ipsilesional M1?; iv) does the application of the anti-Nogo-A antibody treatment influence the mechanism of functional recovery from M1 lesion, as assessed by reversible inactivation experiments. To this aim, seven monkeys subjected to a unilateral permanent lesion of M1 were subjected to reversible inactivation experiments after they reached their post-lesion behavioural plateau, using infusions of muscimol (an agonist of GABA), restricted in a given daily session to M1, PMd or PMv. The effect of transiently inactivating a given motor cortical area (M1, PMd or PMv) is assessed using the manual

dexterity task of the modified Brinkman board (see chapter 3.1 of the thesis for instance, as well as materials and methods of the present thesis manuscript).

The approach of reversibly inactivating a given motor cortical area was already used in the laboratory also in intact animals, without preliminary permanent lesion of M1 as in the present thesis work. In intact monkeys, a reversible inactivation of M1 obtained by infusion of lidocaine (volume ranging between 8 – 18 μl) led to a dramatic decrease of the score in the modified Brinkman board task (Rouiller et al., 1997). In contrast, a reversible inactivation of the supplementary motor area (SMA) with an intact M1 did not affect significantly the score in the modified Brinkman board task (Rouiller et al., 1997). In later reversible inactivation studies, lidocaine was replaced by muscimol. In the modified Brinkman board task, a reversible inactivation of M1 obtained by infusion of muscimol resulted in a highly significant decrease of the score for the contralesional hand (Kermadi et al., 1997). Confirming the lidocaine data, a reversible inactivation of the SMA unilaterally with muscimol did not affect the score of the contralesional hand in the modified Brinkman board (Kermadi et al., 1997). In the latter study, the main goal of reversible inactivation experiments was to test the role played by various motor cortical areas (M1, SMA or PMd) in the control of a bimanual task (the so-called "reach and grasp drawer" task) in intact monkeys. Relevant for the present chapter of the thesis is the observation that a reversible unilateral inactivation of PMd with infusion of muscimol (volume ranging from 12 to 15 μl) did not influence the "reach and grasp drawer" task (Kermadi et al., 1997), which also comprises the prehension of a pellet in a small well, using the precision grip as in the modified Brinkman board task. In the present chapter of the thesis, reversible inactivation of PMd or PMv will also be performed, but here not in intact monkeys, but in animals previously subjected to a permanent lesion of the hand representation in M1.

Materials and Methods

The experiments were conducted on seven adult male macaque monkeys (*Maccaca fascicularis)*, all subjected to a unilateral lesion of the hand representation in M1 (see chapters 3.1 and 3.2 of the present thesis). Immediately after the lesion, three monkeys were treated during four weeks with the anti-Nogo-A antibody (treated group), whereas four monkeys were not treated (control group; see Table 1). All monkeys were tested for their manual dexterity, based on several behavioural tests (see Schmidlin et al., 2011: addendum of the present thesis), more specifically using the modified Brinkman board task. Several months after the lesion, when the animals have reached a post-lesion behavioural plateau of functional

recovery, experiments of intracortical microstimulation (ICMS) were conducted in order to establish the post-lesion somatotopic map in M1 (see chapter 3.2). These ICMS maps were used as a reference to guide the sites of infusion of muscimol to reversibly inactivate M1, PMd or PMv (in some cases, PMd and PMv altogether). All surgical and electrophysiological procedures have been described in the chapter 2 (material and methods) of the present thesis manuscript.

The reversible inactivation experiments were conducted in the form of daily behavioural sessions (3 in general), taking place usually at one week interval. In a given daily session, one specific motor area was inactivated (Table 1). The daily session began with the execution by the monkey of the modified Brinkman board task, successively with the contralesional hand and the ipsilesional hand, as described in chapter 3.1. Based on the post-lesion ICMS map established a few weeks before, several sites where muscimol is injected were selected. For each monkey and for each motor cortical area inactivated (M1 or PMd or PMv), Table 1 indicates the number of sites where muscimol (GABA agonist) was infused (Sigma, 1 µg in 1 µl of saline 0.9% solution), using a Hamilton syringe (10 µl) inserted perpendicularly into the brain at a selected ICMS site. The volume injected at each site, the number of sites, the distance between sites and the position of the sites of infusion were determined based on the study conducted by Martin (1991), on the rat brain. Along an individual penetration with the syringe, muscimol was delivered at usually 1 to 2 depths, distant from each other by 2-3 mm (Kermadi et al., 1997, Schmidlin et al., 2004). After infusion of muscimol along several penetrations, the monkey remained quiet in its primate chair for about 30 minutes. Then, the modified Brinkman board task was repeated for each hand at three time points: 30, 45 and 60 minutes after offset of muscimol injections. To assess the impact of the reversible inactivation of a given motor cortical area, two parameters were measured, as previously described in chapter 3.1: i) the **score**, given by the number of pellets retrieved during the first 30 seconds of the test, separately for the vertical and horizontal slots; a good manual performance is indicated by a high score; ii) the **contact time**, representing the time of contact in seconds between the fingers and the pellet before retrieval from the slot (as defined in Figure 1); a good manual performance is indicated by a short contact time. The contact time was measured for the five first vertical slots and the five first horizontal slots visited by each hand, as previously reported (Kaeser et al., 2010, 2011). The contact time was determined by visualization of the video sequences frame by frame, corresponding to a time resolution of 40 ms (25 images per second). The score is a unique observation at each time point (30, 45 and 60 minutes) and therefore it cannot be compared statistically with the score

of reference obtained at a single time point before infusion of muscimol. In contrast, as the contact time is assessed from five values for each slot orientation, statistical comparisons between the reference (before infusion of muscimol on the same day) and at each time point (30, 45 and 60 minutes) was undertaken using the non parametric Mann and Whitney test. The results of the statistical comparisons are shown on Figures 2 to 8, with the corresponding p value or “n.s.” (for statistically non significant differences).

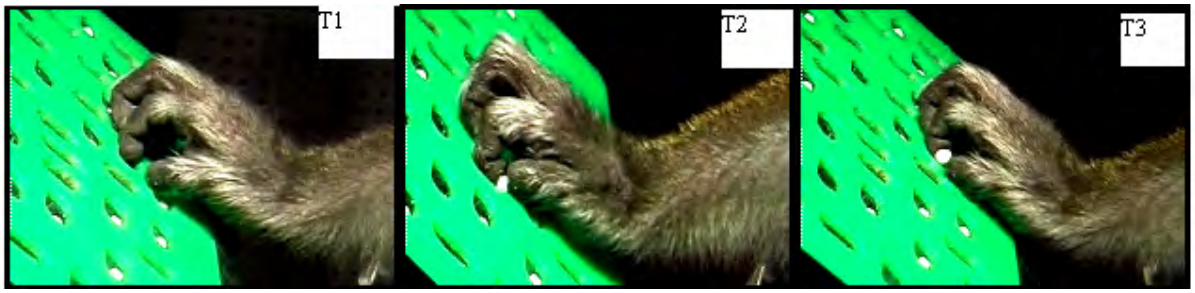


Figure 1: Illustration of the parameter «Contact time» to analyse the manual performance in the modified Brinkman board task. The contact time is defined for each slot of the board visited by the monkey’s hand as the time interval between the moment of insertion of the first finger (usually the index finger) in the slot (T1) and the moment at which the pellet is grasped out of the slot (T3). The contact time is thus given by T3 – T1 in seconds.

Table 1: Survey of the monkeys involved in the reversible inactivation experiments, with indication of the number of sites infused with muscimol for each of the seven monkeys and for each daily session (corresponding to the inactivation of a given motor cortical area).

		Control monkeys				**Anti-Nogo-A antibody treated monkeys**		
	Motor cortical area inactivated	**Mk-BI**	**Mk-CE***	**Mk-RO**	**Mk-JU***	**Mk-MO**	**Mk-VA**	**Mk-SL &**
Number of infusion sites with muscimol	**M1**	12	9	10	6	20	11	11
	PMd	6	8	7	-	7	5	10
	PMv	5	8	6	-	4	4	-
	PMv/PMd	-	-	-	16	-	-	-
Total volume of muscimol injected (µl)	**M1**	12	9	10	6	20	11	11
	PMd	7	8	7	-	7	7.5	10
	PMv	5.4	8	6	-	4.4	4.4	-
	PMv/PMd	-	-	-	16	-	-	-

* Two pilot monkeys taken from Liu and Rouiller, 1999

& only the contralesional hand was tested in Mk-SL

See Table 1 in the first chapter of the present thesis for general parameters of the seven monkeys included in the reversible inactivation experiments.

The infusion of muscimol at each site took roughly 3 minutes (1 minute to inject and then 2 minutes of pause before moving to the next site). As a result, the duration of the infusion procedure ranged between 20 and 40 minutes.

In Mk-SL, a third inactivation session took place in which muscimol was infused in M1 targeting a few ICMS sites representing digits in the transition zone towards PMd (referred to as “M1 penumbra” session: see Fig. 8). A total volume of 9 µl muscimol was infused at 9 sites along 9 syringe penetrations.

Results

A reversible inactivation session represents a clearly more challenging experiment as compared to a standard behavioural session performed by the monkeys every day during the whole experimental protocol. For that reason, reversible inactivation experiments were scheduled near the end of the experimental protocol, after the monkey reached a stable post-lesion behavioural plateau and after the post-lesion ICMS mapping. The reversible inactivation of a given motor cortical area was performed via multiple syringe penetrations to deliver muscimol, possibly creating cortical tissue damage. To restrict such tissue damage to preserve the subsequent neuroanatomical investigations, only a single reversible inactivation experiment was conducted for each of the 3 motor cortical areas accessible via the chronic chamber, namely M1, PMd or PMv (Table 1). In one animal (Mk-JU), only two reversible inactivation sessions were conducted, one to inactivate M1 and the second aimed at PMd and PMv taken altogether (Table 1). In one monkey (Mk-SL), in contrast to the other animals, only the contralesional hand was tested, due to a decrease of motivation of the animal at that step of the entire protocol, prompting for a reduction of the duration of the session by skipping the ipsilesional hand assessment. The data derived from the seven monkeys subjected to reversible inactivation sessions (usually 3 in each animal) are shown in Figures 2 to 8, where the score and the contact time were plotted for comparison between the manual performance of reference before infusion of muscimol and the manual performance at usually 3 time points following muscimol infusion (30 min, 45 min and 60 min).

For each monkey, several positions on the brain surface, where muscimol is injected, were selected based on the post-lesion ICMS maps established when the post-lesion plateau was reached. The unfolded post-lesion ICMS maps used for such selection of infusion sites are displayed for each monkey at the bottom left of Figures 2 to 8. As reported in chapter 3.2 of the thesis, in some monkeys there was a preservation in M1 after the permanent lesion of a few hand territories (e.g. Mk-BI, Mk-RO, Mk-MO, Mk-VA and Mk-SL). Such specific zones were targeted for muscimol infusion. In other monkeys (Mk-CE, Mk-JU), although there was no hand representation left, in some ICMS penetrations hand effects were observed at higher intensities (not shown in the ICMS maps), where muscimol was infused. The locations selected for penetrations with the syringe were also consistent with zones delineated in the pre-lesion ICMS maps.

3.3.1.1 Score of manual performance

3.3.1.1.1 Effect on the ipsilesional hand

The ipsilesional hand is controlled mainly by the intact hemisphere. As a result, it was hypothesized that infusion of muscimol in the lesioned hemisphere (in M1, PMd or PMv) should not affect the manual performance expressed by the **score** for the ipsilesional hand (number of pellets retrieved in 30 seconds). This hypothesis was verified in the monkeys Mk-BI (Fig. 2), Mk-RO (Fig. 3), Mk-CE (Fig. 4), Mk-JU (Fig. 5) and Mk-MO (Fig. 6), where the score was largely unchanged as a result of reversible inactivation, except some variability inherent to the behavioural task itself, especially considering the exceptional duration of the session (about twice long than the standard behavioural sessions without pharmacological inactivation). As a consequence, the motivation of the animal was most likely less constant. In Mk-VA (Fig. 7), there was a trend towards a progressive decrease of score as a function of time post-infusion of muscimol for the 3 motor areas inactivated, but to a limited extent however. The ipsilesional hand was not tested in the seventh monkey (Mk-SL).

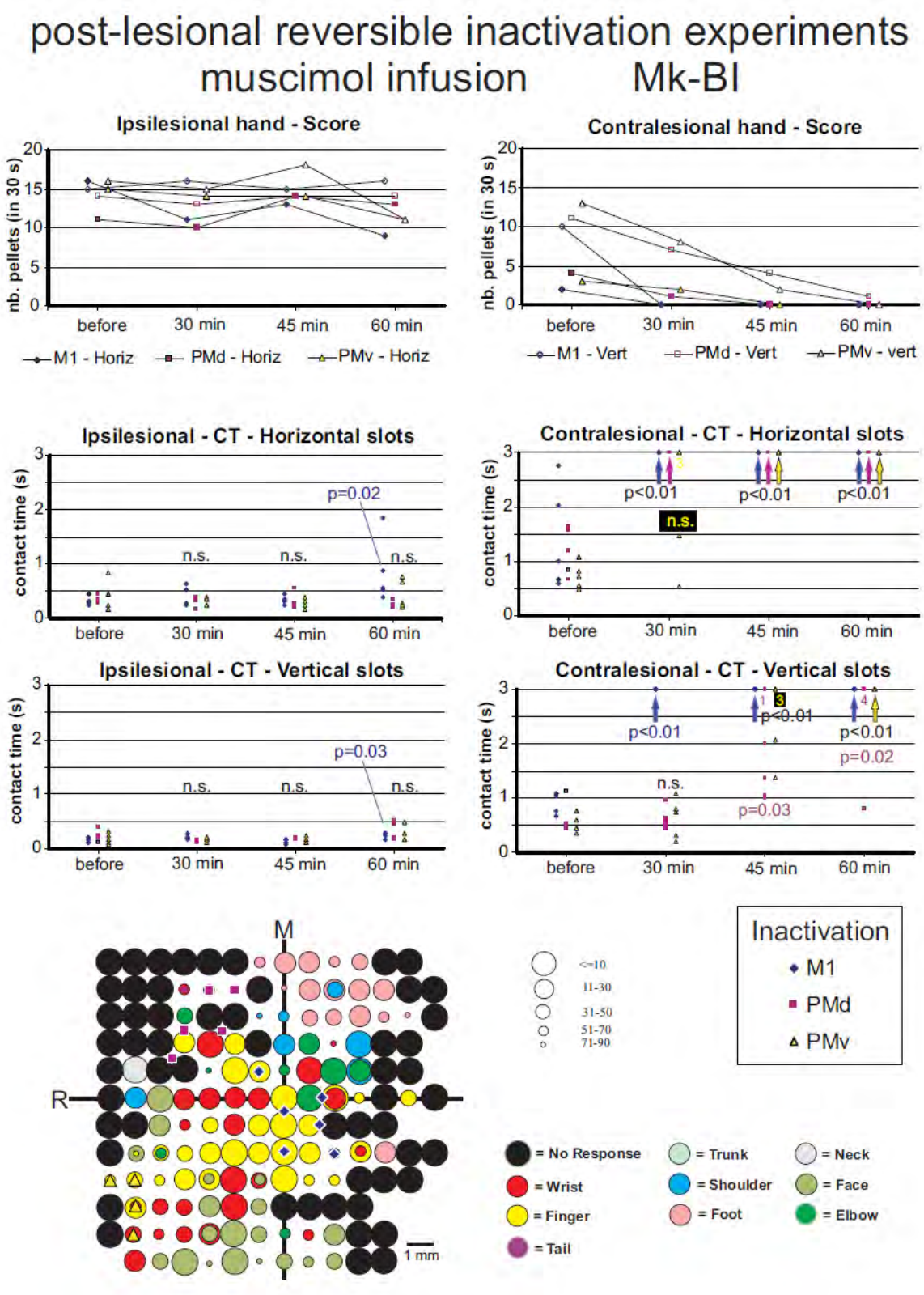
The next pages display Figures 2 to 8, showing the quantitative data of the reversible inactivation experiments conducted in the seven monkeys. See below the legend to Figure 2. The same conventions were applied to Figures 3 to 8.

Figure 2: The top two graphs are plots of the manual dexterity score (number of pellets retrieved in 30 seconds) as a function of time, namely the four repetitions of the modified Brinkman board task, performed with the ipsilesional hand (left) or the contralesional hand (right) in a given inactivation session (M1, PMd or PMv). The 4 time points are: before infusion of muscimol, 30 minutes after (infusion of muscimol), 45 minutes after and 60 minutes after. The scores are shown separately for the vertical (*Vert*) slots and for the horizontal (*Horiz*) slots, as well as for the 3 sessions in which M1, PMd or PMv were inactivated, respectively (6 different symbols, as coded below the 2 graphs). As the score is a unique observation at each time point, no statistical comparison could be conducted.

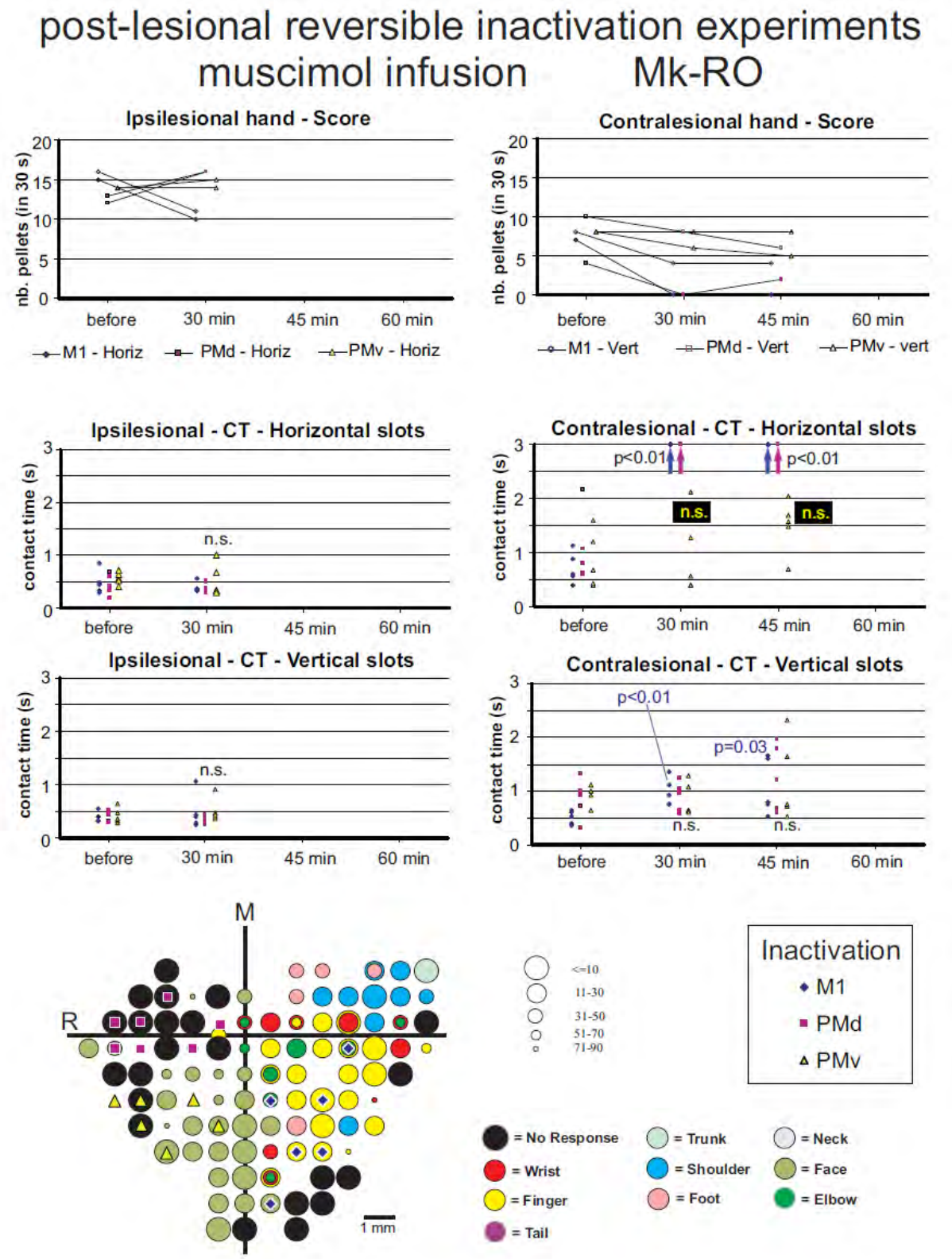
The four graphs in the middle are plots of the contact time (in seconds) measured from the five first horizontal slots (middle two graphs) and from the five first vertical slots (bottom two graphs) attempted by the monkey in the 3 sessions (inactivation of M1, PMd or PMv, as shown by the symbols in the bottom right inset). The data for the ipsilesional hand are in the left column, whereas those for the contralesional hand are in the right column. Data points saturated at 3 seconds are for contact times indeed equal or longer than 3 seconds or even infinitely long (in case the monkey was unable to grasp the pellet from the aimed slot). Vertical arrows below the data points at 3 seconds indicate that all five first attempts were unsuccessful (or took more than 3 seconds): in such a case, the difference with the performance of reference (before infusion of muscimol) was highly statistically significant ($p<0.01$; Mann and Whitney test). In some cases, numbers below the data points saturated at 3 seconds indicate how many observations were indeed equal or longer than 3 seconds. At each time point and for

each inactivation session (M1, PMd or PMv: see colour code in the bottom inset), there are 5 data points when the monkey performed the task successfully (in some cases, two data points may overlap). The contact time values at each of the three time points after infusion of the muscimol (30, 45 and 60 minutes) were statistically compared with those obtained before infusion of muscimol (manual performance of reference). Using the same colour code as the symbols, when the difference was statistically significant, the corresponding p value is indicated, whereas "n.s." means that the difference was not statistically significant. When a single p value or "n.s." is indicated, it means that it holds true for the three inactivation sessions (M1, PMd or PMv).

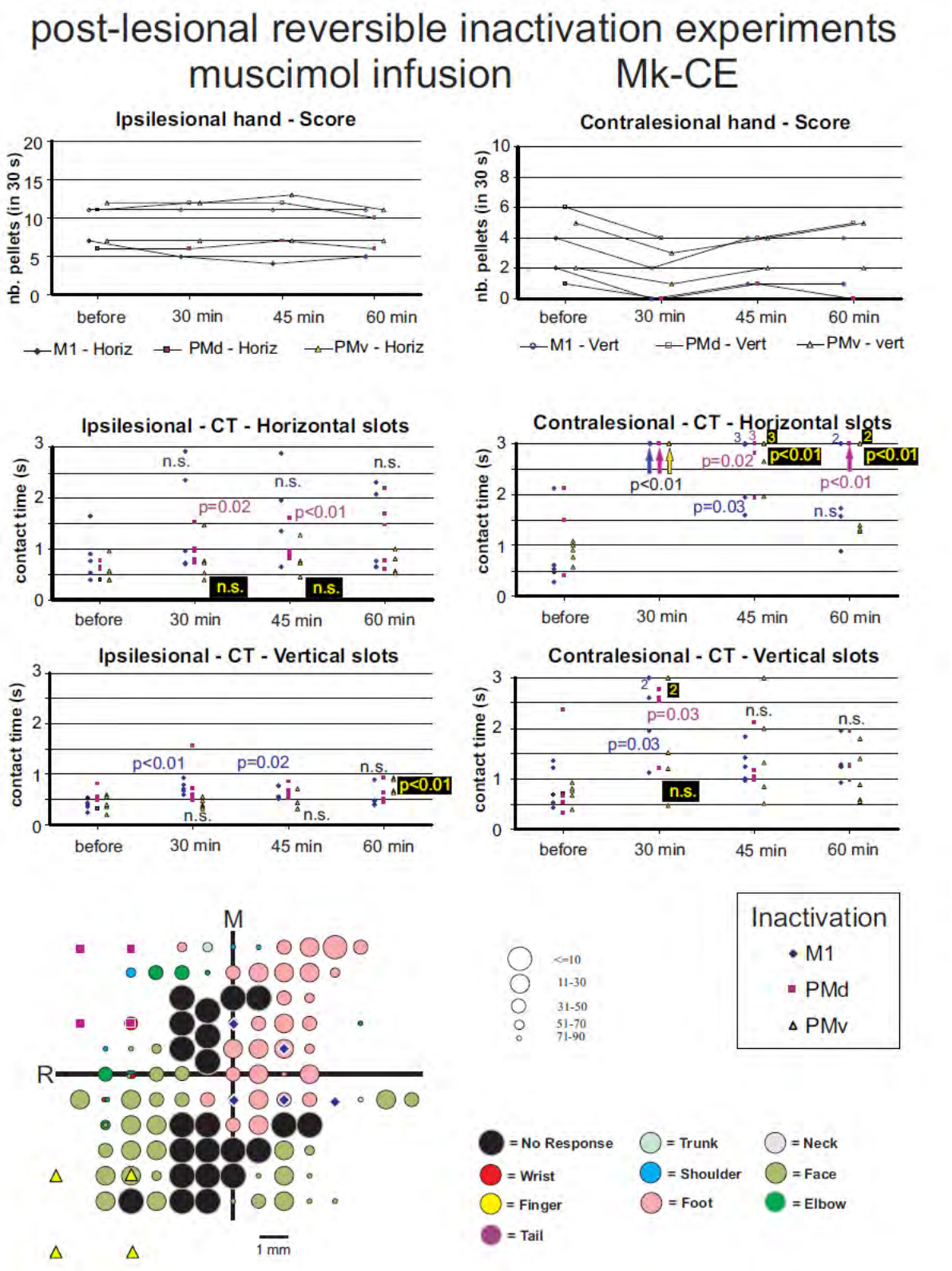
At the bottom left, the unfolded ICMS map established post-lesion is shown, as it represented the basis to identify positions where to perform penetrations with the syringe to infuse muscimol in M1, PMd or PMv (same symbols as in the inset on the right). Along the axes, "R" is for rostral and "M" is for medial. In all monkeys (except Mk-JU), the M1 permanent lesion was in the left hemisphere and therefore the sites of infusion of muscimol were ipsilesional in M1, PMd or PMv. For Mk-JU, in which the M1 lesion was in the right hemisphere as well as the infusion sites of muscimol, the ICMS map was flipped so that it appears as a left hemisphere map, for better comparison with the other monkeys. The circles represent the positions of electrodes penetrations for ICMS, with colour code to represent the body territory activated at the lowest threshold along the corresponding penetration. The threshold value is given by the size of the circle in microAmps (see on the right of the ICMS map). The corresponding unfolded maps post-lesion can be seen in chapter 3.2 of the thesis. The blue diamonds are for the site of infusion of muscimol in M1, the violet squares for the sites of infusion in PMd and the yellow triangles for the sites of infusion in PMv. The choice of the infusion sites was also based on a confrontation with the unfolded pre-lesion map, as shown for Mk-BI in chapter 3.2 of the thesis (Fig. 1). The diamonds, squares and triangles symbols show the position of the penetrations with the syringe. Note however that the number of infusion sites may be bigger, as in some individual penetrations muscimol was infused at more than one depth. See Table 1 for the total number of infusion sites and the total volume of muscimol injected.



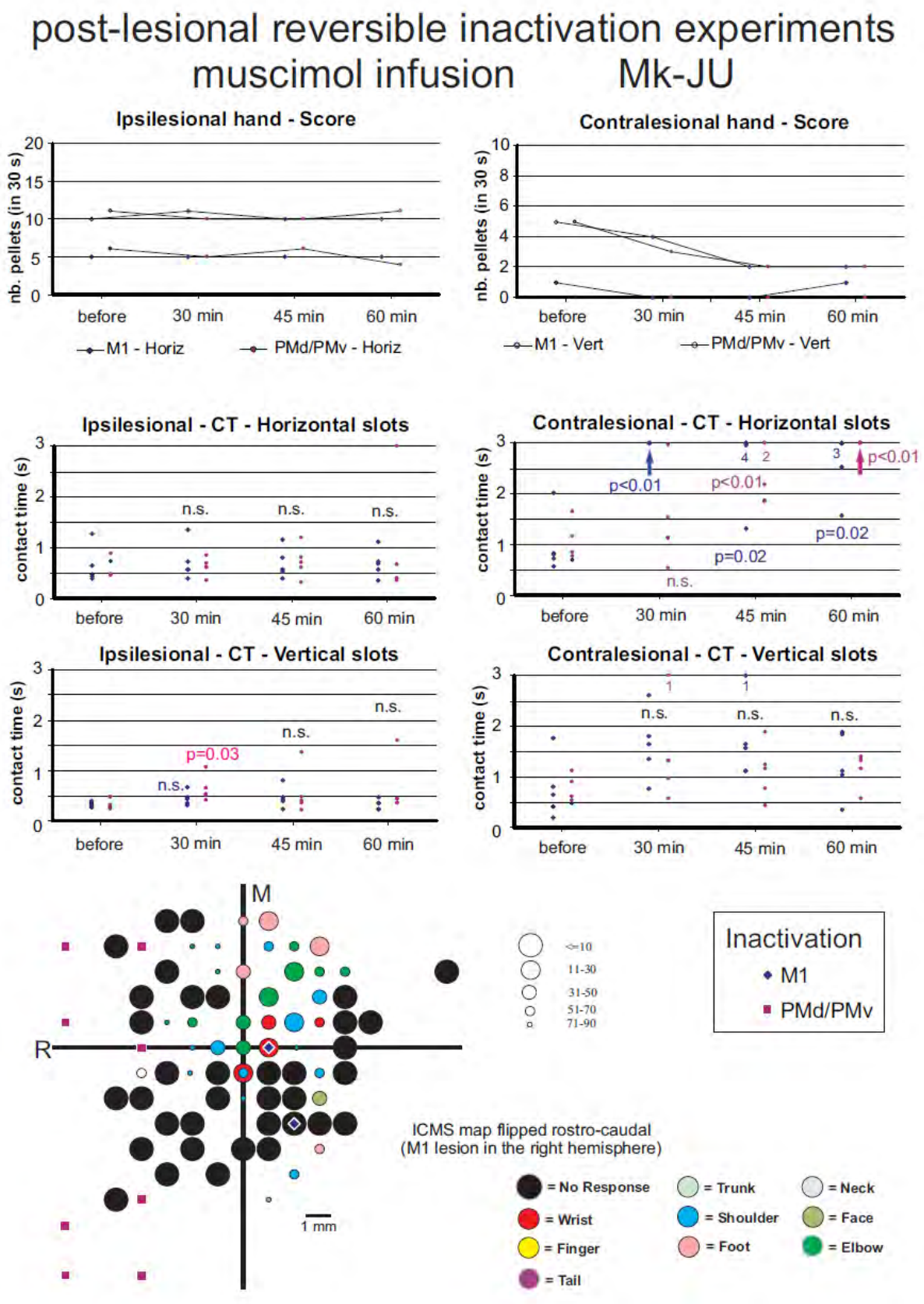
<u>Figure 2</u>: reversible inactivation data and post-lesion ICMS data for Mk-BI (see legend on previous page).



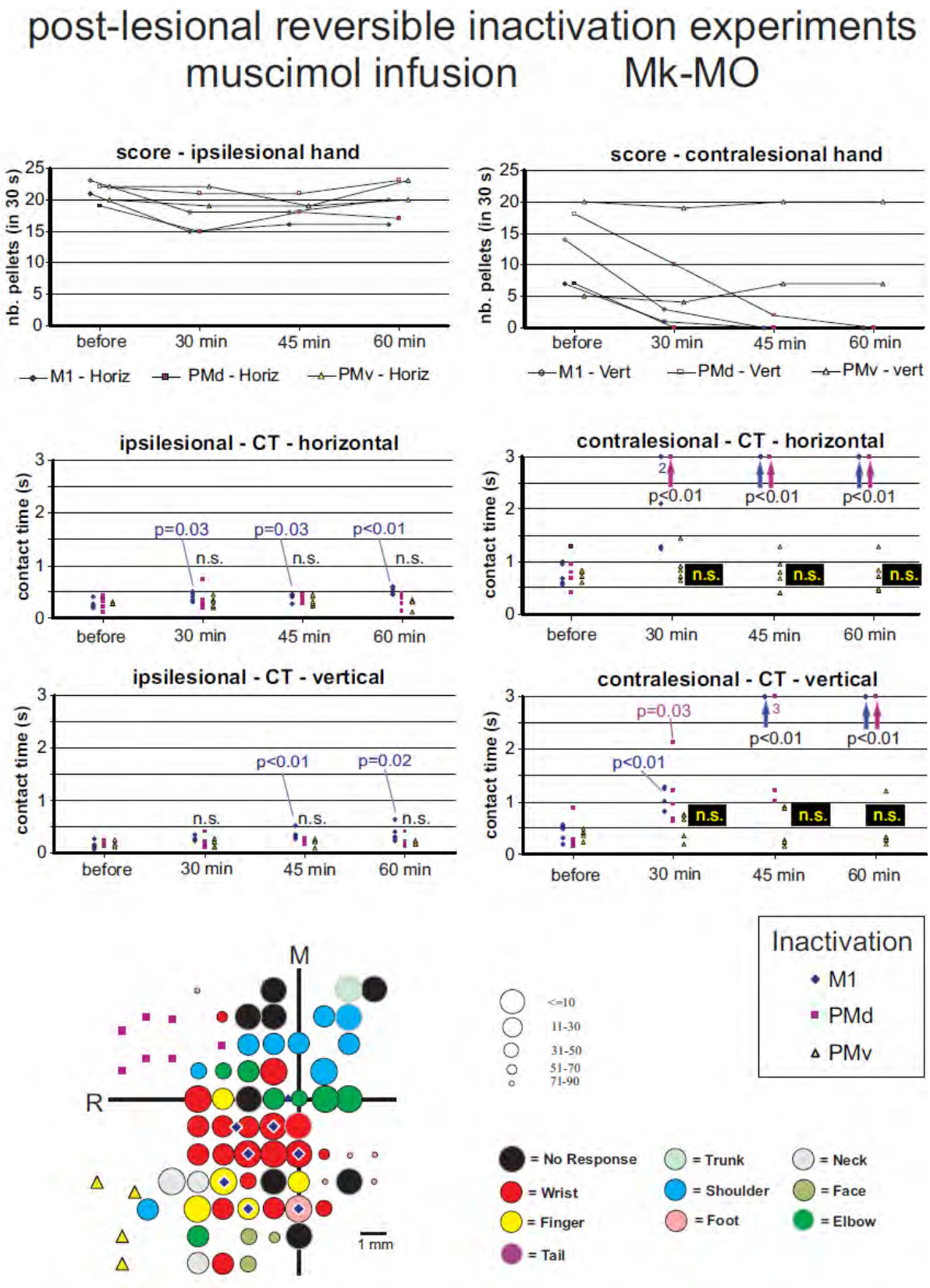
<u>Figure 3</u>: inactivation data and post-lesion ICMS data for Mk-RO (same conventions as in Figure 2).



<u>Figure 4</u>: inactivation data and post-lesion ICMS data for Mk-CE (same conventions as in Figure 2).



<u>Figure 5</u>: inactivation data and post-lesion ICMS data for Mk-JU (same conventions as in Figure 2).



<u>Figure 6</u>: inactivation data and post-lesion ICMS data for Mk-MO (same conventions as in Figure 2).

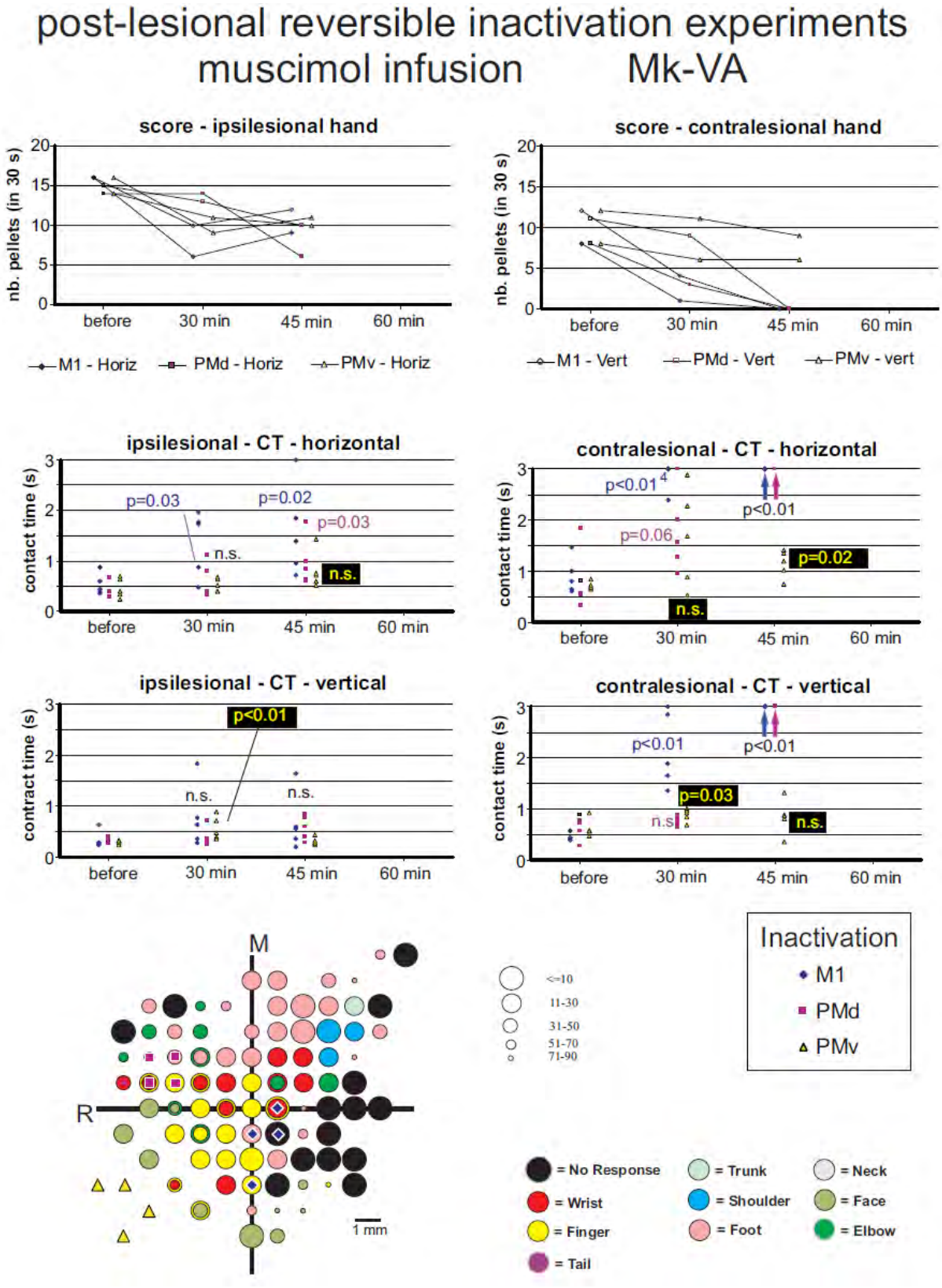
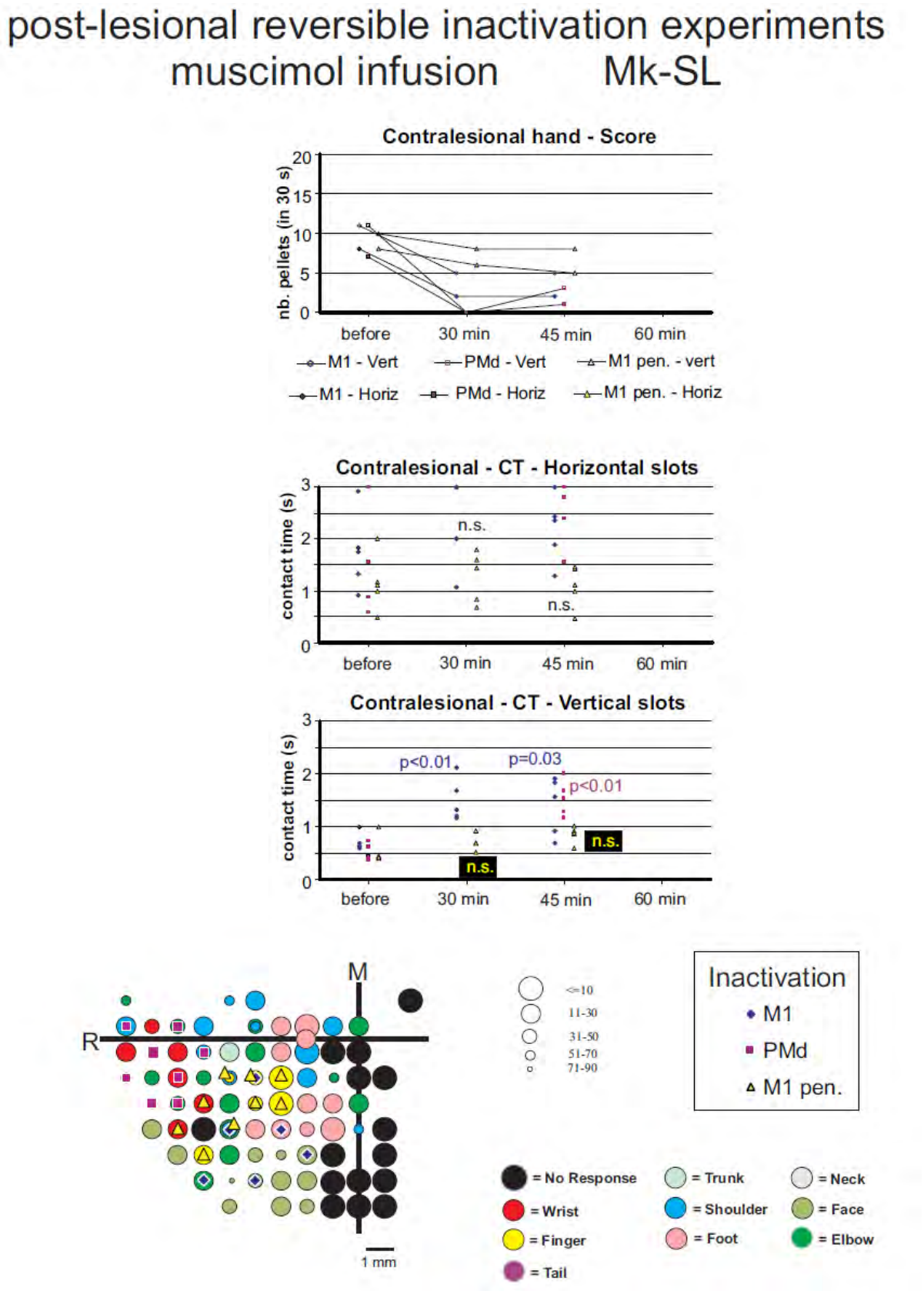


Figure 7: inactivation data and post-lesion ICMS data for Mk-VA (same conventions as in Figure 2).



<u>Figure 8</u>: inactivation data and post-lesion ICMS data for Mk-SL (same conventions as in Figure 2).

3.3.1.1.2 Effect on the contralesional hand

The hypothesis for the contralesional hand is that a transient inactivation of a given motor cortical area (M1, PMd or PMv) leads to a decrease of the recovered motor performance after permanent lesion of M1 expressed by the score if the corresponding motor cortical area is contributing to the functional recovery of manual dexterity. This hypothesis of a decrease of score was largely verified for the three motor cortical areas inactivated (M1, PMd or PMv) in Mk-BI (Fig. 2, top right panel), whereas in monkeys Mk-MO, Mk-VA and Mk-SL, the decrease of score was observed after inactivation of M1 or PMd, but not PMv (Figures 6, 7 and 8, top right panel). In Mk-RO (Fig. 3, top right panel), the effect was limited to the horizontal slots, after inactivation of M1 or PMd, but not PMv. Finally, in monkeys Mk-JU and Mk-CE subjected to the largest lesions, there was also a trend towards a decrease of score, but less obvious due to low scores of reference before infusion of muscimol (Figures 4 and 5).

3.3.1.2 Contact time

As mentioned above, a clear limitation of the score data is that a single observation was obtained at each time point, preventing a statistical analysis. In contrast, the contact time based on the grasping duration in five vertical and five horizontal slots is more meaningful as it allows a statistical evaluation, as described below.

3.3.1.2.1 Effect on the contact time for the ipsilesional hand

In line with the hypothesis that the ipsilesional hand is not affected by reversible inactivation of M1, PMd or PMv, in most cases the contact time was not different at various time points after infusion of muscimol, as compared to the contact time of reference before infusion (middle and bottom left column graphs in Figs. 2 – 8). In a few cases, there was a statistically significant difference, although a closer visual inspection of the plots shows that the clusters of data points are nevertheless not so remote from each others. The episodic differences found for the ipsilesional hand may rather reflect the constraint imposed by the reversible inactivation experiment imposing exceptionally long daily sessions, thus decreasing the motivation of the monkey at time points after the long process of muscimol infusion. Furthermore, the contact time values of reference obtained at the onset of the daily session before infusion of the muscimol are very short and homogeneous, which favours a statistically significant difference with somewhat more variable data points obtained after reversible inactivation (see for instance Mk- BI in Fig. 2 and Mk-MO in Fig. 6). In spite of these few

statistically significant differences the contact time data are generally consistent with the score data, indicating an absence (or limited) effect of reversible inactivation of M1, PMd or PMv on the contact time for the ipsilesional hand. Indeed, small volumes of muscimol infused (e.g. 4.4 μl in Mk-Mo and Mk-VA; see Table 1) may have yielded a statistically significant difference, although the post-infusion manual performance with the ipselesional hand was excellent (Figs. 6 and 7).

3.3.1.2.2 Effect on the contact time for the contralesional hand

In contrast to the modest effect on the ipsilesional hand, the reversible inactivation of M1, PMd or PMv had generally more dramatic (and higher levels of statistical significance) effects on the contralesional hand (Figs. 2 – 8, middle and bottom right column graphs). In Mk-BI (Fig. 2), in line with the score data, the contact time for the contralesional hand increased substantially (with high statistical significance) 45 and 60 minutes after reversible inactivation of all three motor areas (M1, PMd or PMv). The effect was already largely present after 30 minutes post infusion of muscimol. Also consistent with the score data, Mk-MO (Fig. 6) and Mk-VA (Fig. 7) exhibited a clear increase of contact time for the contralesional hand after inactivation of M1 and PMd, but less so after infusion of muscimol in PMv. In Mk-RO (Fig. 3), the contact time obtained for the horizontal slots also showed a strong effect on the contralesional hand of the inactivation of M1 and PMd; the effect was clearly less pronounced for the vertical slots and limited to inactivation of M1. These four monkeys clearly support the notion that ipsilesional motor cortical areas (mainly M1 and PMd) play a role in the incomplete functional recovery of manual dexterity with the contralesional hand after unilateral permanent lesion of M1.

The contact time data appear somewhat less straightforward to interpret in the other three monkeys. Unexpectedly, Mk-SL exhibited an effect of reversible inactivation of M1 or PMd on the contralesional hand for the vertical slots but not for the horizontal slots (Fig. 8). This observation may be explained, at least in part, by the relatively large difficulty for the animal to perform the grasping from the horizontal slot already before reversible inactivation. The results are opposite in Mk-CE (Fig. 4), in which the effect of the reversible inactivation was prominent (and generally significant) for the horizontal slots and much less for the vertical slots (except maybe 30 minutes after infusion of muscimol). In Mk-CE, the increase of contact time in the horizontal slots was found after reversible inactivation of all three motor cortical areas (M1, PMd or PMv), with the exception of M1 after 60 minutes (Fig. 4). In Mk-JU (Fig. 5), subjected to reversible inactivation of M1 or PMd/PMv taken together, there was

also an increase of contact time (statistically significant in most cases) for the horizontal slots, but not for the vertical ones.

In conclusion, in spite of some variability inherent to such challenging reversible inactivation experiments (long daily session for the monkeys including penetrations with the syringe in the cerebral cortex), the data are generally consistent with the notion that M1 comprising the lesioned territory and the perilesional area, as well as PMd are playing a role in the functional recovery of manual dexterity following unilateral lesion of the hand area in M1. PMv may also contribute, to a clearly lesser extent and less systematically however than PMd. The effects of muscimol inactivation of M1, PMd or PMv, as assessed by the contact time data, are summarized in the Table 2, together with a reminder of the percentages of recovery (post-lesion plateau compared to pre-lesion plateau) for the horizontal and vertical slots in each individual monkey. It appears that the inactivation experiments with muscimol infusion had a slightly stronger impact on the horizontal slots than on the vertical ones, in other words the corresponding areas may be a bit more involved in the functional recovery from the most difficult task (horizontal slots) than in the less difficult one (vertical slots). Moreover, the effects observed in the muscimol inactivation sessions do not appear to be different between the control group (spontaneous recovery) and the group of monkeys treated with the anti-Nogo-A antibody treated, suggesting that the corresponding cortical areas contribute to the functional recovery in a comparable manner irrespective of the treatment.

Table 2: Survey of the reversible inactivation data for the contralesional hand.

Contralesional hand only		**Control monkeys**				**Anti-Nogo-A antibody treated monkeys**		
	Motor cortical area inactivated	**Mk-BI**	**Mk-CE***	**Mk-RO**	**Mk-JU***	**Mk-MO**	**Mk-VA**	**Mk-SL**
Effect of muscimol infusion on horiz slots	**M1**	Yes	Yes	Yes	Yes	Yes	Yes	No
	PMd	Yes	Yes	Yes	-	Yes	Yes	No
	PMv	Yes	Yes	No	-	No	Y/N	-
	PMv/PMd	-	-	-	Yes	-	-	-
Effect of muscimol infusion on vert slots	**M1**	Yes	No	Yes	No	Yes	Yes	Yes
	PMd	Yes	No	No	-	Yes	Yes	Yes
	PMv	Yes	No	No	-	No	Y/N	-
	PMv/PMd	-	-	-	No	-	-	-
Volume M1 lesion (mm^3)		20.13	112.8	14	63.01	41.8	20	78.2
% recovery horiz slots		36 %	9 %	90 %	29 %	60 %	91 %	77 %
% recovery vert slots		94 %	59 %	100 %	46 %	84 %	87 %	77 %

The effect of muscimol (Yes or No) after injection in M1, PMd or PMv on the horizontal slots or on the vertical slots for the contralesional hand are based on the contact time data displayed in Figures 2-8, reflecting here the general tendencies derived from the time points 15, 30 and 45 minutes post-infusion. Y/N is for divergent observations at 2 time points (e.g. Mk-VA; see Fig. 7).

* Two pilot monkeys taken from Liu and Rouiller, 1999

See Table 1 in the first chapter of the present thesis for general parameters of the seven monkeys included in the reversible inactivation experiments

Discussion

In contrast to intact monkeys in which PMd was reversibly inactivated without effect on the manual dexterity for the contalesional hand (Kermadi et al., 1997), the present study demonstrates that reversible inactivation of PMd in monkeys subjected several months before to a permanent lesion of the hand representation in M1 indeed provokes a loss of the recovered manual dexterity, irrespective of whether the recovery is spontaneous or presumably enhanced with an anti-Nogo-A antibody treatment. In other words, PMd contributes to the incomplete functional recovery from M1 lesion, thus confirming the pilot data of Liu and Rouiller (1999), derived from two monkeys. In the latter report, only data derived from the monkeys Mk-JU and Mk-CE (both untreated) were reported. A major difference in the analysis was that in the report by Liu and Rouiller (1999), the data were restricted to the score (no contact time measurement) and the number of pellets was given only by a total value, without distinction between the vertical and horizontal slots (as in the present study). Furthermore, the score values in Liu and Rouiller (1999) were the number of pellets retrieved in 60 seconds whereas, in the present study, a period of 30 seconds was considered, justified by the observation that in some cases after the cortical lesion the monkeys did not work as long as 1 minute. As a consequence, a shorter period of 30 seconds was found more appropriate to perform a more reliable comparison pre- versus post-lesion. The distinction between vertical and horizontal slots is also of considerable importance, due to the different degree of difficulty to grasp the pellet depending on the slot orientation (see e.g. Freund et al., 2009; Kaeser et al., 2010).

As compared to the pilot data of Liu and Rouiller (1999), a further major advance of the present study was to introduce the analysis of the contact time, allowing statistical analysis. Furthermore, in spite of low post-lesional scores, the contact time yield pertinent data as long as the monkey performs the task for at least five vertical and five horizontal slots, which was usually the case (see Figs. 2-8). The contact time is also more pertinent to address specifically the issue of manual dexterity, namely the prehension of the pellets with the index finger and the thumb (precision grip). Indeed, the score comprises not only the precision grip phase, but also the transport of the pellet from the board to the mouth and the transport of the empty hand back to the board to aim towards the next slot. The transport phase of the hand involves more proximal muscles than the precision grip itself. As a consequence, to assess specifically the manual dexterity, the contact time appears to be a more valid parameter than the score, in addition to its statistical value.

The main conclusion of the present reversible inactivation study is that, after unilateral permanent lesion of the hand area in M1, the same area (without distinguishing the lesion territory from the peri-lesional one still in M1) played a significant and systematic role in the functional recovery of the contralesional hand (Figs. 2-8; Table 2). The same was largely true for the ipsilesional PMd, with however a few exceptions for the vertical slots at one or the other time point in the corresponding daily session (Table 2). On the other hand, evidence for a role played by PMv was less systematic and less prominent (Table 2): a contribution of PMv to the functional recovery of manual dexterity was found in Mk-BI (vertical and horizontal slots), in Mk-CE (horizontal slots only), in Mk-JU (horizontal slots only), in Mk-VA (both slots orientation but at a single time point), but not in the monkeys Mk-RO and Mk-MO. This observation may be explained by smaller volumes of muscimol injected in PMv (Table 1), because a more restricted extent of PMv than PMd was accessible via the chronically implanted chamber. Indeed, small volumes of muscimol (4.4 μl) were injected in Mk-MO and Mk-VA, in which the inactivation of PMv was ineffective and an effect of PMv inactivation was found in Mk-CE, in which the volume infused in PMv was as large as that injected in PMd (8 μl; Table 1). However, this interpretation is not straightforward, as shown by the case of Mk-BI, in which the inactivation of PMv affected the contact time in spite of a relatively small volume of muscimol injected (5.4 μl; Table 1). Mk-RO, in spite of a larger volume of infusion in PMv (6 μl; Table 1), did not exhibit an effect on contact time after inactivation of PMv.

Two monkeys involved in the present analysis (Mk-CE and Mk-JU) were reported in the pilot study of Liu and Rouiller (1999), as the data are derived from the same muscimol infusion sessions. A detailed comparison shows the clear benefit to have introduced here the contact time analysis, the distinction between horizontal and vertical slots and, but to a lesser extent, the score in 30 seconds instead of 60 seconds. The latter parameter (score in 60 seconds cumulated for both slot orientations) did not allow Liu and Rouiller (1999) to dissociate the contribution of PMd and PMv in Mk-CE. Indeed, their data based on this single parameter showed an effect of reversible inactivation of PM only when both PMd and PMv were inhibited simultaneously, but not when they were inactivated separately (see Figure 4 of Liu and Rouiller, 1999 for Mk-CE). In the present re-examination of the same data, the reversible inactivation of PMd alone in Mk-CE was followed by a dramatic loss of manual dexterity for the horizontal slots, as shown by the increase of contact time (Fig. 4). Moreover, still in Mk-CE (Fig. 4), the reversible inactivation of PMv alone also led to a highly

significant loss of manual dexterity for the horizontal slots only. It can be concluded that the distinction between vertical and horizontal slots is crucial. The distinction between PMd and PMv is not possible in Mk-JU, as they were not inactivated separately in this particular animal (see Table 1).

Along the same line, the data as analyzed in Liu and Rouiller (1999) for the same two monkeys (Mk-CE and Mk-JU) were inconsistent regarding the effect of reversibly inactivating the lesioned territory in M1 and/or its surrounding zone. There was no effect in Mk-CE, whereas there was a decrease of score (in 60 seconds for both slot orientations) in Mk-JU (see Figure 4 of Liu and Rouiller, 1999). The analysis conducted in the present work led to different results in Mk-CE, in which an effect of inactivation of the lesioned territory in M1 provoked a significant increase of contact time 30 and 45 minutes after infusion of muscimol, whereas the effect was less significant on the vertical slots and limited to a single time point (Fig. 4). The present data in Mk-JU confirm statistically the effect of inactivating the lesioned M1 as seen on the score by Liu and Rouiller (1999), but the increase of contact time occurs for the horizontal slots only (Fig. 5). In the other monkeys (Mk-BI, Mk-RO, Mk-MO, Mk-VA and Mk-SL; not yet available in Liu and Rouiller, 1999), the effect of reversible inactivation of the lesioned M1 was more systematic and more pronounced than in Mk-CE and Mk-JU. Indeed, in all of them (except Mk-SL), there was a statistically significant increase of contact time, both for the horizontals slots and the vertical slots. A more prominent effect of inactivating the lesioned M1 in these more recent monkeys (Mk-BI, Mk-RO, Mk-MO, Mk-VA) than in the pilot monkeys Mk-CE and Mk-JU is consistent with the notion that the permanent lesion in M1 is smaller in the recent monkeys than in the 2 pilot ones. The consequence is that in case of a smaller lesion, there is more preserved territory in M1 most likely involved in the functional recovery. When inactivated with muscimol, then the effect on the recovered manual dexterity is larger than in animals with less territory in M1 involved in the functional recovery. This interpretation is supported by the case of Mk-SL, subjected to a large permanent lesion (comparable to that in Mk-JU): the reversible inactivation of the lesioned M1 provoked a moderate effect limited to the vertical slots (Fig. 8), consistent with a less strong effect observed in the other two monkeys with a large permanent lesion of M1 (Mk-CE and Mk-JU). In other words, it seems that the importance of the role played by the lesioned M1 in the functional recovery depends on the size of the permanent lesion, in the sense of a more prominent role in case of small lesion. Reciprocally, in case of a large permanent lesion in M1, the mechanisms of functional recovery are more

dependent on non-primary motor cortical areas, such as PM (Liu and Rouiller, 1999; present study) or SMA (McNeal et al., 2010).

As far as the reversible inactivation technique with infusion of muscimol is concerned, the control experiment of injecting saline instead of muscimol has not been conducted in the course of the present study in the seven monkeys involved (to avoid additional sessions with the risk to damage the cortex with multiple syringe penetrations). Nevertheless, this control experiment has been conducted several times in our laboratory in previous studies on intact monkeys (Rouiller et al., 1997; Kermadi et al., 1997): the infusion of saline instead of muscimol in M1, or in SMA or in PM (same volume and same number of sites) did not affect the motor performance of the monkey in the modified Brinkman board task or in the reach and grasp drawer task.

An important issue concerns the possibility that the reversible inactivation with muscimol of PMd or PMv for instance does not inhibit per se the functional role of PM in the functional recovery but rather that muscimol may diffuse passively from the injections sites in PM to M1, thus inhibiting M1 in a second step. Again, previous studies conducted in intact monkeys argue against such interpretation. As shown in Kermadi et al. (1997), the deficits observed after infusion of muscimol in SMA or PM were clearly different from the deficits resulting from infusion in M1, up to at least one hour post-injection of muscimol. If muscimol would diffuse massively from the infusion sites in SMA or PM to M1, then after some time (15 or 30 minutes), one would expect a deficit resembling that observed after direct infusion in M1. As this was clearly not the case, one can assume that the diffusion of muscimol from PM or SMA to M1 is limited, which should also be the case in the present study. The post-lesion ICMS maps (Figs. 2-8), illustrating the position of the sites of infusion of muscimol in PMd or PM, show that the sites in PMd or PMv closest to M1 are located in most cases at 2-3 mm from the initial hand representation in M1, the other sites of infusion in PMd or PMv being more distant. Based on the estimated distance of diffusion of the inactivating agent (1-2 mm, see Martin 1991), the concern about direct diffusion from PM to M1 is most likely minor.

The present reversible inactivation data confirm the role played by the ipsilesional PM (PMd and, but to a lesser extent, PMv) in the functional recovery from a permanent lesion of the hand representation in M1. The question is how PM does exert its influence in the functional recovery of manual dexterity. In intact animals, the influence of PM on manual dexterity, especially PMv, is exerted in a large part via its corticocortical projection to M1 (Schmidlin et al., 2008). In other words, the motor effects from PMv depend for some part on

the projection from PMv to M1, then leading to the activation of the corticospinal outputs to hand motoneurons in the cervical cord. This observation is consistent with the notion that the corticospinal projection originating from PM terminates mostly in cervical segments (C3-C4) located higher than the hand motoneurons at spinal levels C8-T2. One cannot however exclude that some motor effects from PMv may be exerted via its corticospinal projection, then relayed to lower spinal segments via propriospinal projections. In the present monkeys subjected to a permanent lesion of M1, the role played by PM (PMd or PMv) in the functional recovery of manual dexterity is exerted via different scenarios. First of all, in monkeys in which some lesion and the peri-lesion territories in M1 are preserved, PM may enhance its indirect influence via M1, following the mechanisms reported by Schmidlin et al. (2008) in intact animals. Second, the influence of PM in the functional recovery may be exerted via its corticospinal projection, which may be enhanced in the hypothetic case of sprouting of the axons terminals to reach more caudal hand motoneurons. Third, as demonstrated anatomically by Dancause et al. (2005), after permanent lesion of M1, there is an enhancement of the projection from PM (actually PMv) to the primary somatosensory cortex (S1), which may improve motor control.

The present reversible inactivation sessions based on infusion of muscimol are challenging experiments, and therefore there are limitations in their interpretation. First, the daily session in which the monkey had to perform repetitively the manual task with each hand was very long, much longer than the usual purely behavioural session. Indeed, the modified Brinkman board task had to be repeated at 3-4 time points (before infusion of muscimol, 30 min, 45 min and sometimes 60 minutes post-infusion). Considering the time needed to infuse the muscimol at several sites, the overall reversible inactivation session was 2 to 3 times longer than the regular behavioural sessions. As a result, a decrease of motivation may have occurred, which may perturb the assessment of manual dexterity. In most cases, the data obtained at the 2nd or 3rd times points post-infusion are generally comparable, indicating that the drop of motivation was not a major concern. Another issue is the unfolded post-lesion ICMS maps available at the time of muscimol infusions. Due for instance to limitations in the extent of territories accessible from the chronic chamber, PMd and PMv could not be fully characterized (see ICMS maps in Figs. 2-8) and therefore the choice of the sites where to infuse muscimol was more difficult than in M1. As a consequence (see ICMS maps in Figs. 2-8), PMd or PMv were better identified in some monkeys (Mk-BI, Mk-VA, Mk-SL) than in others (Mk-RO, Mk-CE, Mk-JU, Mk-MO). In the latter monkeys, the position of PMd and PMv were to some extent guessed based on their known distance from the hand area in M1,

previously determined based on the pre-lesion ICMS map. As a consequence, one cannot be certain that the most pertinent region in PM has been inactivated with muscimol. Based on the pilot data of Liu and Rouiller (1999), with evidence for a role played by PM in the functional recovery, the chronic chamber implanted in the subsequent monkeys was aimed to the lesioned M1 and PM. It would however have also been interesting to have inactivated SMA (see McNeal et al., 2010), as well as CMA, plus the motor cortical areas in the intact hemisphere. Such a broader investigation of many more cortical areas would require not only a larger chronic chamber, but also more of them, with the inherent risk of complications during the very long overall duration of the experiment. To preserve the cortical tissue for the tracing studies and histological analysis (see tracing experiments in the next chapters of the thesis), the inactivation of a given cortical area was limited to a single session. Indeed, the repeated insertion of the syringe may damage the cortical area infused with muscimol. It would however be important to repeat several times the very same inactivation protocol to assess how much this method is reproducible, a study to conduct in the future in a monkey not used later on for tracing studies. In spite of these technical difficulties and limitations of interpretation, the reversible inactivation approach based on muscimol infusion remains a precious tool to test whether a given brain area plays a role or not in a process of functional recovery from a permanent lesion.

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3.4 Chapitre 4:

Influence of anti-Nogo-A antibody treatment on the reorganization of callosal connectivity of the premotor cortical areas following unilateral lesion of primary motor cortex (M1) in adult macaque monkeys.

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Abstract

Adjacent motor cortical areas (e.g. the premotor cortex=PM) play a role in functional recovery from lesion of the primary motor cortex (M1). Nevertheless, the role of callosal projections from the intact hemisphere following unilateral M1 lesion remains unknown. The reorganization of callosal projections from the intact hemisphere to the ipsilesional PM was investigated in 8 monkeys subjected to unilateral M1 lesion, in absence of treatment (control; n=4) or treated with function blocking antibodies against the neurite growth inhibitory protein Nogo-A (n=4). After functional recovery, though incomplete, the tracer BDA was injected in the ipsilesional PM. Retrogradely labelled neurons were plotted in the intact hemisphere and their number was normalized with respect to the volume of the core of BDA injection sites. For comparison, BDA was also injected in PM in three intact monkeys.

1) The callosal projections to PM in the controls originate mainly from homotypic PM areas and, but to a somewhat lesser extent, from the mesial cortex (cingulate and supplementary motor areas).

2) In the lesioned anti-Nogo-A antibody treated monkeys, the normalized number of callosal retrogradely labelled neurons was up to several folds higher than in controls, especially in the homotypic PM areas.

3) Except one control with a small lesion and a limited, transient deficit, the anti-Nogo-A antibody treated monkeys recovered to nearly baseline levels of performance (73 to 90%), in contrast to persistent deficits in the control monkeys.

These results are consistent with a sprouting and/or sparing of callosal axons promoted by the anti-Nogo-A antibody treatment after lesion of the primary motor cortex, as compared to untreated monkeys.

Introduction

The motor cortical areas of macaque monkeys comprise four main distinct regions: the primary motor cortex (M1), the premotor cortex (PM), the supplementary motor area (SMA) and the cingulate motor area (CMA). These four cortical regions have been subdivided further into sub-areas on the basis of anatomical and functional criteria. For instance, the premotor cortex comprises a dorsal (PMd) area separated from the ventral (PMv) area (e.g. Kurata 1991; Kurata and Hoffman 1994; Kurata 1994a,b). The SMA has been subdivided into a rostral zone (pre-SMA) and a caudal zone (SMA-proper), based also on anatomical and functional criteria (e.g. Matsuzaka et al. 1992; Matsuzaka and Tanji 1996; Preuss et al. 1996; Inase et al. 1999; Shima and Tanji 1998, 2000; Liu et al. 2002; Fuji et al. 2002; Russo et al. 2002; Akkal et al. 2007; Nakajima et al. 2009). Based essentially on the topographic distribution of corticospinal neurons, three distinct sub-areas have been recognized in the CMA (Dum and Strick 1991; He et al. 1995). Finally, M1 exhibits different properties in its caudal versus rostral portion (e.g. Strick and Preston 1982; Friel et al. 2007; Rathelot and Strick 2009). In intact monkeys, the corticocortical connectivity of the motor cortical areas is well established, both within the same hemisphere (e.g. Luppino et al. 1990, 1993, 1999, 2003; Kurata 1991; Matelli et al. 1998; Tanné-Gariépy et al. 2002) and with the other hemisphere (e.g. Jenny 1979; Rouiller et al. 1994a; Liu et al. 2002; Marconi et al. 2003; Boussaoud et al. 2005). The multiplicity of motor cortical areas in primate represents a basis for a (partial) vicarious substitution of one area by others in case of a restricted lesion affecting a specific motor zone. Along this line, it was shown that PM plays a crucial role in the spontaneous (incomplete) functional recovery of hand dexterity after M1 lesion (e.g. Liu and Rouiller 1999; Frost et al. 2003; Dancause et al. 2006), for instance based on an enhancement of the homolateral connectivity of PM with the postcentral gyrus as a result of M1 lesion (Dancause et al. 2005).

The study of Dancause and collaborators (2005) demonstrates that the intra-hemispheric connectivity of a motor cortical area (PM in the present case) is subjected to spontaneous and significant changes, such as rewiring, as a result of M1 lesion. Such re-arrangement is most likely, at least in part, the anatomical support for (incomplete) spontaneous recovery from the lesion. If this is the case, one may predict that a treatment enhancing functional recovery after motor cortical lesion is likely to promote adaptive changes of cortico-cortical connectivity. In this context, the present study aims at testing the hypothesis that, after a lesion of M1, the callosal connectivity of PM is modified, possibly preserved and/or enhanced as a result of a

treatment promoting better functional recovery, by neutralizing the neurite growth inhibitor Nogo-A. Such intervention has been shown to enhance functional recovery and sprouting of the corticospinal tract in rats (see Gonzenbach and Schwab 2008; Schwab 2010 for review) and in monkeys subjected to spinal cord injury Fouad et al. 2004; (Freund et al. 2006, 2007, 2009). More specifically, we hypothesize that after M1 lesion, in the absence of treatment, there is a limited spontaneous sprouting of the callosal projections reaching the ipsilesional PM. In contrast, the administration of an anti-Nogo-A antibody may preserve connectivity and/or promote local sprouting of callosal fibers reaching PM rostral to the lesioned M1. To test this hypothesis, adult macaque monkeys were subjected to a unilateral lesion of M1, subdivided then in a group of monkeys without treatment and a group of monkeys treated with an anti-Nogo-A antibody. In both groups of monkeys, after (incomplete) functional recovery, the anatomical tracer BDA was injected in the ipsilesional PM to quantify the number of callosal retrogradely labelled neurons in the opposite (intact) hemisphere projecting to PM. Although the BDA injection sites were located mainly in PMd, some sites also involved PMv. For this reason, we will use below the common nomenclature PM to designate these two premotor areas. We hypothesize that, after M1 lesion and due to preservation of connectivity and/or increase of axonal sprouting, more labelled callosal neurons will be found in the anti-Nogo-A antibody treated monkeys than in the untreated monkeys, after injection of BDA in the ipsilesional PM.

Materials and Methods

The inter-hemispheric connectivity of PM has been derived from 8 adult macaque monkeys (*Macaca fascicularis*) subjected to lesion of M1, plus three intact monkeys for comparison. The experiments were conducted on three groups of monkeys: i) four control monkeys (lesion of M1, no treatment); ii) four treated monkeys (lesion of M1 and anti-Nogo-A antibody treatment); iii) three intact monkeys. All monkeys were subjected to injections of the neuroanatomical tracer BDA (Biotinylated Dextran Amine) in PM. In the present report, the analysis was restricted to the neurons retrogradely labelled with BDA in the hemisphere opposite to the injected PM. Although BDA is usually used for anterograde tracing (to study for instance efferent projections of PM), it was however found to provide retrograde labelling as well, yielding consistent and reliable data, as seen in previous studies in which several retrograde tracers, including BDA, were switched around across cases (e.g. Tanné-Gariépy et al., 2002; Morel et al., 2005; Boussaoud et al., 2005). Based on these previous data supporting the pertinence of BDA for retrograde tracing, the present analysis was conducted to assess the

origin of callosal projections reaching PM. Table 1 shows a survey of the parameters of BDA injections in the premotor cortex (in 11 monkeys) and ibotenic acid injections to induce a permanent lesion in M1 (in 8 monkeys). Surgical procedures and animal care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and were approved by local (Swiss) veterinary authorities.

In the animal facility, monkeys were housed in rooms of 12 m^3, in which usually 2-4 monkeys were free to move and to interact among each other[2]. Before daily behavioural testing in the morning, the animal caretaker transferred the monkeys to temporary cages, for subsequent transfer to a primate chair, in which the monkeys were transported to the behavioural laboratory. The monkeys had free access to water and were not food deprived. The reward (pellets) obtained during the behavioural tests was the first daily access to food. After completion of the behavioural tests, the monkeys received additional food (fruits and cereals). The body weight of the animals was monitored on each working day. In case the body weight dropped by 10% or more, the experiment was interrupted until the monkey regained the lost weight (this criterion for interruption was not met in the course of the present experiments).

The complete experimental protocol described below applies to the group of treated monkeys (Mk-VA, Mk-SL, Mk-MO, Mk-LA), whereas for the group of control monkeys (Mk-GE, Mk-RO, Mk-CE and Mk-BI) the anti-Nogo-A antibody treatment was omitted. The cases Mk-GE, Mk-RO, Mk-CE, Mk-BI, Mk-VA, Mk-MO and Mk-SL have already been considered in recent reports dealing with the influence of the lesion of M1 on the manual performance of the ipsilesional hand or in relation to adult progenitor cells transplantation (Kaeser et al. 2010, 2011; Bashir et al. 2011). In the present study, one additional lesioned monkey was considered (Mk-LA). The three intact monkeys were only subjected to BDA injections in PM.

[2] A new Swiss regulation has been introduced in September 2010 requesting now a volume of 45m^3 at least to be given to a group of up to 5 macaque monkeys.

Table 1: Summary of protocols of BDA and ibotenic acid injections.

Monkey groups	**Intact**			**Untreated**				**Treated with Anti-Nogo-A antibody**			
	Mk-CH	**Mk-R13**	**Mk-R12**	**Mk-GE**	**Mk-RO**	**Mk-CE**	**Mk-BI**	**Mk-VA**	**Mk-SL**	**Mk-MO**	**Mk-LA**
Species	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.
Sex of animals	Male	Male	Male	Female	Male	Male	Male	Female	Female	Male	Female
Lesion of M1 hand area	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Treatment	None	None	None	None	None	None	None	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Anti-Nogo-A antibody
Age at time of lesion (rounded 0.5 year)	10	4.5	5	5	4	4.5	5	5.5	5.5	5.5	5
Weight at time of lesion (kg)	6#	4#	4#	2.8	3.2	3.8	5	4.9	4.6	5.6	2.6
Volume of ibotenic acid injected (µL)	-	-	-	13	18	40	29.74	15.5	18	20	13.5
Nb. of ICMS sites injected with ibotenic acid	-	-	-	13	12	21	29	11	11	20	9
Time interval (days) between lesion and post-lesion ICMS mapping	-	-	-	97	80	315	220	210	132	97	137
Volume of BDA injected (µL)	8	8.8	7.2	2.1	4.8	16	7.2	5	10	10.8	2
Nb. of BDA sites injected	10	11	9	5	6	16	11	5	10	12	5
Hemisphere in which BDA was injected *	LH	LH	LH	LH	LH	LH	LH	LH	LH	LH	LH
Duration of survival time (days)				29	21	21	22	22	18	21	27
Cortical areas of BDA injections	PMd/PMv	PMd/PMv	PMd	PMd/PMv	PMd	PMd/PMv	PMd/PMv	PMd/PMv	PMd/PMv	PMd/PMv	PMd/PMv
Total volume of lesion (mm^3) Gray matter (motor cortex+post central gyrus)	**0**	**0**	**0**	**48.7**	**14**	**112.8**	**20.13**	**20**	**78.2**	**41.8**	**3.12**
Volume of lesion in post central gyrus (mm^3)	0	0	0	7.6	0	10.1	0	5.8	1.8	0	0
Volume of lesion spread to sub-cortical white matter (mm^3)	0	0	0	0	0	86.5	0	0	130.6	0	0
Absolute number of BDA-labelled cells	**4542**	**4503**	**1383**	**167**	**89**	**84**	**958**	**927**	**24**	**1805**	**73**

Under species, "fasc" is for *macaca fascicularis*

* Ipsilesional hemisphere

LH=left hemisphere

Surgery

The monkeys subjected to M1 lesion were implanted unilaterally with a chronic, stainless steel or tecapeek chamber giving access to M1, the dura mater being however left in place (see Schmidlin et al. 2004 for detail). The monkeys were sedated with i.m. injection of ketamine (Ketalar, 5 mg/kg) and pre-medicated as previously described, in particular with injection of 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 deep anesthesia, maintained 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). The surgery was carried out under continuous monitoring of the following parameters: heart rate, respiration rate, expired CO_2, arterial O_2 saturation and body temperature. After surgery, the animals were treated with antibiotics (ampicilin 10%, 30 mg/kg, s.c.) and analgesics (pills of Rymadil mixed with food) during several days. The chambers were fixed to the skull with titanium screws and orthopedic cement (Palacos). The inside of the chronic chamber was cleaned daily with Betadine and an antibiotic ointment was spread on the dura mater surface to reduce the risk of infection.

Behavioural assessment: Brinkman box

The monkeys subjected to M1 lesion were initially trained to perform a large palette of manual dexterity tasks, as previously described in detail (e.g. Rouiller et al. 1998; Freund et al. 2009; Kaeser et al. 2010, 2011; Schmidlin et al. 2011; see also http://www.unifr.ch/neuro/rouiller/motorcontcadre.htm). Our main behavioural test was based on a modified version of the Brinkman board task (e.g. Brinkman and Kuypers, 1973; Brinkman, 1984), in which the monkeys had to grasp food pellets from wells using the precision grip (opposition of thumb and index finger). The behavioural data for all monkeys subjected to motor cortex lesion will be presented in a comprehensive manner in future reports (based on the complete palette of tests and on a larger populations of monkeys, including those not subjected to BDA injections in the premotor cortex). The present report will show behavioural data only for monkeys subjected to a lesion of the motor cortex and to BDA injection in the premotor cortex. In the present report, the behavioural assessment is focused on a further variation of the modified Brinkman board task (Schmidlin et al. 2011), consisting of a “box” containing only 20 wells (instead of 50) but confined to a restricted

volume, limiting the movements and orientations of the hand to approach the wells (Fig. 1C). The dimensions of the "box" are 23 cm (length) x 13 cm (width) x 10 cm (height). Only one side of the "box" is open to allow access with the hand to the pellets inside the "box". The board inside the "box" is made of 10 wells oriented vertically and 10 wells oriented horizontally. Under visual control, the monkey had to grasp the food pellets. The total time (in seconds) needed to empty all 20 wells was measured (Fig. 2). After several weeks of training, the monkeys reached a plateau of hand performance, after which a pre-lesion performance of reference was established (Fig. 2), before the motor cortex was electrophysiologically mapped and lesioned. Mk-CE is not included in the behavioural data (Fig. 2) because, when the experiment was conducted, the Brinkman box was not introduced yet in our behavioural palette of manual dexterity tasks.

Electrophysiology: intracortical microstimulation (ICMS)

In the eight monkeys subjected to M1 lesion, electrophysiological intracortical microstimulation (ICMS) sessions were performed twice, first before lesion in order to guide the lesion procedure by mapping the M1 hand representation and, second, several months after the lesion when the monkeys reached a stable manual performance in the motor tasks (see e.g. Rouiller et al. 1998; Liu and Rouiller 1999; Schmidlin et al. 2004; Freund et al. 2006, 2009). A tungsten microelectrode (0.1 - 1 MΩ impedance, FHC Inc, USA.) was used to micro-stimulate the motor cortex accessible from the chronic chamber, along penetrations performed at 1 mm distance from each others (see e.g. Schmidlin et al. 2004, 2005). Along each electrode track, ICMS was applied below the surface of the dura at intervals of 1 mm, along a variable distance depending on the position of the penetration with respect to the central sulcus (see Kaeser et al. 2010). The effect of ICMS was assessed by visual inspection and/or palpation of the body part (articulation) at which a movement was elicited. The minimal current (ICMS threshold) producing this movement was determined at each stimulation site. The ICMS map was finally represented in the form of an unfolded map, as previously reported (Kaeser et al. 2010: supplementary Fig. 1; Park et al. 2001, 2004) and represented the basis to guide injections of ibotenic acid to produce a permanent lesion of M1 targeted mainly on the hand area (pre-lesion ICMS sessions) and BDA injections in PM (post-lesion ICMS sessions).

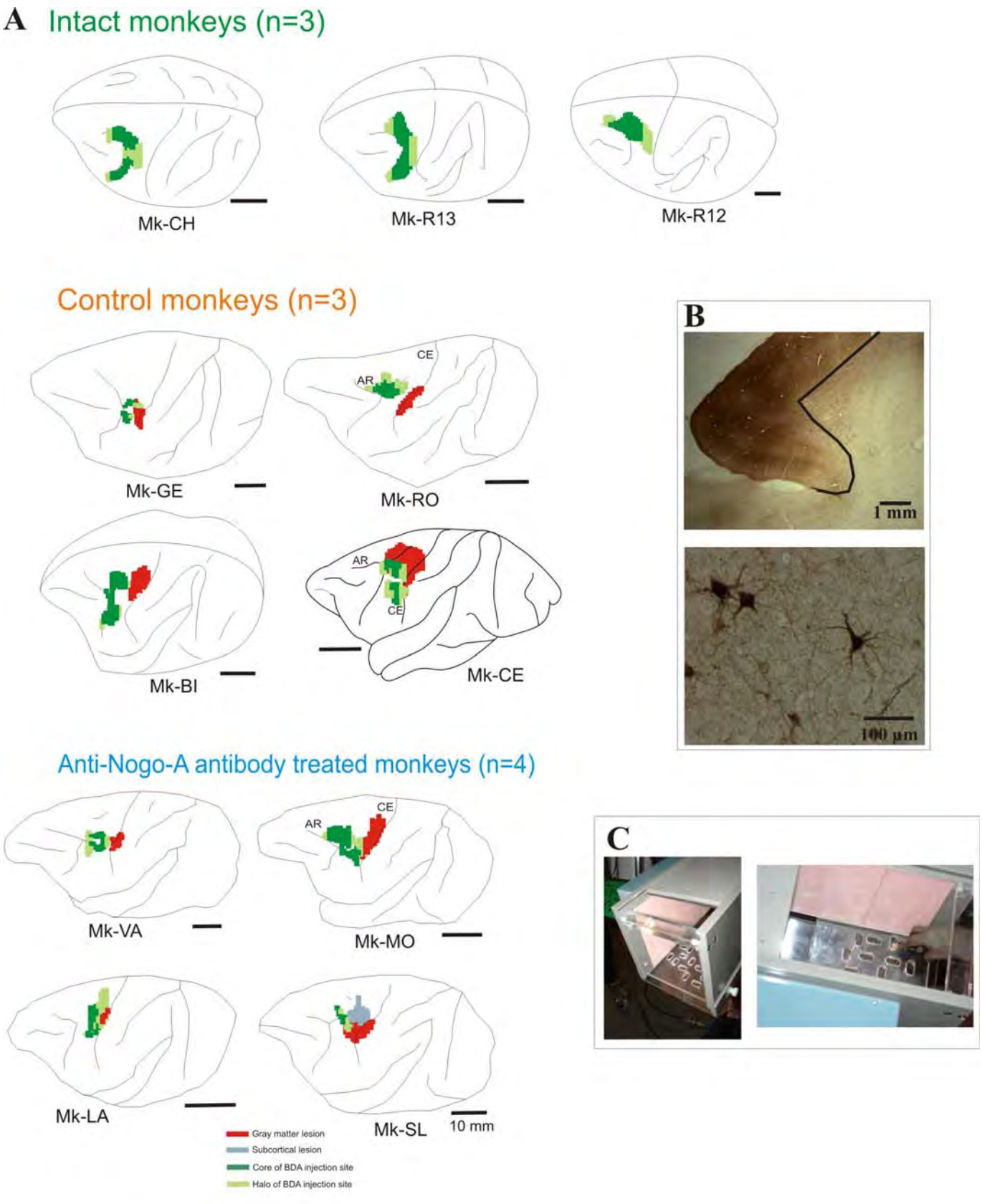

Figure 1

<u>Figure 1</u>: <u>Panel A</u> represents a reconstruction on a lateral view of the left hemisphere in each monkey of the location and extent of the lesion aimed at the M1 hand representation (red territory) and BDA

injection sites (territory in dark green for the core of the injection site and light green for the halo), as seen in transparency of the cortical surface. In three intact monkeys, BDA was injected unilaterally in M1 (top line). The actual volume of the lesions and BDA injections sites are given in Figure 7B for the eight monkeys subjected to M1 lesion. Panel B represents a photomicrograph of a typical BDA injection site in PMd and resulting BDA retrogradely labelled callosal cells in the opposite hemisphere, with the boundary of the gray matter in black line. Panel C is a view of the "Brinkman box" used for behavioural assessment of manual dexterity, representing a variation of our modified Brinkman board task (see text). The left picture shows the open facet of the "box" allowing access to the 20 wells, in which the pellets are placed. The right picture shows that "box" from above with the monkey's hand aimed to a well filled with a pellet to be grasped using the precision grip between the index finger and the thumb. At the beginning of the test, the 20 wells are each filled with a pellet. As the top facet of the "box" is transparent, the hand movements are executed under visual control. The behavioural test was taped with a video camera placed below the "box".

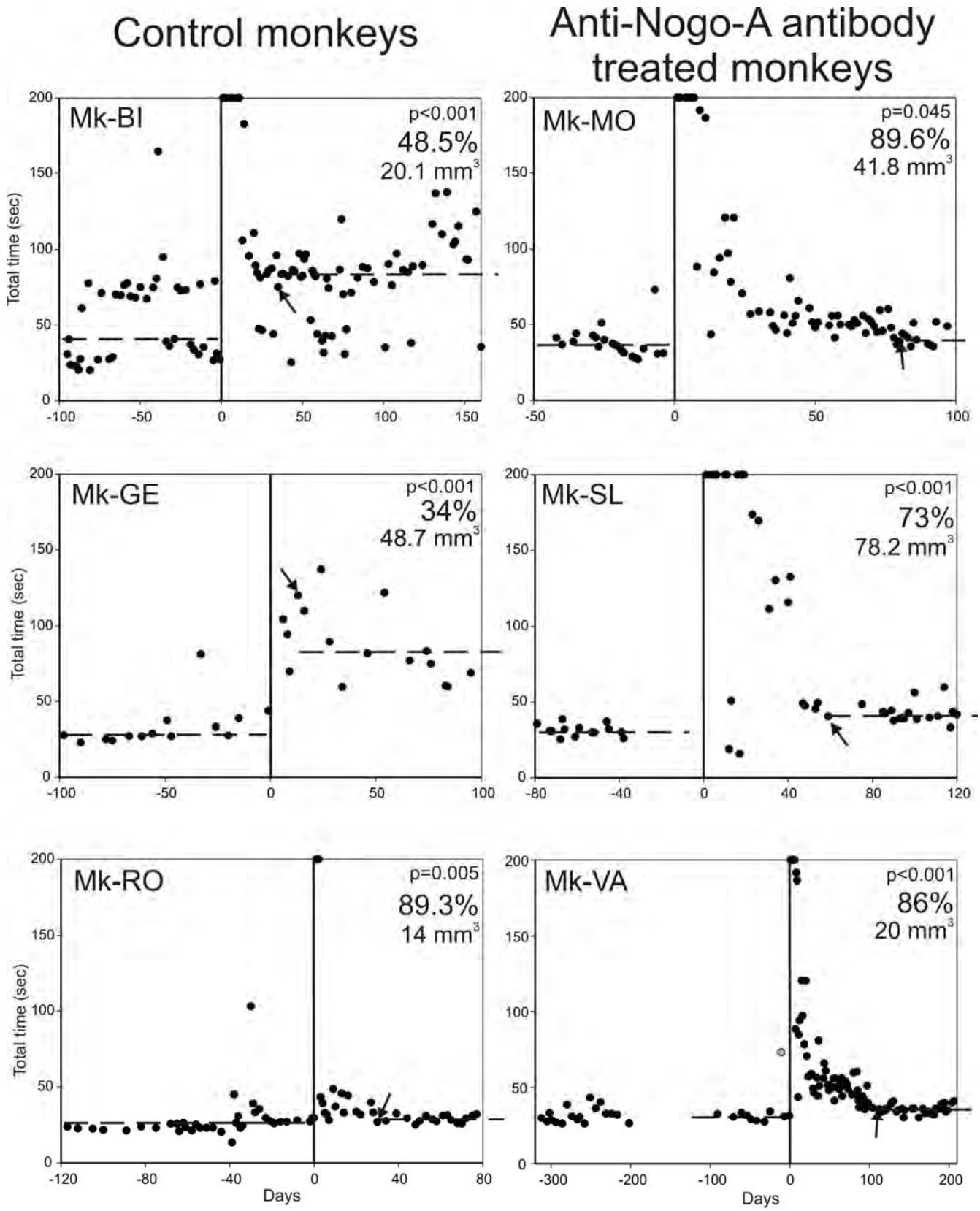

Figure 2

Figure 2: Behavioural data showing the manual dexterity of six monkeys before the lesion (negative days on the abscissa) and post-lesion (positive days on the abscissa). The day of the lesion (day zero) is indicated

by a vertical line. Each dot value corresponds to the total time (in seconds) needed by the monkey in a given session to empty the board containing 20 wells using the contralesional hand (see Fig. 1C). The median total time pre-lesion is given by the horizontal dashed line on the left part of each graph. Note the dramatic increase of total time immediately after the lesion, reflecting the deficit (increased difficulty to grasp the pellet using the precision grip). The values saturated at 200 correspond to sessions immediately post-lesion in which the monkey was unable to complete the task (empty the 20 wells). Then, there is a progressive recovery of manual dexterity. The recovery was considered as complete on the day indicated by the arrow, corresponding to the time point at which the monkey reached a plateau in the modified Brinkman board task (see Kaeser et al., 2010, 2011). The median total time at plateau of recovery is indicated by the horizontal dashed line on the right. In all monkeys, the post-lesion median total time was significantly longer than the pre-lesion median total time (p value given for each graph). The percentage of functional recovery was calculated by dividing the pre-lesion median total time by the post-lesion median total time at plateau, multiplied by 100. The percentage of functional recovery is given in each graph. For interpretation of the data (see text), the volume of the cortical lesion in mm^3 is indicated also in the graphs for each monkey. Mk-CE and Mk-LA (see Table 1) were not considered here as the former did not perform this task at the time of the experiment (Liu and Rouiller, 1999) whereas, in the latter (Mk-LA), there was nearly no lesion of M1.

Permanent lesion of M1 hand representation with ibotenic acid

In order to induce a permanent lesion of the motor cortex (M1) in eight monkeys, the neurotoxic ibotenic acid (10μg/μl in phosphate-buffer) was infused using a Hamilton micro-syringe at selected intracortical microstimulation (ICMS) sites of the hand area in M1 unilaterally, as previously reported in detail (Liu and Rouiller 1999; Kaeser et al. 2010, 2011; Basjir et al. 2011; Peuser et al. 2011). The number of ICMS sites injected and the total volume of ibotenic acid infused in M1 were determined based on the extent and shape of the hand area established for each monkey on the basis of the ICMS map. The parameters of ibotenic acid injections are indicated for each monkey in Table 1. As a result of ibotenic acid infusion, the contralateral hand exhibited after a few minutes a dramatic flaccid paralysis. The location and extent of the lesion are shown on lateral views of the left hemisphere in the four untreated (control) and the four treated monkeys (Fig. 1A).

Anti-Nogo-A antibody treatment

As outlined in Table 1, four of the eight monkeys subjected to unilateral permanent lesion of the motor cortex (M1) were treated with anti-Nogo-A antibody, delivered immediately after the lesion during four weeks (except in Mk-LA in which the treatment lasted only 2 weeks; see discussion). The anti-Nogo-A antibody treatment was similar to that applied to monkeys subjected to spinal cord injury (Freund et al. 2006, 2007, 2009), except

that two osmotic pumps were implanted instead of one. The tube delivering the antibody from one osmotic pump was placed intrathecally in the cervical cord whereas the tube of the second osmotic pump was placed below the dura in the vicinity of the lesion of M1. The anti-Nogo-A antibody treatment was tested here for motor cortex lesion in monkeys, as it was found to significantly enhance functional recovery after cortical lesion in rats (Papadopoulos et al. 2002; Emerick et al. 2003, 2004; Markus et al. 2005; Seymour et al. 2005; Tsai et al. 2007; Cheatwood et al. 2008; Gillani et al. 2010). Delivery of the anti-Nogo-A antibody in the cervical cord, in addition to the cerebral cortex, was motivated by possible enhancement of sprouting of corticospinal axons originating from PM at segmental level, in line with evidence for an increase of functional recovery and sprouting of corticospinal axons after cervical cord injury in macaques treated with anti-Nogo-A antibody (Freund et al. 2006, 2007, 2009).

BDA injection and histology

Following a surgical procedure and anesthesia protocol as described above, Biotinylaled Dextran Amine (BDA, MW 10'000, 10% in phosphate-buffered saline, Molecular Probes, Eugene, OR) was injected unilaterally into PM (mainly in PMd and to a lesser extent in PMv) on the lesioned hemisphere of the 8 lesioned monkeys (except in Mk-RO in which the injection was restricted to PMd), using a Hamilton micro-syringe, as previously described (e.g. Rouiller et al. 1994a,b, 1996, 1999, 2003; Liu et al. 2002; Tanné-Gariépy et al. 2002; Boussaoud et al. 2005; Morel et al. 2005; Cappe et al. 2007, 2009). PMd and PMv areas were identified partly on the basis of the ICMS sessions performed post-lesion. The detailed parameters of BDA injections are given for each monkey in Table 1. In addition, BDA was injected in Pm unilaterally in 3 intact monkeys (Fig. 1). The survival time after BDA injection ranged from 18 to 29 days, to allow anterograde transport of the tracer down to the cervical cord, as assessed in previous experiments (Rouiller et al. 1994a,b, 1996). Such duration of survival time is also sufficient for retrograde transport of BDA to the intact hemisphere. The animals were then 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 (Paraformaldehyde 4% in phosphate buffer), and 10, 20, 30% solutions of sucrose in phosphate buffer. The brains were placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection during 3-5 days. Frontal sections (50 µm thick) of the brain were prepared using a cryostat and collected in five series. BDA staining was revealed in one series of sections, as previously described (Rouiller et al., 1994a,b). A second series of sections was Nissl stained with cresyl violet whereas a third series of sections was processed to visualize

the marker SMI-32, as previously described (Liu et al. 2002; Wannier et al. 2005; Beaud et al. 2008). The two remaining series of sections were kept in reserve.

Data analysis

Nissl and SMI-32 stained sections were used to reconstruct on consecutive sections the position and extent of the permanent lesion in M1. BDA stained sections were used to reconstruct the position and extent of BDA injection sites in PM. The lesion and the BDA injection sites were positioned on a lateral view of the lesioned hemisphere (Fig. 1A). An individual BDA injection site appears as a column of about 1-3 mm of diameter. As BDA was injected at multiple sites adjacent to each other, a composite BDA injection site was obtained, corresponding to a dense and dark center zone ("core" of the injection site), surrounded by a less dense zone ("halo" of the injection site). The core and "halo" of the composite BDA injection site are represented for each monkey by the dark green and light green areas in Fig. 1A, respectively. As previously reported (Kaeser et al. 2010), using an ad-hoc function of the Neurolucida software (based on the Cavalieri method), the volume of the cortical lesion (in mm^3) was extrapolated from the reconstructions of the lesion on consecutive histological sections of the brain taken every 0.25 mm intervals (see Table 1). The same procedure was used to determine the volume of the BDA injections sites, separately for the core and the halo territories.

The distribution of retrogradely BDA-labelled neurons on the opposite hemisphere was plotted using Neurolucida software, on sections taken at 1 mm interval. Drawings with plots of labelled neurons were then printed in the form of pdf files for later processing using the software CorelDraw 14 (X4). As previously reported in tracing studies in which all labelled neurons were counted on the analyzed sections (see e.g. Lavenex et al. 2000; Geuna 2000; Benes and Lange 2001), stereological technique is not adequate. Stereology is appropriate when the number of cells to be counted is so large that samples have to be considered (with the condition that each object has the same probability to be counted). In the present study, all labelled neurons were systematically chartered (as the number of labelled neurons in the intact hemisphere was relatively low). This approach is considered as adequate because all samples were analyzed using the same procedure.

To account for variability due to the size of BDA injection sites and BDA uptake, the number of BDA-labelled neurons was normalized based on the volume of the core of the corresponding BDA injection site: the number of BDA labelled cells was divided by the volume of the core of the injection site expressed in mm^3. Figure 1B illustrates a typical BDA

injection site in PMd (top panel) and retrogradely BDA-labelled callosal neurons in the opposite hemisphere (bottom panel). The parcellation of the cerebral cortex in the intact hemisphere, in which the labelled neurons were distributed into distinct cortical areas, was based on criteria previously defined (Liu et al. 2002; Boussaoud et al. 2005).

Results

In eight monkeys, the lesion of the motor cortex was targeted to the hand representation of M1 (as determined on the basis of ICMS data), which varied in size and precise position across monkeys. As a result, there was also inter-individual variability in terms of size and location of the lesion, as represented in transparency of the brain surface in the left hemisphere of the eight monkeys subjected to a lesion (the red areas in Fig. 1A correspond to the extent of gray matter affected by the lesion).

1) Preliminary behavioural data

The behavioural consequence of the lesion affecting the hand area in M1 was a dramatic paralysis of the opposite hand, within the minutes following the infusion of ibotenic acid (see Liu and Rouiller, 1999). One exception was Mk-LA, as the lesion of M1 turned out to be very small (Table 1; Fig. 1A) and only a modest deficit took place for only a few days, after which the manual dexterity came back to normal. Mk-LA was thus not considered further for behavioural analysis (considered as an outlier), but only for the tracing data. In the present study, the behavioural assessment based on the Brinkman box task was conducted on six other monkeys subjected to a substantial lesion of M1, followed by a significant deficit of manual dexterity (Fig. 2). In the group of control monkeys (Mk-BI, Mk-GE and Mk-RO), two of them showed a considerable increase of the total time needed to empty the 20 wells containing the pellets after the lesion, a deficit that was long lasting: the post-lesion recovery was clearly incomplete, representing 48.5% of the original (pre-lesion) performance in Mk-BI and 34% in Mk-GE (Fig. 2 left column). The control monkey Mk-RO exhibited a very good spontaneous recovery post-lesion, but the volume of the lesion was clearly smaller than in the other two control monkeys. In the subgroup of the anti-Nogo-A antibody treated monkeys (Mk-LA excluded; see above), there was a post-lesion recovery ranging from 73 to 89.6% (Fig. 2 right column), although the volume of the cortical lesion affecting the gray matter was comparable (Mk-VA; Mk-MO) or even larger (Mk-SL) than in the two control monkeys (Mk-BI; Mk-GE) exhibiting clearly incomplete functional recovery (below 50%). Taking into account that the good spontaneous recovery observed in Mk-RO is most likely related to a restricted lesion,

the behavioural data for the other five lesioned monkeys (Fig. 2) provide preliminary evidence for a better functional recovery in the subgroup of anti-Nogo-A antibody treated monkeys (to be confirmed on a larger number of monkeys and additional behavioural tests).

2) Callosal connectivity of PM in intact monkeys

The pattern of callosal connectivity of PM has been described in two recent studies (Marconi et al., 2003; Boussaoud et al., 2005). To extent these basic data to the present experimental conditions, BDA was injected unilaterally in PM in three intact monkeys (Fig. 1A). The precise position and size of the multiple BDA injection sites were variable across monkeys (Fig. 1A) and the parameters of injection sites are summarized in Table 1.

The normal distribution of labelled callosal neurons, as assessed based on BDA injection in PM in three intact monkeys, is illustrated in Figure 3 for Mk-CH. As expected, after injection of BDA in PM unilaterally, most labelled callosal neurons were located in the opposite PMd and PMv. Labelled neurons, but to a lesser extent, were also located in pre-SMA, S1-S2 and CMA, whereas even fewer labelled neurons were observed in the prefrontal cortex (Pfc) and SMA. Nearly no labelled neuron was found in M1. Very similar results were obtained in Mk-R13 (supplementary Fig. 1), where the major callosal connections of PM originate from PMd and PMv. Again, in fairly comparable proportions as in Mk-CH, labelled neurons were also found in pre-SMA, CMA, Pfc, SMA and S1-S2 areas. Very few labelled neurons were detected in M1.

In the case Mk-R12, the BDA injections were targeted to PMd only (supplementary Fig. 2), yielding a dominant retrograde BDA-labelling in the homotypic PMd area in the opposite hemisphere. Additional labelling was found mainly in PMv and pre-SMA, whereas fewer neurons were found in CMA, Pfc and SMA. Few labelled neurons were also present in M1 and no labelling was detected in S1-S2.

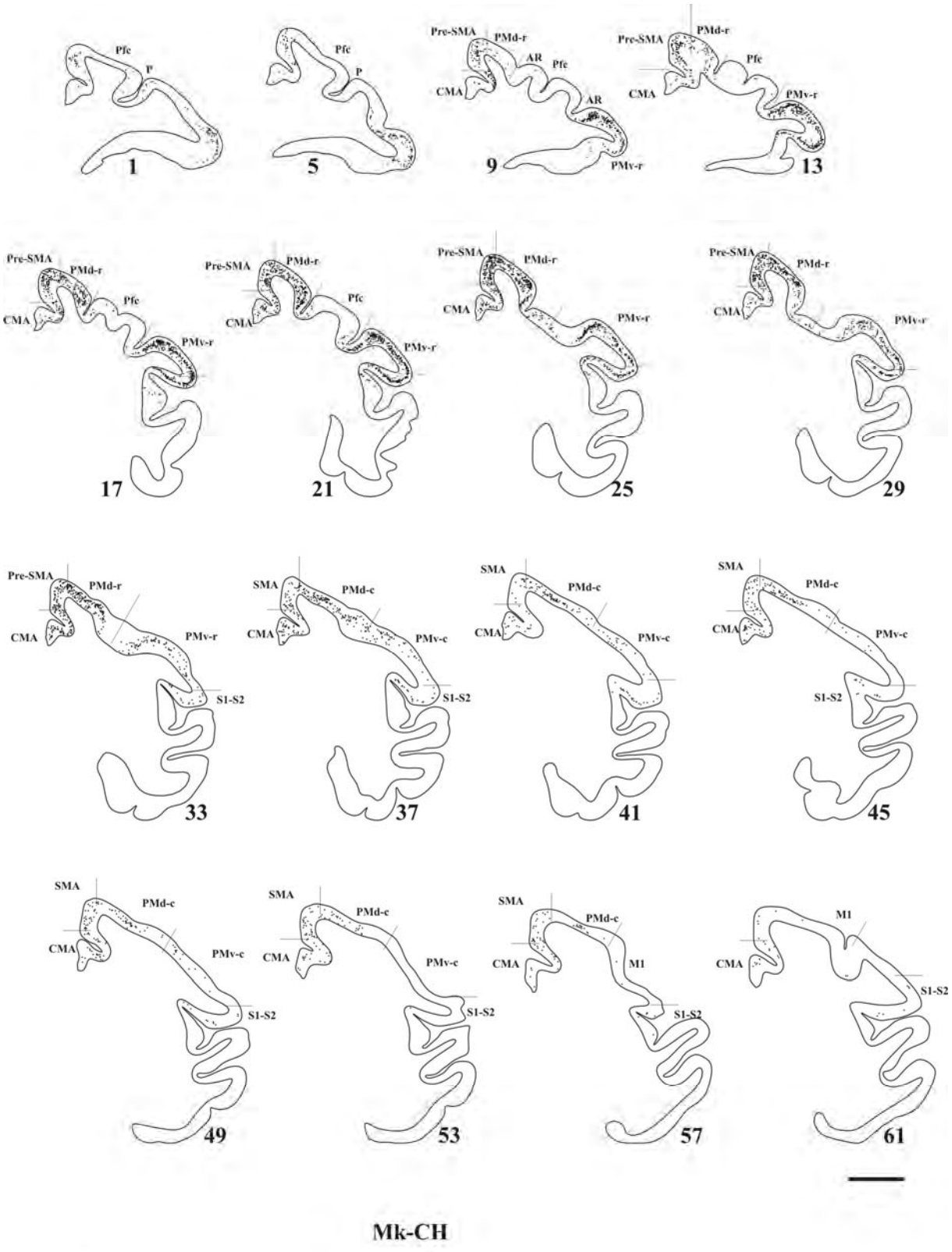

Figure 3

<u>Figure 3</u>: Frontal sections of the right hemisphere in Mk-CH (intact monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Scale bar: 5 mm. The inset on the top right is a section of the brain stem showing the position and density of labelled CS axons in the pyramid (above the pyramidal decussation). P=principal sulcus; AR=arcuate sulcus. See text for other abbreviations.

3) Callosal connectivity of PM in the untreated (control) monkeys subjected to M1 lesion

To investigate whether the possible, preliminary enhancement of functional recovery promoted by the anti-Nogo-A antibody treatment illustrated in Fig. 2 is correlated with a change of pattern of callosal connectivity of PM, BDA was injected in PM in all monkeys subjected to a lesion of M1. The extent and location of the BDA injections are represented by the green territories in Figure 1A. As a result of BDA injection in PM homolateral to the M1 lesion, the distribution of retrogradely BDA-labelled callosal neurons was established in four untreated (control) monkeys, with typical results illustrated in Figure 4 (top panel) for Mk-BI (see also Fig. 7A). PMv, pre-SMA and PMd contained the highest number of labelled neurons, although labelling was also substantial in CMA (Fig. 7A). Clearly fewer labelled neurons were present in SMA, S1-S2, prefrontal cortex (Pfc) and M1. In line with BDA injections sites covering a smaller extent of PMv as compared to PMd, the main region of origin of the callosal projections in two other control monkeys originated from the homotypic PMd in the opposite hemisphere (Fig. 4, bottom panel for Mk-RO; supplementary Figure 3 for Mk-GE). In Mk-GE, the other labelled neurons were distributed in largely comparable amounts in PMv and pre-SMA; fewer labelled neurons were found in CMA, SMA and M1. In Mk-RO (Fig. 4; bottom panel), the additional labelling outside the homotypic PMd was found in pre-SMA, whereas there was no labelling in Pfc, M1 and S1-S2. The last control monkey (Mk-CE) was subjected to a large lesion and BDA was injected in both PMd and PMv (Fig. 1A). The reconstruction of BDA retrograde labelling in the intact hemisphere (not shown) exhibited a small number of labelled callosal neurons (see Fig. 7), most of them in the homotypic region (PM) and, but to a lesser extent, in SMA.

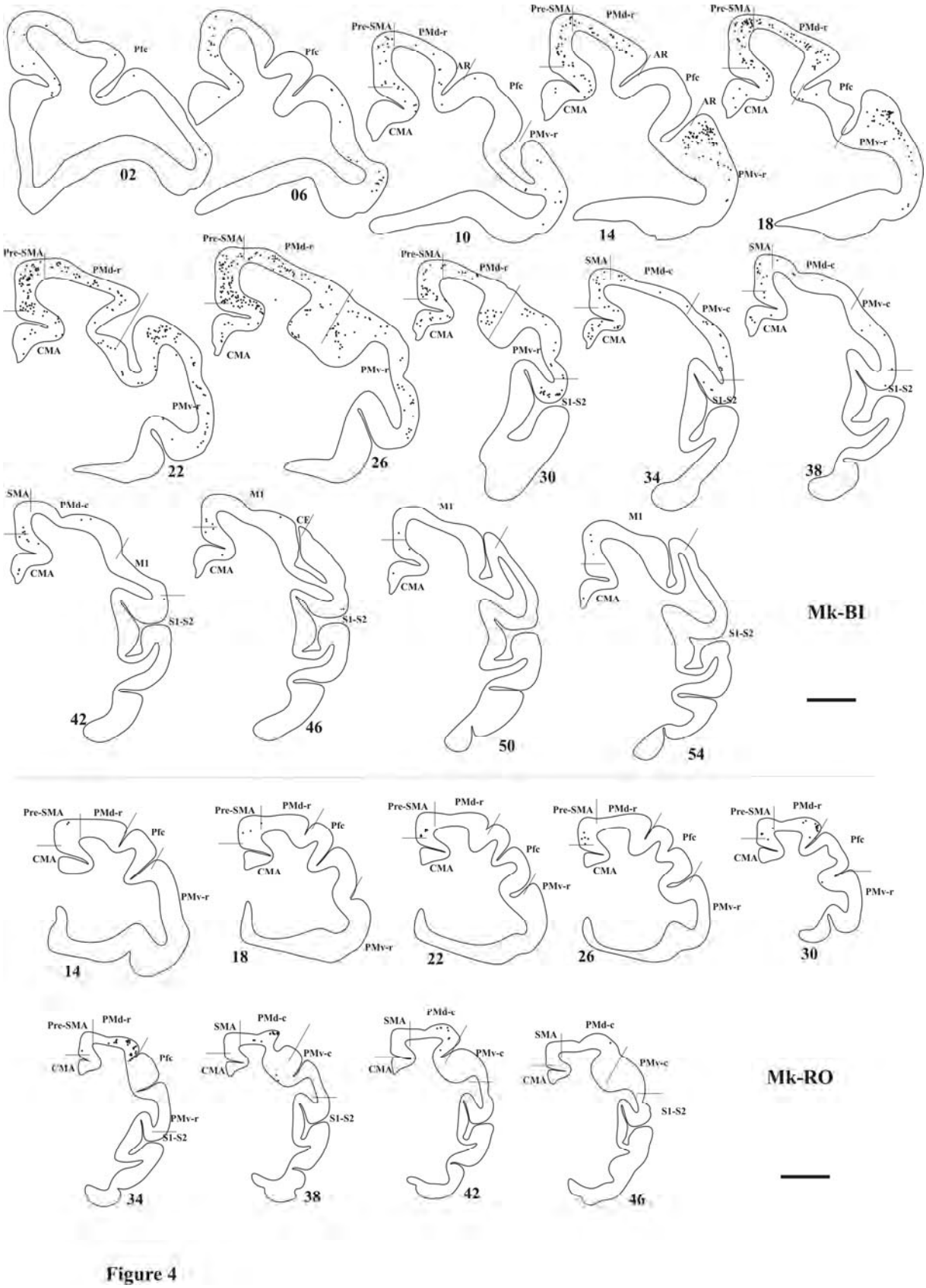

Figure 4

<u>Figure 4</u>: <u>Top panel</u>: Frontal sections of the right hemisphere in Mk-BI (control, untreated monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. The number next to each reconstruction is the serial number

of the corresponding histological section. Scale bar: 5 mm. P=principal sulcus; AR=arcuate sulcus; CE=central sulcus.

<u>Bottom panel</u>: Frontal sections of the right hemisphere in Mk-RO (control monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Same conventions as in top panel.

4) <u>Callosal connectivity of PM in the anti-Nogo-A antibody treated monkeys subjected to M1 lesion</u>

The distribution of BDA-labelled cells resulting from injection of BDA in the opposite PM homolateral to the lesioned M1, assessed in four anti-Nogo-A antibody treated monkeys, is illustrated for Mk-MO in Figure 5. Consistent with BDA injections covering more PMd than PMv (Fig. 1A), the main zone of origin of the callosal projections was the homotypic PMd (Fig. 5), although substantial labelling was also present in pre-SMA and PMv. Additional labelled neurons were observed in SMA and CMA, besides weak labelling in Pfc, M1 and S1-S2.

In Mk-VA, in which the BDA injections were better balanced between PMd and PMv, the retrograde labelling was predominantly found in the homotypic PMd and PMv (Fig. 6). Other labelled neurons were located in fairly comparable amounts in pre-SMA, M1, S1-S2 and, but to a lesser extent, in SMA, Pfc and CMA.

The BDA injection sites performed in the ipsilesional PM in two other anti-Nogo-A antibody treated monkeys (Mk-SL and Mk-LA; see supplementary Figure 4) yielded smaller numbers of labelled neurons. In the case of Mk-SL, most callosal projections originated from PMv, although PMd was also involved. Fewer labelled neurons were located in M1, whereas no labelling was found in CMA, Pfc, pre-SMA, SMA and S1-S2. In Mk-LA, most labelled neurons were seen in PMd, together with few neurons in PMv, SMA and pre-SMA, whereas labelling was rare in CMA and M1.

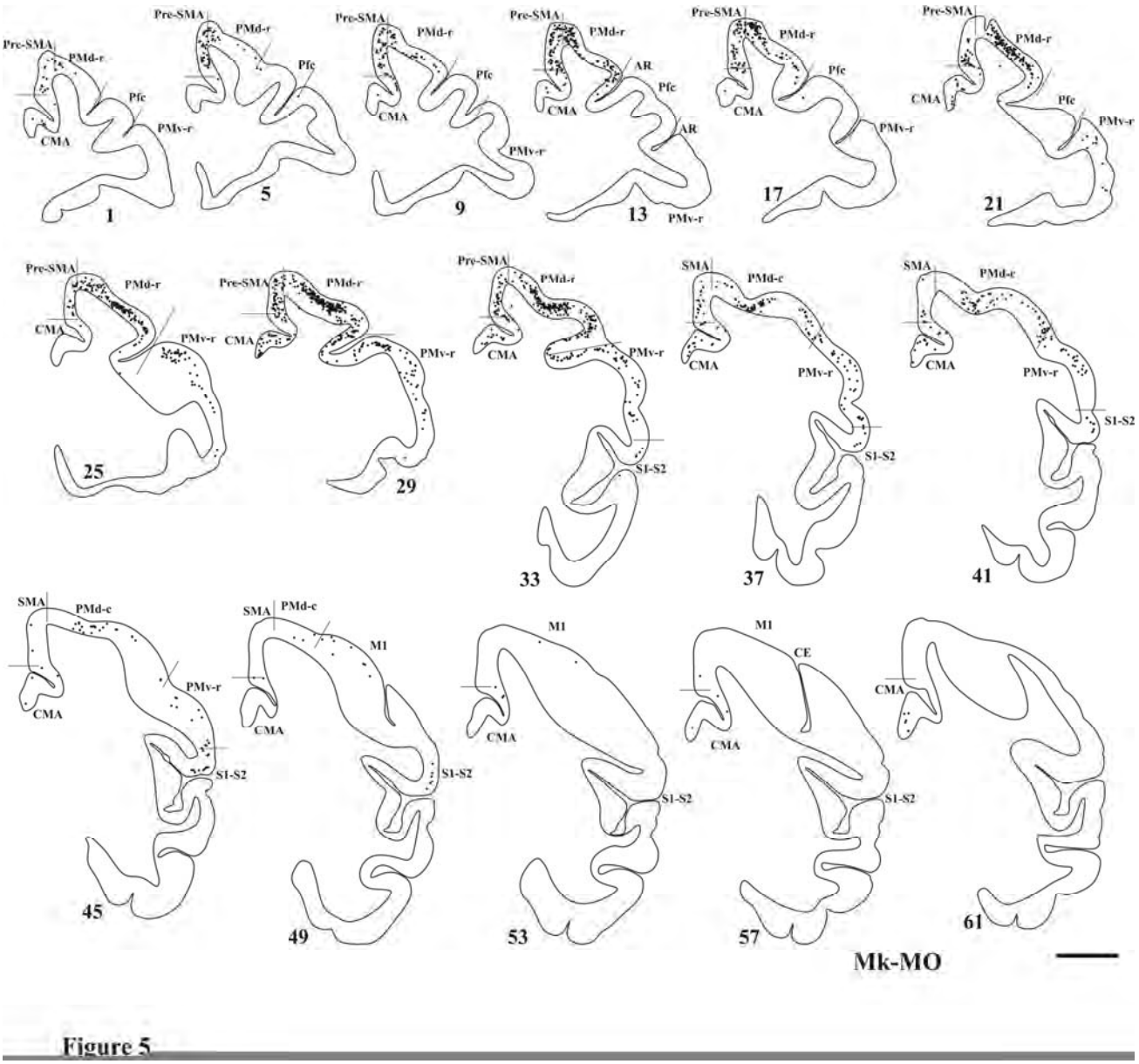

Figure 5

Figure 5: Frontal sections of the right hemisphere in Mk-MO (anti-Nogo-A antibody treated monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Scale bar: 5 mm. Same conventions as in Figures 3 and 4.

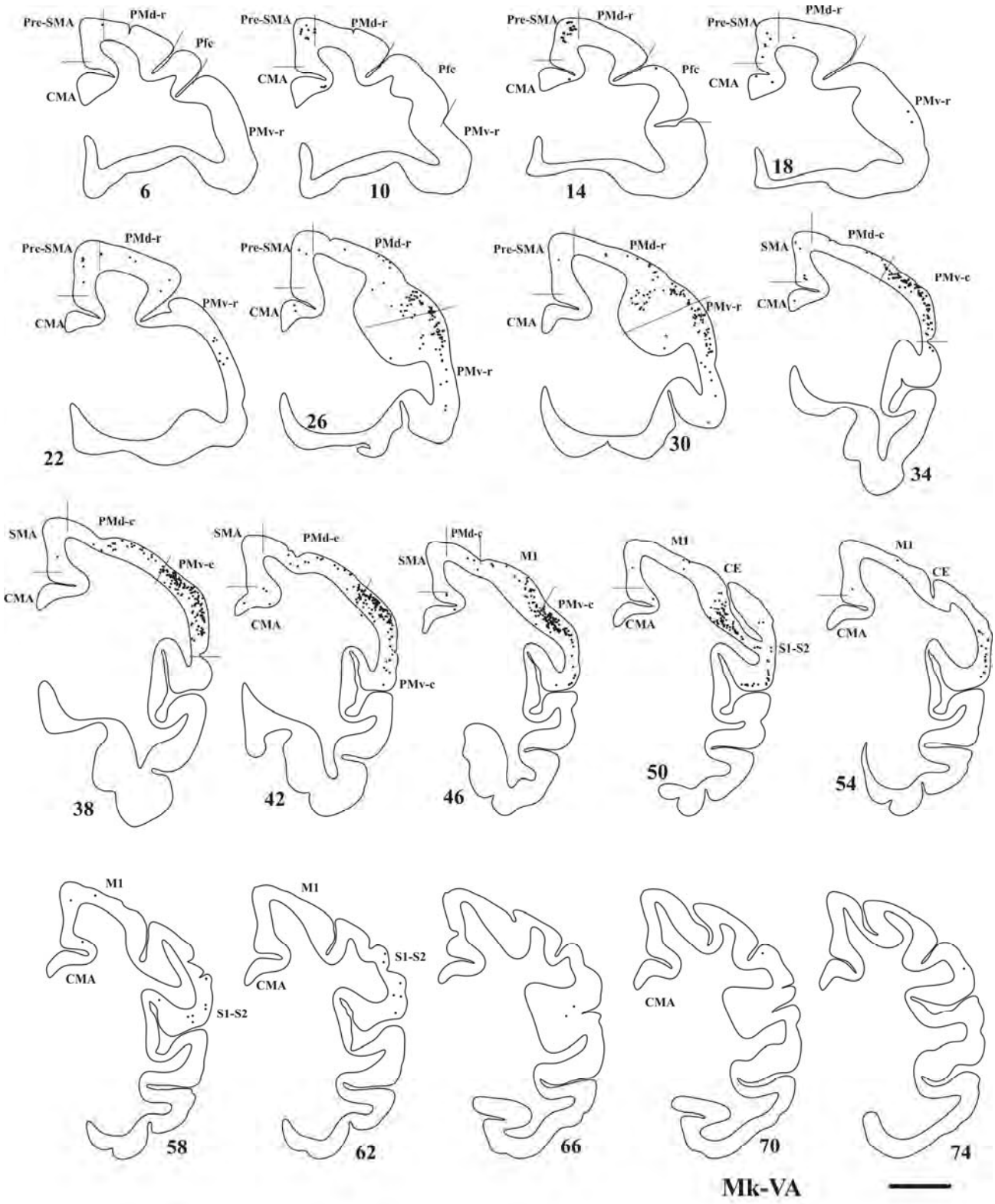

Figure 6

Figure 6: Frontal sections of the right hemisphere in Mk-VA (anti-Nogo-A antibody treated monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Same conventions as in Figures 3 and 4.

5) Normalization of the number of labelled callosal neurons with respect to the volume of injection site

The main goal of the present study is to test the possible influence of the anti-Nogo-A antibody treatment on the callosal connectivity after lesion of M1. Therefore, focus will be placed on the comparison between the two groups of monkeys subjected to M1 lesion, namely on the control group (no treatment) and on the group treated with anti-Nogo-A antibody (Fig. 7).

As there was some variability across monkeys with respect to the sites selected (based on ICMS maps post-lesion) for BDA injections, distributed mainly in PMd (and in some cases additional sites in PMv), a specific analysis of the distribution of labelled cells in the distinct sub-regions of the various motor cortical areas would be meaningless. For this reason, only three large cortical zones were considered (Fig. 7A): the mesial cortex (pre-SMA, SMA and CMA), the premotor cortex (PM, including few neurons labelled in the prefrontal cortex) and the sensorimotor cortex (M1, post-central gyrus).

To account for the inevitable variability across monkeys due to the size of BDA injection sites (guided by ICMS maps) and the uptake of the tracer, the callosal data were normalized based on the volume of the core of the corresponding BDA injection site. The normalization was obtained by dividing the number of retrogradely labelled callosal neurons in each cortical area by the volume of the core of the injection site in mm^3. The normalized numbers of callosal BDA-labelled neurons could thus be better compared across monkeys, in spite of the individual variability of the BDA uptake (Fig. 7A). In addition, Figure 7A provides an estimate of the contribution of the premotor area (PMd and PMv), the mesial cortex (pre-SMA, SMA and CMA) and the sensorimotor cortex (M1, S1 and S2) to the overall callosal connectivity reaching the ipsilesional PM. The premotor area also comprises the few labelled neurons observed in the prefrontal cortex, ranging across monkeys between 0 and about 2% of the total number of labelled cells.

In the group of control monkeys, the majority of callosal projections reaching the ipsilesional PM originated from the opposite PM, although a substantial number of projections came from the mesial cortex (Fig. 7A). The callosal projections originating from the opposite sensorimotor cortex (M1, S1 and S2) represented a fairly modest proportion. In the group of anti-Nogo-A antibody treated monkeys, one animal (Mk-LA) exhibited a normalized number of callosal labelled neurons close to those observed for untreated monkeys (Fig. 7A): however, the treatment was shorter than in the other monkeys and the lesion in M1 was very small, without persisting deficit (Mk-LA is thus discarded from further

analysis). In contrast, the other three anti-Nogo-A antibody treated monkeys exhibited quantitatively higher normalized numbers of callosal labelled neurons than the untreated monkeys. For instance, the anti-Nogo-A antibody treated monkeys Mk-MO and Mk-SL yielded about twice more labelling in PM in the intact hemisphere than the untreated monkey with the highest normalized values (Mk-BI), although the BDA injection site was comparable (Mk-MO) or even smaller (Mk-SL; see Fig. 1A). In the treated monkeys Mk-VA, the amount of normalized callosal labelled neurons reached a level close to seven times higher than in the control monkey Mk-BI. The difference was even larger when comparing the anti-Nogo-A antibody treated monkeys Mk-VA, Mk-SL and Mk-MO with the other two control monkeys Mk-RO and Mk-GE (Fig. 7A). Overall, the data presented in Figure 6A are consistent with an increase of labelled callosal neurons in the anti-Nogo-A antibody treated monkeys as compared to control (untreated) monkeys, all subjected to a lesion in M1. The difference is mainly due to an increase of labelling in the homotypic region (PM) of the anti-Nogo-A antibody treated monkeys.

Although the volume of BDA injected is a major parameter influencing the present tracing data, the size of the M1 lesion may also impact on the retrograde labelling. To address this issue, the normalized total numbers of callosal neurons in the intact hemisphere were plotted for each monkey in a 3D plot, as a function of the volume of the core of the BDA injection sites and the volume of the cortical lesion affecting the gray matter (Fig. 7B). The monkey Mk-LA is represented in red as it can be considered as an outlier (very small lesion, nearly no functional deficit post-lesion, anti-Nogo-A antibody treatment restricted to 2 weeks; see discussion). In the anti-Nogo-A antibody treated monkeys (blue dots in Fig. 7B), the data indicate an increase of the normalized total number of labelled callosal neurons (especially in Mk-VA), as compared to the control, untreated monkeys. This effect is not due to larger BDA injection sites in the anti-Nogo-A antibody treated monkeys as their sizes overlap those in the control monkeys (Fig. 7B). Similarly, the volumes of lesion in the motor cortex were in a fairly comparable (overlapping) range for the two groups of monkeys.

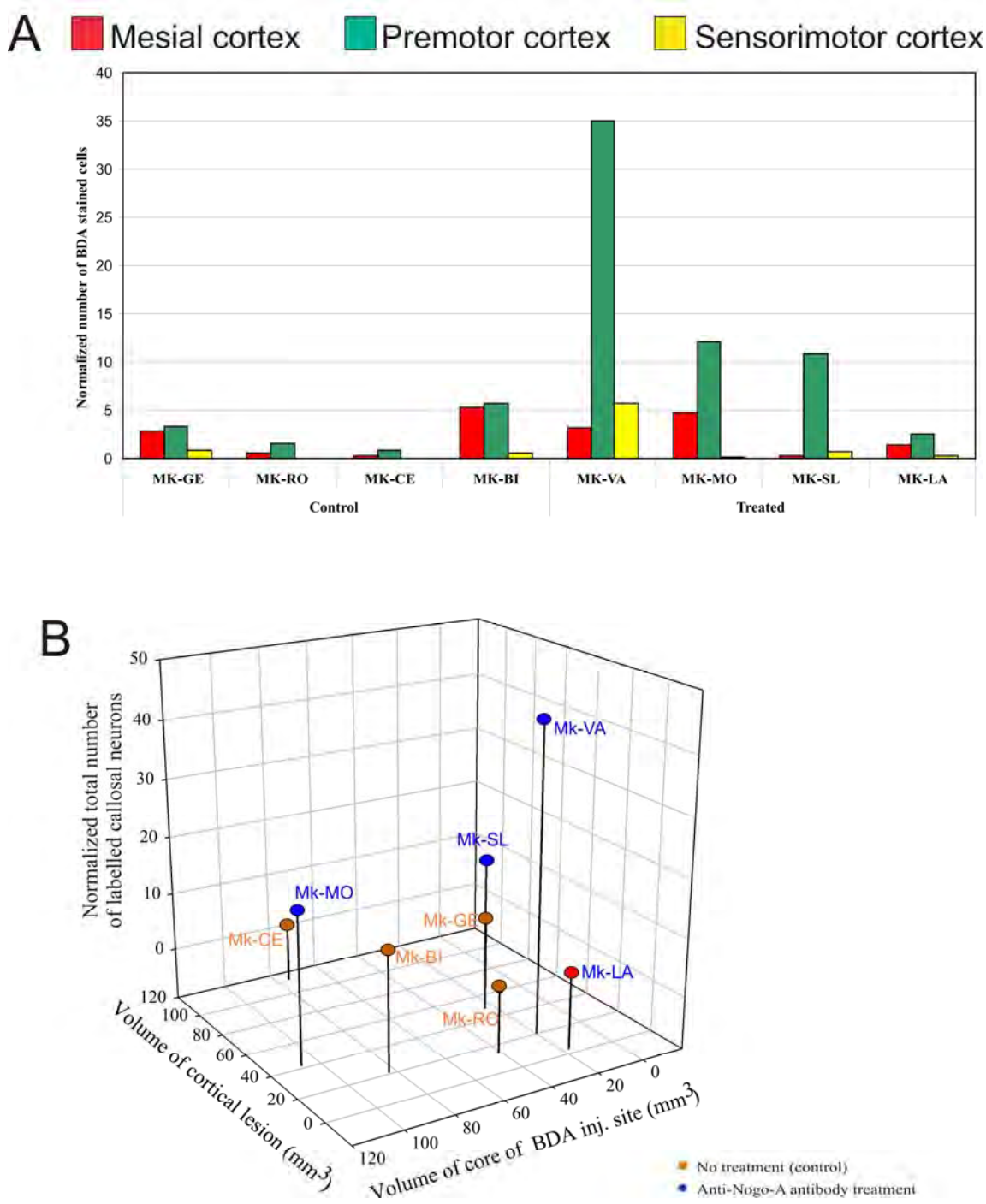

Figure 7

<u>Figure 7</u>: <u>A</u>: Normalized number of BDA labelled callosal neurons in the two groups of monkeys, observed in the intact hemisphere, in each case and across three main cortical zones (premotor cortex, mesial cortex and sensorimotor cortex). See text for the description of the procedure of normalization with respect to the volume of BDA injection site (core). The distribution of the absolute numbers of cells is shown in Table 1 and in supplementary Figure 5.

<u>B</u>: 3D plots showing the relationship of the normalized total number of labelled callosal neurons with the lesion volume and the volume of the core of the BDA injection sites (see text). Mk-LA (in red) represents an outlier (see text).

Discussion

Survey of data

In the present study, we have investigated the reorganization of callosal connectivity of the ipsilesional PM following a unilateral lesion of M1 in adult macaques. The monkeys treated with the anti-Nogo-A antibody exhibited a highly enhanced callosal connectivity reaching the ipsilesional PM, based on the normalized numbers of retrogradely labelled neurons in the intact hemisphere, in comparison to the control (untreated) monkeys. As depicted in Figure 7A, the enhancement of the callosal connection from the intact hemisphere to PM induced by the anti-Nogo-A antibody treatment was essentially present for the homotypic projection from PM of the intact hemisphere to the ipsilesional PM. The latter conclusions hold true for 3 anti-Nogo-A antibody treated monkeys (Mk-VA, Mk-MO and Mk-SL), but not for the fourth animal (Mk-LA). However, the latter monkey differed from the other three treated monkeys by a much smaller lesion in M1, an absence of substantial and persisting deficit of manual performance in the contralesional hand in the days and weeks post-lesion. Even more importantly, Mk-LA received the anti-Nogo-A antibody only during two weeks, instead of four weeks in the other three monkeys (Mk-LA can thus be considered as an outlier: see Fig. 7B). One may interpret the low normalized number of labelled callosal neurons in Mk-LA as an insufficient duration of treatment to produce a significant sprouting of callosal axons. In addition, if the lesion is especially small, the treatment may be less efficient in promoting a re-organization of connectivity.

The difference in the normalized numbers of retrogradely BDA-labelled callosal cells (Fig. 7A) between the groups of control (Mk-GE, Mk-RO, Mk-BI, Mk-CE) and treated (Mk-VA; Mk-SL, Mk-MO) monkeys is not due to differences related to the duration of the survival time after BDA injection (Table 1). Indeed, the survival time was comparable for 5 of these monkeys (21 or 22 days), whereas it was longer in the control monkey Mk-GE than in the treated monkey Mk-SL, which would have provided the control animal more time for retrograde transport.

Re-organization of corticocortical connectivity after lesion of M1

In a recent, very extensive and quantitative study, Dancause et al. (2005) have investigated the re-arrangement of the intra-hemispheric connectivity of PM after lesion of the homolateral M1 in squirrel monkeys. It was found that projections originating from PM and directed to M1 in the intact animal were re-directed to the post-central sulcus, namely to S1. Such intra-hemispheric redirection of axons was not investigated in the present study,

focussed on inter-hemispheric connections. However, in line with the data of Dancause et al. (2005), one may speculate that the callosal axons originating from the intact hemisphere and directed to the lesioned M1 and perilesional territories may have been redirected in the lesioned hemisphere, maybe towards the post-central gyrus as well. This possibility could not be tested in the present material, as injections in the intact hemisphere are needed to test this hypothesis (based on anterograde labelling).

The originality of the present study is essentially that the reorganization of the connectivity of PM, callosal in the present case, has been investigated in relation to a treatment (anti-Nogo-A antibody), that enhances fiber growth and plasticity in the injured and intact brain (Montani 2009; Schwab 2010). We observed a better functional recovery of manual performance in the anti-Nogo-A treated animals as compared to untreated monkeys (see preliminary data in Fig. 2). As illustrated in Figure 8 for control (untreated) monkeys (left panel), the lesion of M1 may be followed by a limited spontaneous regenerative sprouting of axons affected by the lesion (blue axon terminal). As such sprouting may be very limited in distance, injection of BDA in the ipsilesional PM may not label the corresponding neuron of origin. In contrast, in the anti-Nogo-A antibody treated monkeys (Fig. 8, right panel), the regenerative sprouting may extend on a longer distance (green axon terminal prolonging the blue axon terminal). In addition, compensatory sprouting of intact axon terminals originating from a larger cortical zone in the intact hemisphere may take place (other green axon terminals in right panel of Fig. 8), some of which may be labelled after BDA injection in the ipsilesional PM. As a result, more retrogradely labelled callosal neurons are labelled in the intact hemisphere than in control monkeys (Fig. 7). As the callosal connectivity is predominantly homotypic, the intact PM gives rise to more axon terminals in the ipsilesional PM than projections originating from other cortical areas of the intact hemisphere (Fig. 8). As a result, in the injected ipsilesional PM, more compensatory sprouting is likely to occur for the axons originating from the opposite PM than for axons coming from other cortical areas of the intact hemisphere. This interpretation is in line with the present observation (Fig. 7A) that the enhancement of callosal connectivity observed in the anti-Nogo-A antibody treated monkeys (as compared to control monkeys) was found predominantly for the homotypic projection from the intact PM to the ipsilesional PM. Furthermore, although speculative, it may be hypothesized that reactive events in the lesion and peri-lesion territories stimulate sprouting, by activation of an injury-associated molecular program (e.g. Li et al. 2010). Such sprouting event may be limited in control monkeys, but enhanced in treated monkeys, a

phenomenon possibly more effective in territories close by the lesion (e.g. PM) than in more distant cortical areas (e.g. SMA, CMA).

As an alternative explanation to enhanced sprouting, one cannot exclude that the lesion of M1, in particular its consequences during the following hours and days (inflammation, scar formation, etc), interrupts callosal axons reaching the ipsilesional PM, especially if the lesion encroached the white matter. A possible effect of anti-Nogo-A antibody treatment could have been to preserve some of the callosal axons from such damage, resulting in an increase of callosal connectivity in the treated monkeys. Such mechanism of preservation might have taken place in Mk-SL, in which the lesion affected significantly the white matter (sub-cortical territory represented in gray in Fig. 1). As indicated in Table 1, the lesion encroached the white matter in only one other monkey (Mk-CE), but to a lesser extent than Mk-SL. Overall, there is no systematic relation between the effect of anti-Nogo-A antibody treatment on callosal connectivity and the absence/presence of impact of the lesion on the white matter (Table 1). However, pro-survival effects of anti-Nogo-A antibodies or other Nogo-A suppressive treatments have never been observed up to now in a variety of different CNS lesion models, in contrast to the well documented effects on nerve fiber sprouting and regeneration in spinal cord injury models demonstrating substantial sprouting (regenerative and compensatory), promoted by anti-Nogo-A antibody treatment (e.g. Gonzenbach and Schwab 2008; Fouad et al. 2004; Freund et al. 2007). Although not excluded, this explanation of preservation appears less likely therefore at present.

At that step, a relationship between the enhanced callosal connectivity of PM promoted by anti-Nogo-A antibody (Fig. 7B) and an improved functional recovery remains speculative, although most likely probable. To demonstrate such an effect, it would be necessary to transiently inactivate PM in the intact hemisphere of the anti-Nogo-A antibody treated monkeys several months after the lesion and hopefully obtain a loss of the enhanced functional recovery of the manual performance (an experiment not done in the present study). Such reversible inactivation of PM in the intact hemisphere after unilateral lesion of M1 was however performed with muscimol infusion in two monkeys not subjected to any treatment in our original study introducing this model of motor cortex lesion (Liu and Rouiller 1999): in line with the hypothesis of a modest sprouting of the callosal projections in control monkeys (Fig. 8, left panel), the reversible inactivation of the contralesional PM did not affect the incomplete spontaneous recovery of manual performance in the two untreated monkeys.

In spite of the lesion of M1, the general pattern of callosal connectivity of PM found in the present study in both control and anti-Nogo-A antibody treated monkeys remains

generally consistent with previous reports in intact monkeys (Marconi et al. 2003; Boussaoud et al. 2005 for macaque monkeys; see also Fang et al. 2008 in a prosimian primate), with a predominance of an origin in the homotypic motor cortical area PM, a feature even accentuated as a result of the anti-Nogo-A antibody treatment. In addition, in line with these previous studies in intact monkeys, callosal inputs to the ipsilesional PM also originate from other source areas, such as pre-SMA, CMA, Pfc, SMA and, to a lesser extent, M1 and S1-S2 (Fig. 7A).

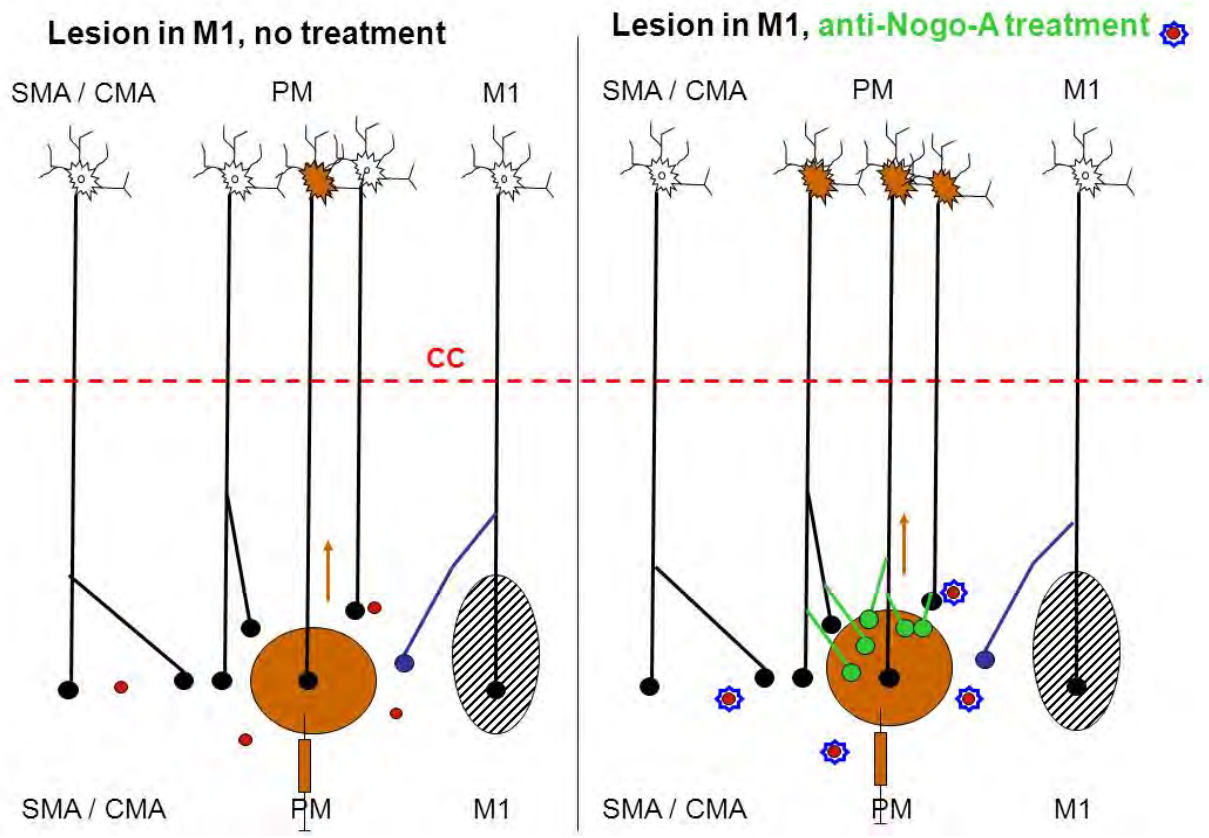

Figure 8: Schematic and speculative representation of the pattern of callosal projection reaching PM in monkeys subjected to a permanent lesion of M1 (black hatched area) and interpretation of the present results. In the left panel, the control (untreated) monkeys exhibit a modest, spontaneous regenerative axonal sprouting of the callosal projection reaching the ipsilesional PM (blue axon terminal). In the right panel, in contrast, the anti-Nogo-A antibody treatment promotes local compensatory sprouting of the callosal axons (green axon terminals), in particular in PM where BDA was injected. In other words, the results of the present study are consistent with an enhanced number of callosal neurons in the homotypic PM of the intact hemisphere in the anti-Nogo-A antibody treated monkeys as compared to the control (untreated) monkeys. The horizontal dashed red line represents the corpus callosum, with the intact hemisphere above and the lesioned hemisphere below. Neurons in the intact hemisphere send callosal projections to the lesioned hemisphere, terminating with axonal boutons (small filled circles). The red

circles represent Nogo-A, inhibiting axonal growth in the adult central nervous system. In the right panel (treated monkeys), Nogo-A is neutralized with an anti-Nogo-A antibody. The brown syringe represents the injection of the tracer BDA in the ispilesional PM, whereas the brown arrows are for the retrograde axonal transport of BDA from the injected PM to the intact hemisphere. As a result, some neurons are retrogradely labelled with BDA (brown soma in the intact hemisphere). The area "SMA" comprises both pre-SMA and SMA-proper, whereas the area "CMA" includes the three subdivisions of CMA.

Limitations in the interpretation of the data

First of all, as it is generally the case for non-human primate studies, the number of animals is limited, mainly for ethical reasons. Second, as each case was performed one after the other during several years, there were some adjustments in the protocol, mainly in relation to the main objective of the study dealing with the degree of functional recovery. There is an inevitable variability across monkeys with respect to the lesion size and location (Fig. 1A; see Kaeser et al. 2010 for a more detailed discussion on that issue). Other parameters are the location and size of BDA injections, as well as the efficacy of BDA uptake, which were in part compensated in the present study by normalizing the number of retrogradely labelled callosal neurons by the volume of the injection site (core). As shown in Figure 7, a further difficulty of interpretation is the multiplicity of parameters which may influence the results, such as lesion size and volume of the core of the BDA injection sites. In Mk-SL for instance, the lesion spread largely sub-cortically in the white matter (see Fig. 1A), more than in the other monkeys, having possibly interrupted callosal fibers. As a result, the number of labelled callosal neurons in Mk-SL was especially low in spite of a large volume of BDA injected.

In spite of the above limitations of interpretation, the present study supports the hypothesis that, after lesion of M1, the callosal projections reaching the ipsilesional PM are enhanced after anti-Nogo-A antibody treatment. The proposed mechanism is an enhancement of sprouting of callosal axons near their target, promoted by the neutralization of the neurite growth inhibitor Nogo-A (see Fig. 8). Such a mechanism of axonal sprouting near a lesioned territory (PM in that case) is comparable to the increased sprouting of CS axons observed in spinal cord injured monkeys treated with the anti-Nogo-A antibody, as compared to control antibody treated monkeys (Fouad et al. 2004; Freund et al. 2006, 2007). Future analyses are needed to determine whether projections to PM, other than callosal ones, are also promoted by the anti-Nogo-A antibody treatment in monkeys with lesion of M1, such as the thalamocortical projection for instance.

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Conflict of interest

The antibodies were provided by Novartis Pharma AG.

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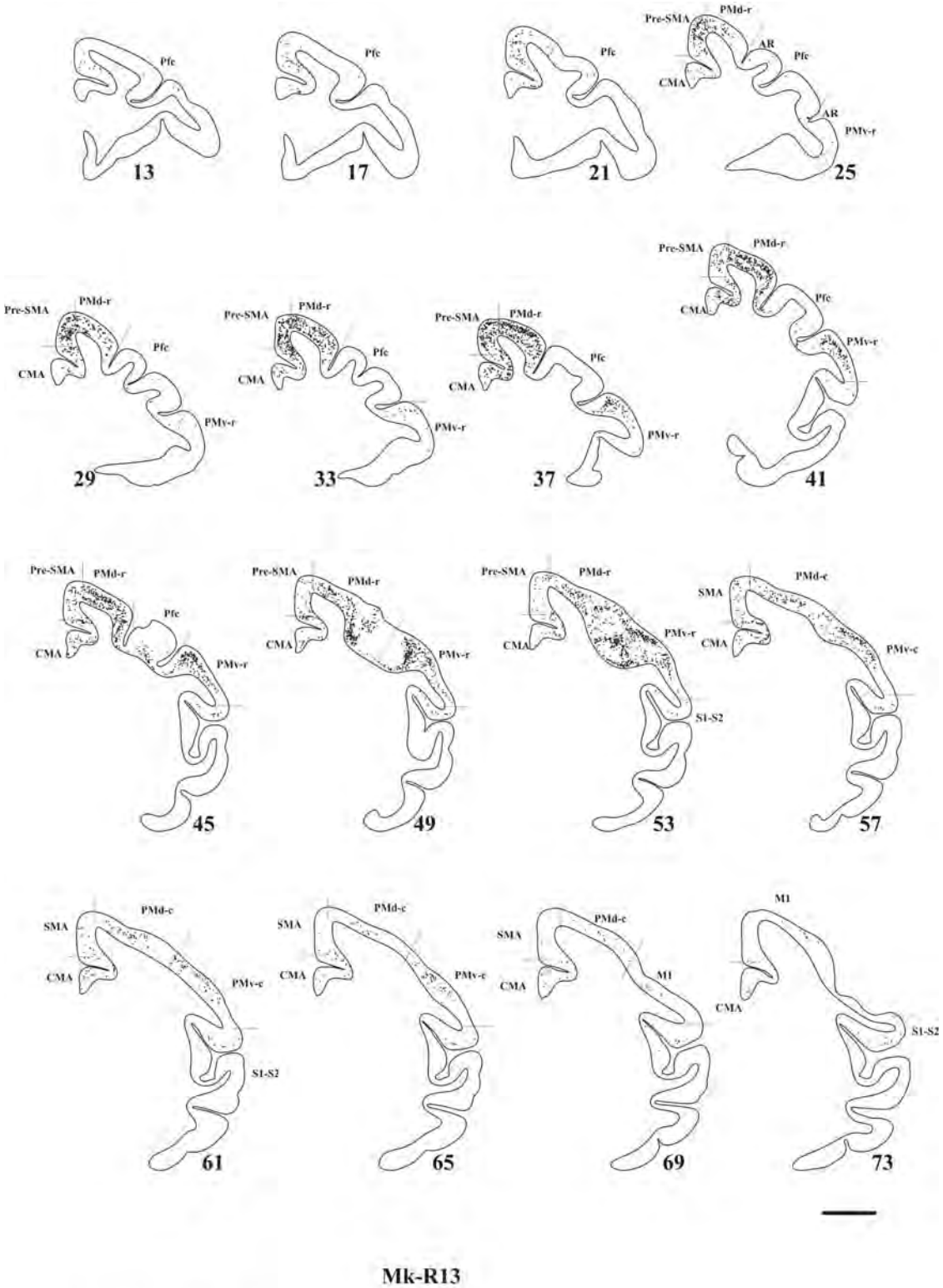
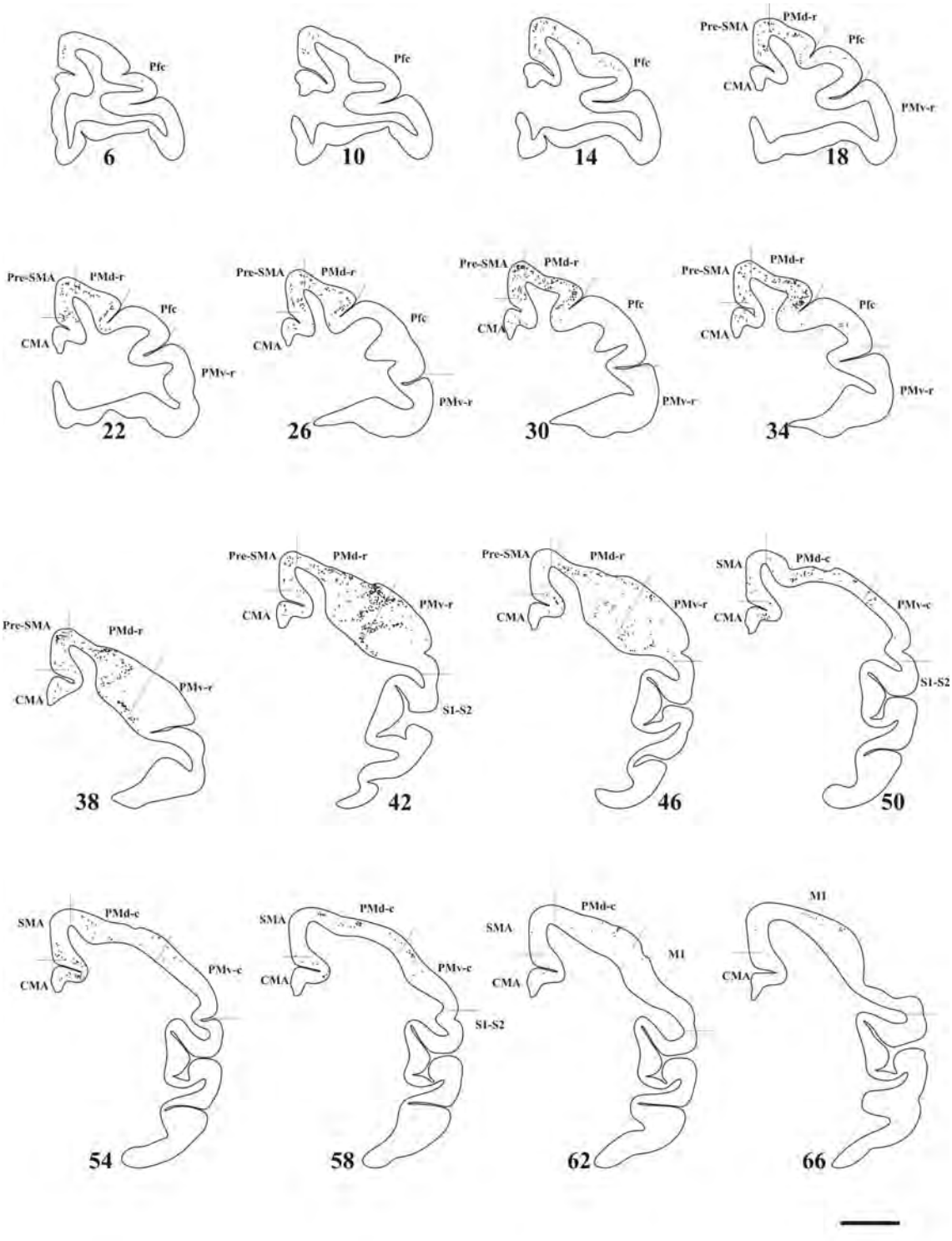
Supplementary figures



Supplementary Figure 1

<u>Supplementary Figure 1:</u>

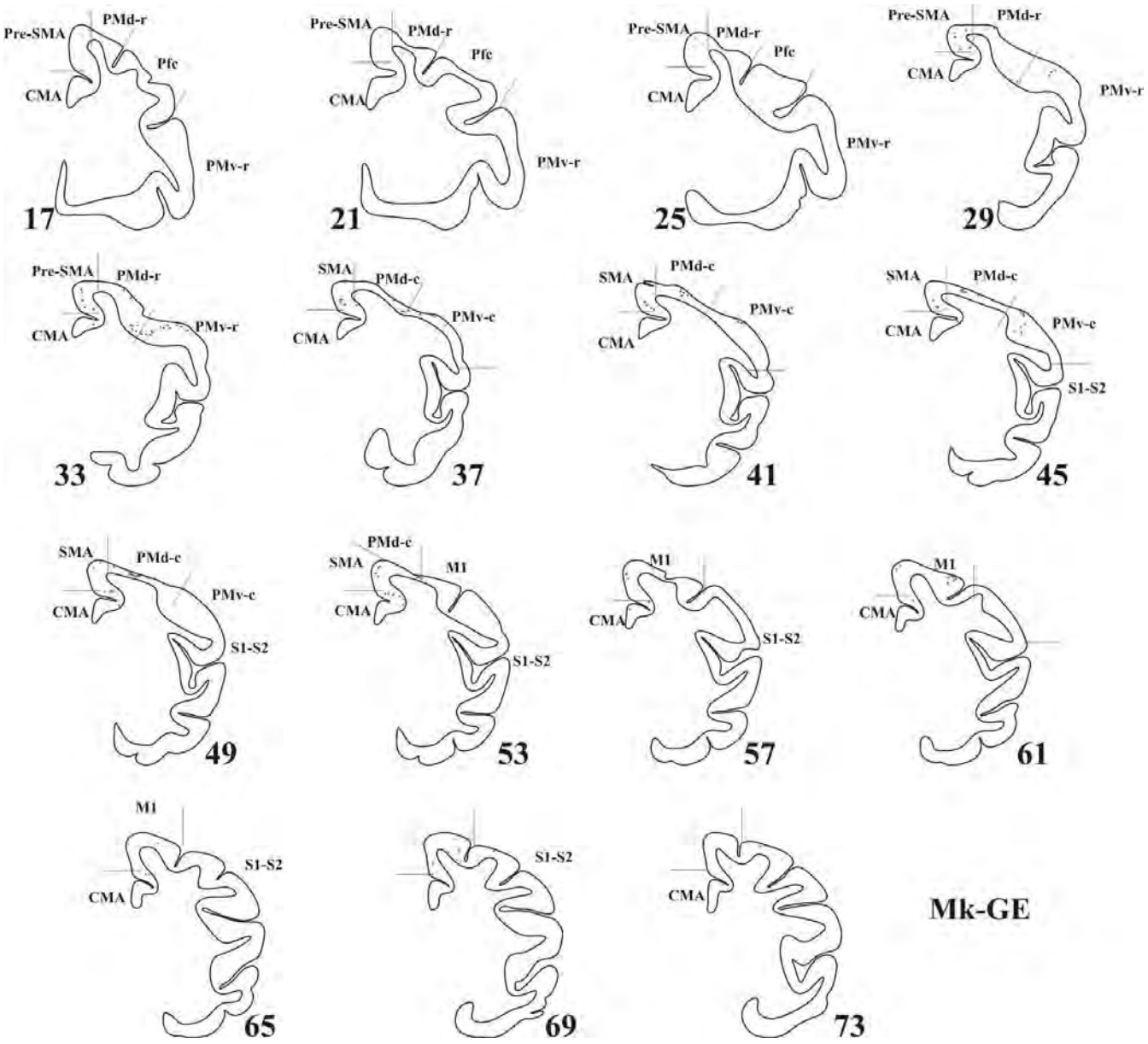
Frontal sections of the right hemisphere in Mk-R13 (intact monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Same conventions as in Figures 3-5.



Supplementary Figure 2

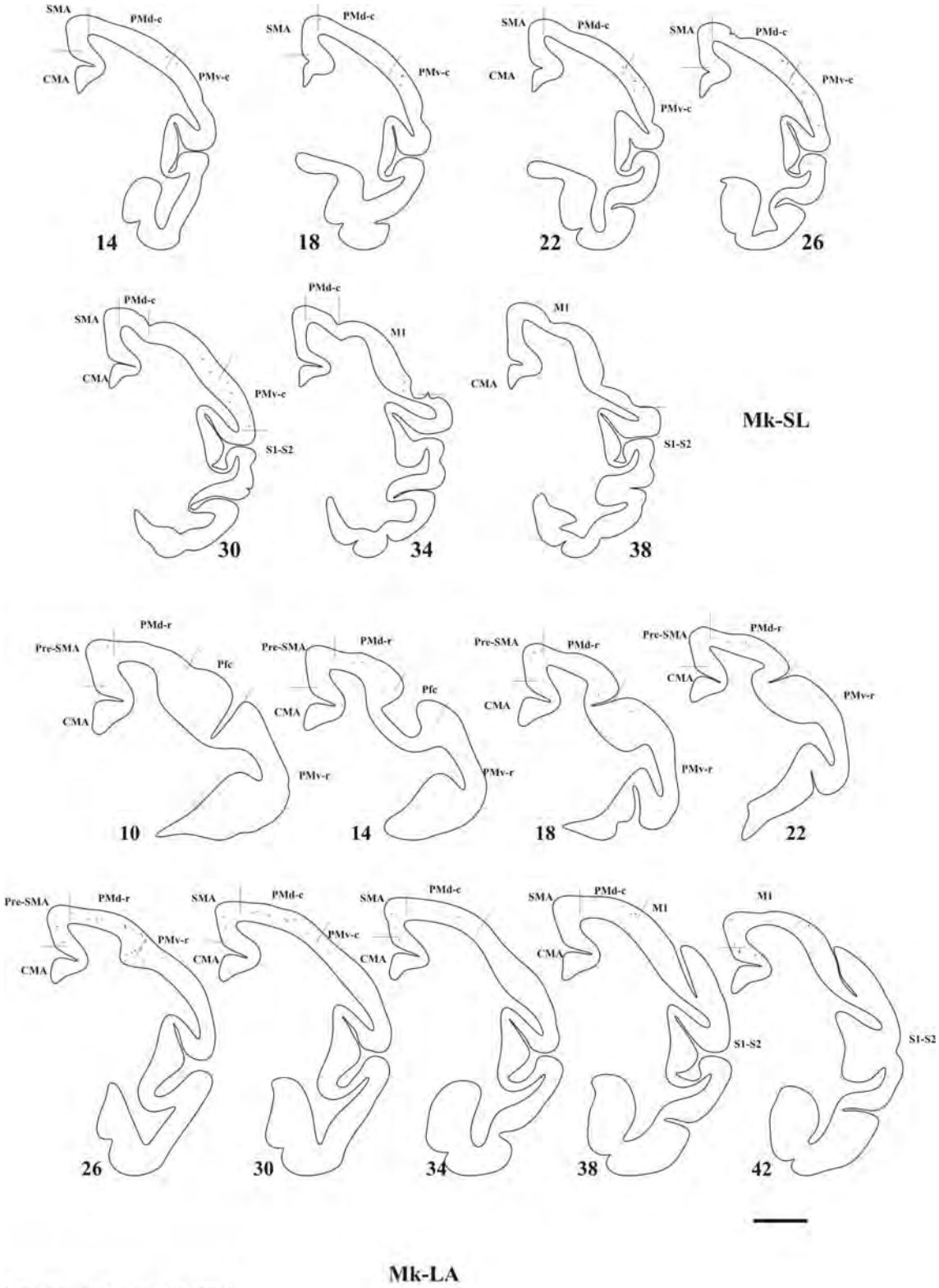
Supplementary Figure 2

Frontal sections of the right hemisphere in Mk-R12 (intact monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Same conventions as in Figures 3-4.



Supplementary Figure 3:

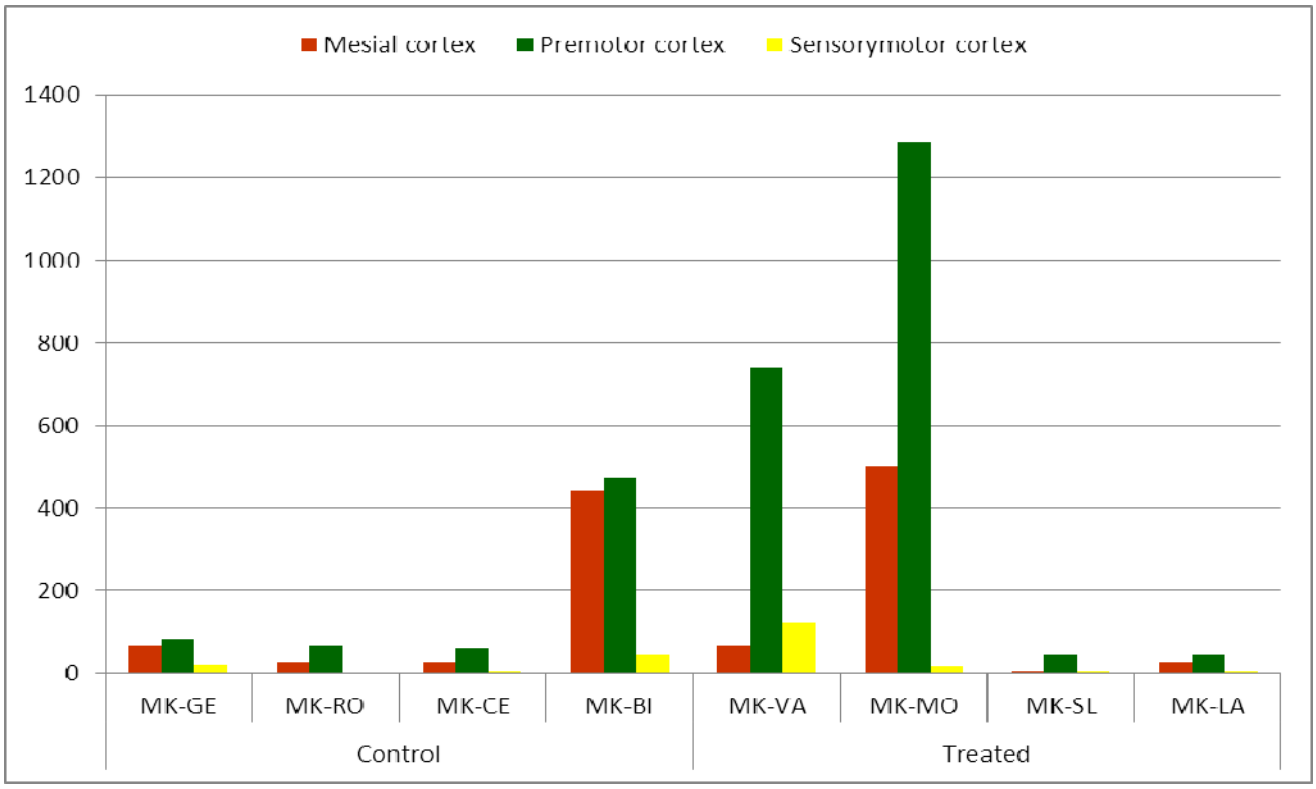
Frontal sections of the right hemisphere in Mk-GE (lesioned, control monkey), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Same conventions as in Figures 3-4.



Supplementary Figure 4

Supplementary Figure 4

Frontal sections of the right hemisphere in two monkeys with M1 lesion and treated with anti-Nogo-A antibody (Mk-SL and Mk-LA), arranged from rostral to caudal, showing the distribution of retrogradely labelled callosal neurons as a result of BDA injection in the opposite PM. Same conventions as in Figures 3-4.



<u>Supplemetary Figure 5</u>:

Distribution of the absolute number of BDA labelled callosal neurons in the two groups of monkeys, observed in the intact hemisphere for each monkey and across the three main cortical zones (premotor cortex, mesial cortex and sensorimotor cortex).

3.5 Chapitre 5:

Thalamocortical projections to the premotor cortical areas following unilateral lesion of primary motor cortex (M1) in the macaque monkeys in presence or absence of anti-Nogo-A antibody treatment

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Abstract

The premotor cortical area (PM) contributes to the preparation and programming of skilled movements. While the thalamocortical projections to M1, the dorsal premotor cortex (PMd) and the ventral premotor cortex (PMv) have been extensively described in intact macaques, almost nothing is known about the patterns of thalamocortical projections to PM after unilateral lesion of M1, representing an important issue as PM was shown to contribute to the incomplete functional recovery (Liu and Rouiller, 1999). The goal of the present study was to investigate the thalamocortical projections to PM in macaque monkeys after unilateral lesion of M1 and to assess the influence of anti-Nogo-A antibody treatment.

The origin of the thalamic projections to PM was derived from injections of the retrograde neuroanatomical tracer BDA into PM in ten adult monkeys. Three monkeys were intact and seven monkeys were subjected to a unilateral cortical lesion produced by microinfusion of ibotenic acid in the hand area of M1. In the lesioned monkeys, three monkeys were treated with the anti-Nogo-A antibody and four monkeys were untreated. The anti-Nogo-A antibody treatment was delivered immediately after the lesion during 4 weeks.

Following hand dexterity recovery, BDA was injected in PM of the lesioned hemisphere. The distributions of BDA-labelled neurons in the thalamus was plotted and then superimposed to photomicrographs obtained from the corresponding Nissl and/or SMI-32 stained sections. The number of retrogradely BDA-labeled thalamocortical neurons was normalized based on the volume of the BDA injection sites in PM. The normalized number of labeled thalamocortical neurons was slightly lower in the monkeys subjected to M1 lesion, as compared to the intact monkeys. However, there was no systematic difference between the untreated and the anti-Nogo-A antibody treated monkeys. The distribution of labeled neurons across the thalamic nuclei of origin was comparable in the 3 subgroups of monkeys, with a predominance in the ventral thalamic nuclei, mainly VL.

Introduction

The premotor cortical area (PM) is thought to contribute to the programming of skilled movement and the sequencing of motor tasks. Based of anatomical and functional criteria, the area PM comprises a dorsal (PMd) part separated from a ventral (PMv) part (e.g. Kurata et al., 1991; Kurata and Hoffman, 1994; Kurata et al., 1994). While the thalamocortical projections of M1, PMd and PMv have been extensively described in intact adult macaques (Strick, 1975; Schell and Strick, 1984; Jones, 1987; Matelli et al., 1989; Darian-Smith et al., 1990a,b;

Nakano et al., 1992, 1993; Kurata et al., 1994; Matelli et Luppino, 1996; Rouiller et al., 1999; Morel et al., 2005; Cappe et al., 2007; Stepniewska et al., 2007), almost nothing is known about the patterns of thalamocortical projections to PM after unilateral lesion of M1 and the possible influence of anti-Nogo-A antibody treatment on this projection system.

The study of Dancause and collaborators (2005) demonstrates that the intra-hemispheric connectivity of a motor cortical area (PM in the present case) is subjected to spontaneous and significant changes, such as rewiring, as a result of M1 lesion. Such re-arrangement is likely to be the anatomical support for (incomplete) spontaneous recovery from the lesion. We demonstrate also in our previous study (chapter 4 of the present thesis) that the callosal connectivity of PM is modified and enhanced as a result of a treatment promoting better functional recovery, by neutralizing the neurite growth inhibitor Nogo-A.

The goal of the present study was to assess the thalamocortical projections of premotor cortical areas PM in macaque monkeys after unilateral lesion of primary motor cortex (M1), either in absence or in presence of anti-Nogo-A antibody treatment. In the present study, we reconstructed the origin of the thalamic projections to PM by injecting the retrograde neuroanatomical tracer BDA into PM in 3 subgroups of monkeys: i) intact monkeys; ii) monkeys subjected to M1 lesion without any treatment; iii) monkeys subjected to M1 lesion and treated with anti-Nogo-A antibody.

In sensory (visual and somatosensory) and motor systems, a survey of the literature related to lesion induced plasticity, irrespective of the lesion site (at periphery, in the thalamus or in the cerebral cortex), there is a general trend towards a stability of the thalamocortical projection, which exhibits much less re-organization than corticocortical connections (see e.g. Sharp and Gonzalez, 1986; Miller et al., 1991; Darian-Smith and Gilbert, 1995; Calford et al., 2003; Croquelois et al., 2005). Based on these previous studies, one may predict that the lesion of M1 should not dramatically modify the thalamocortical projections directed to PM. However, considering a more recent study (Oberlaender et al., 2012), some changes in the thalamocortical projection cannot be excluded, as these authors reported reorganization of the thalamocortical projection following trimming of the wiskers.

Materials and Methods

The present retrograde tracing data are derived from experiments conducted on ten adult monkeys (*Macaca fascicularis*; Table 1), with ages ranging between 4 and 10 years and weighting between 4 and 10 kg. All monkeys subjected to M1 lesion, except monkey Mk-SL (no labelling in the thalamus), were the same ones as used in our previous study for the

investigation of the callosal connectivity of the premotor cortex (see chapter 4 of the present thesis). Surgical procedures and animal care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and were approved by local veterinary authorities.

Table 1: Summary of experimental cases

	Intact			Untreated				Treated with Anti-Nogo_A antibody		
	Mk-CH	**Mk-R13**	**Mk-R12**	**Mk-CE**	**Mk-GE**	**Mk-RO**	**Mk-BI**	**Mk-VA**	**Mk-MO**	**Mk-LA**
Species	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.
Lesion	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sex of animals	Male	Male	Male	Male	Female	Male	Male	Female	Male	Female
Treatment	None	None	None	None	None	None	None	Anti-Nogo-A antibody	Anti-Nogo-A antibody	Anti-Nogo-A antibody
Age at time of lesion (rounded 0.5 year)	10	4.5	5	4.5	5	4	5	5.5	5.5	5
Weight at time of lesion (Kg)	6[#]	4[#]	4[#]	3.8	2.8	3.2	5	4.9	5.6	2.6
Volume of BDA injected (µL)	8	8.8	7.2	16	2.1	4.8	7.2	5	10.8	2
Nb. of BDA sites injected	10	11	9	16	5	6	11	5	12	5
Areas where BDA is injected	PMd/PMv	PMd/PMv	PMd	PMd	PMd/PMv	PMd	PMd/PMv	PMd/PMv	PMd/PMv	PMd/PMv

Under species, "fasc" is for *macaca fascicularis*
[#] Weight at time of BDA injection

Note: the monkey Mk-SL (anti-Nogo-A antibody treated) considered in the study on callosal connectivity (see chapter 4 of the present thesis) was not included here, as no thalamocortical labelling was yielded (may due to the subcortical lesion in the white matter which may have interrupted the thalamocortical axons but not the callosal axons).

Surgery

The control and treated monkeys subjected to M1 lesion were implanted unilaterally with a chronic, stainless steel or tecapeek chamber giving access to M1, the dura mater being however left in place (see Schmidlin et al., 2004 for detail). The monkeys were sedated with i.m. injection of ketamine (Ketalar, 5 mg/kg) and pre-medicated as previously described, in particular with injection of 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 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). The surgery was carried out under continuous monitoring of the following parameters: heart rate, respiration rate, expired CO_2, arterial O_2 saturation and body temperature. After surgery, the animals were treated with antibiotics (ampicilin 10%, 30 mg/kg, s.c.) and analgesics (pills of Rymadil mixed with food) during several days. The chambers were fixed to the skull with titanium screws and orthopedic cement (Palacos). The inside of the chronic chamber was cleaned daily with Betadine and an antibiotic ointment was spread on the dura mater surface to reduce the risk of infection.

Intracortical microstimulation (ICMS) procedures

Electrophysiological intracortical microstimulation (ICMS) sessions were performed twice, first before lesion in order to guide the lesion procedure by mapping the M1 hand representation and, second, several months after the lesion when the monkeys reached a stable manual performance in the motor tasks (see e.g. Rouiller et al. 1998; Liu and Rouiller 1999; Schmidlin et al. 2004; Freund et al. 2006, 2009). A tungsten microelectrode (0.1 - 1 MΩ impedance, FHC Inc, USA.) was used to micro-stimulate the motor cortex accessible from the chronic chamber, along penetrations performed at 1 mm distance from each others (see e.g. Schmidlin et al. 2004, 2005). Along each electrode track, ICMS was applied below the surface of the dura at intervals of 1 mm, along a variable distance depending on the position of the penetration with respect to the central sulcus (see Kaeser et al., 2010). The effect of ICMS was assessed by visual inspection and/or palpation of the body part (articulation) at which a movement was elicited. The minimal current (ICMS threshold) producing this movement was determined at each stimulation site. The ICMS map was finally represented in the form of an unfolded map, as previously reported (Park et al. 2001, 2004) and represented

the basis to guide injections of ibotenic acid to produce a permanent lesion of M1 targeted mainly on the hand area (pre-lesion ICMS sessions) and BDA injections in PM (post-lesion ICMS sessions).

Permanent lesion of M1 hand representation with ibotenic acid

Ibotenic acid (10μg/μl in phosphate-buffer) was infused using a Hamilton micro-syringe at selected intracortical microstimulation (ICMS) sites of the hand area in M1 unilaterally, as previously reported in detail (Liu and Rouiller, 1999; Kaeser et al., 2010). As a result of ibotenic acid infusion, the contralateral hand exhibited after a few minutes a dramatic flaccid paralysis. The location and extent of the lesion are shown on lateral views of the left hemisphere for all monkeys (Figure 1).

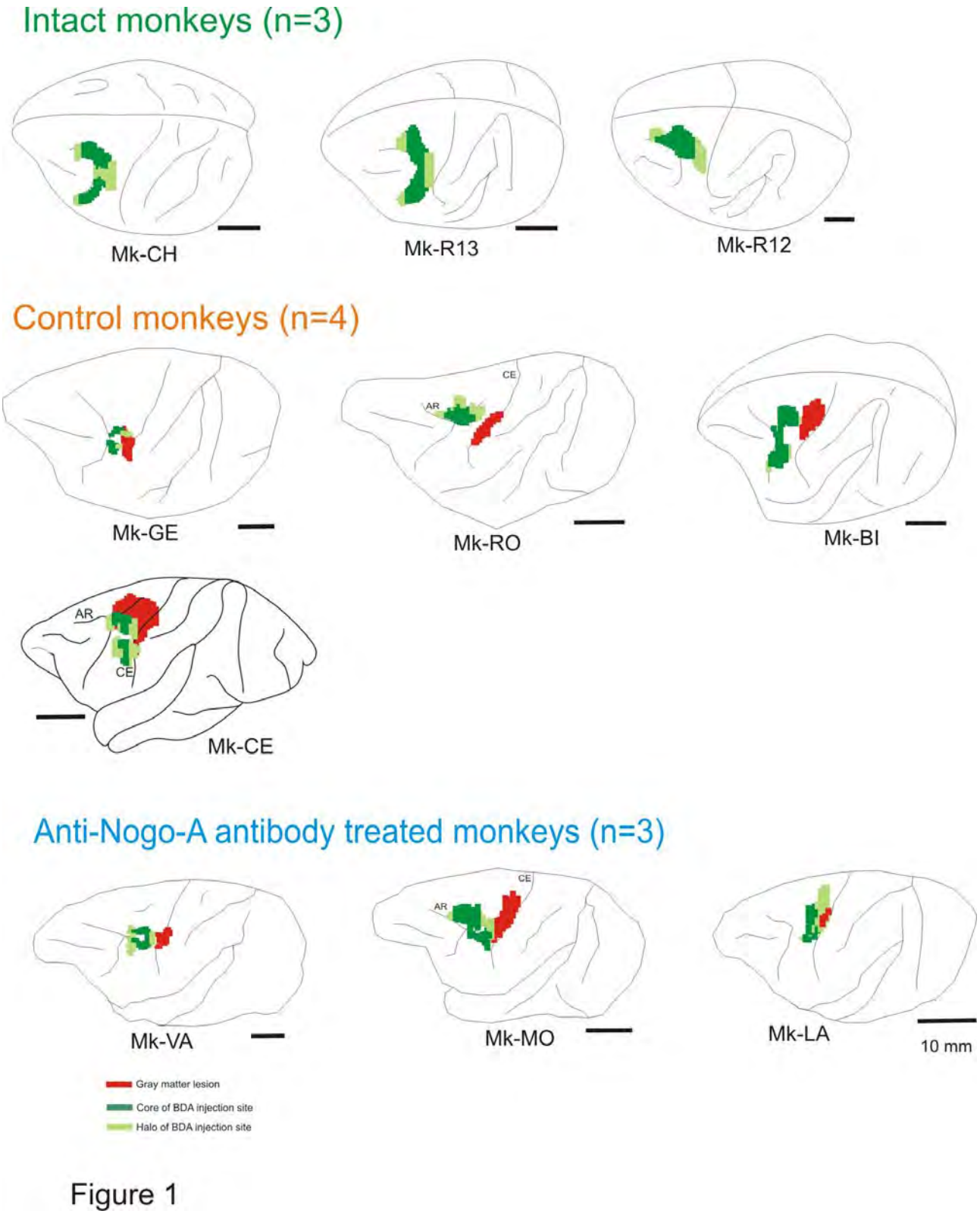


Figure 1

<u>Figure 1</u>: Lateral view of the left hemisphere in each monkey of the location and extent of the lesion aimed at the M1 hand representation (red territory) and BDA injection sites (territory in dark green for the core of the injection site and light green for the halo), as seen in transparency of the cortical surface.

Anti-Nogo-A antibody treatment

As outlined in Table 1, three of the seven monkeys subjected to unilateral permanent lesion of the motor cortex were treated with anti-Nogo-A antibody, delivered immediately after the lesion during four weeks except in Mk-LA in which the treatment lasted only 2 weeks). The anti-Nogo-A antibody treatment was similar to that applied to monkeys subjected to spinal cord injury (Freund et al., 2006, 2007, 2009), except that two osmotic pumps were implanted instead of one. The tube delivering the antibody from one osmotic pump was placed intrathecally in the cervical cord whereas the tube of the second osmotic pump was placed below the dura in the vicinity of the lesion of M1. The anti-Nogo-A antibody treatment was tested here for motor cortex lesion in monkeys, 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), as well as functional recovery after cortical lesion in rats (Papadopoulos et al., 2002; Emerick et al., 2003, 2004; Markus et al., 2005; Seymour et al., 2005; Tsai et al., 2007; Cheatwood et al., 2008; Gillani et al., 2009).

BDA injection and histology

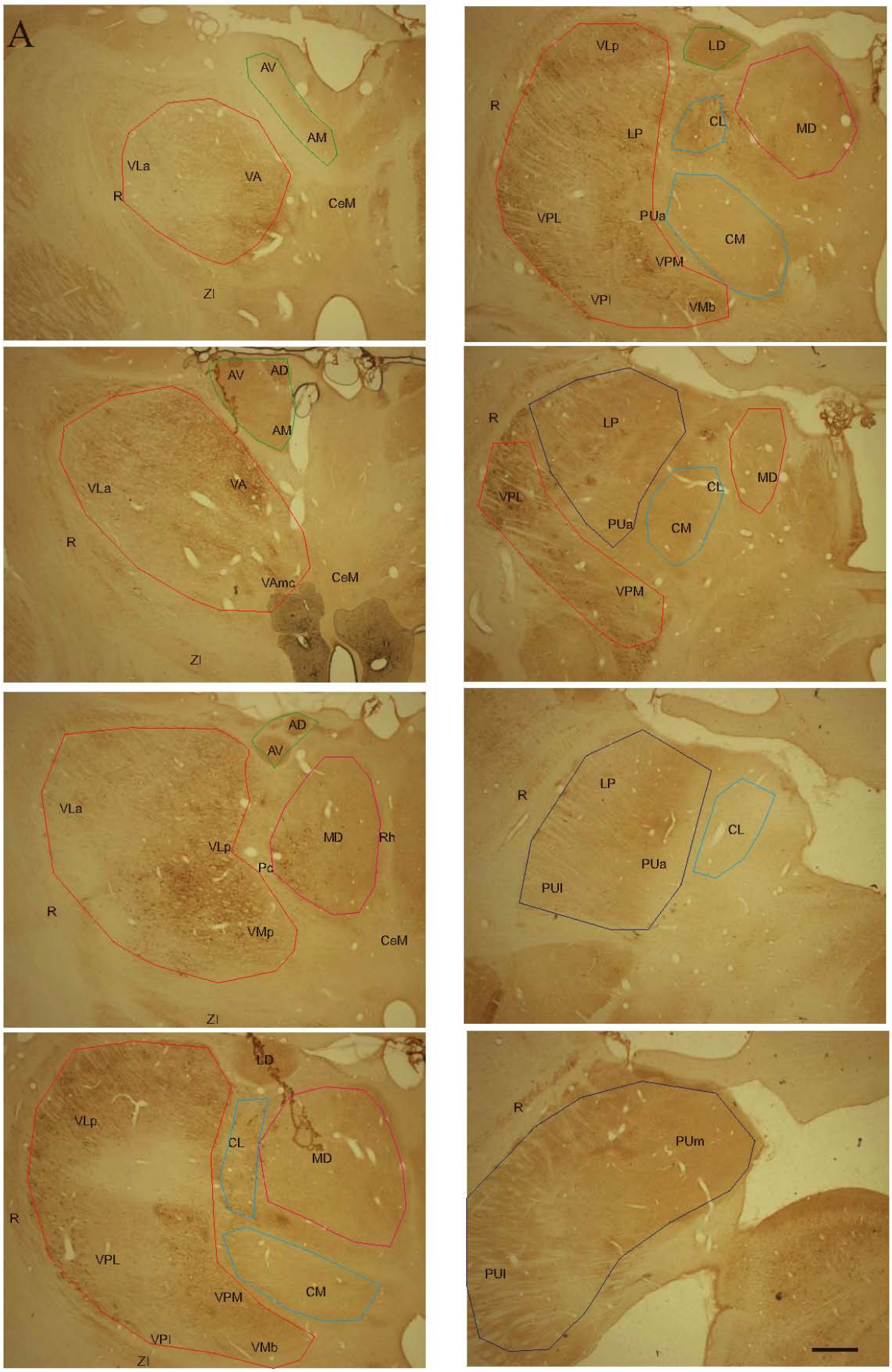
Following a surgical procedure and anaesthesia protocol as described above, Biotinylaled Dextran Amine (BDA, 10% in phosphate-buffered saline, Molecular Probes, Eugene, OR) was injected unilaterally into PM (PMv and PMd) on the lesioned hemisphere of the 7 lesioned monkeys (except in Mk-RO and Mk CE in which the injection was restricted to PMd) using a Hamilton micro-syringe, as previously described (e.g. Rouiller et al., 1994a,b, 1999, 2003; Liu et al., 2002; Tanné-Gariépy et al., 2002; Boussaoud et al., 2005; Morel et al., 2005; Cappe et al., 2007, 2009). PMd and PMv areas were identified partly on the basis of the ICMS sessions performed post-lesion. For comparison, BDA was injected unilaterally into PMd and/or PMv in three additional intact monkeys. The detailed parameters of BDA injections are given for each monkey in Table 1. Three to four weeks after BDA injection, 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 (Paraformaldehyde (4% in phosphate buffer), and 10, 20, 30% solutions of sucrose in phosphate buffer. The brains were placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection during 3-5 days. Frontal sections (50 µm thick) of the brain were prepared and collected in five series. BDA staining was revealed in one series of sections. A second series of sections was Nissl stained with cresyl violet whereas a third series of sections was processed to visualize the marker SMI-32, as previously described (Liu et al., 2002; Wannier

et al., 2005; Beaud et al., 2008 for later histological processing). The two remaining series of sections were kept in reserve.

Data analysis and thalamic parcellation

Nissl and SMI-32 stained sections were then used to reconstruct on consecutive sections the position and extent of the permanent lesion in M1 whereas BDA staining was used to reconstruct the position and extent of BDA injection in PM (see Figure 1). More detailed information on the BDA injections, such as the amounts delivered in each monkey, as well as on the volume of the cortical lesion (in mm^3) is available in Table 1. Distributions of BDA-labelled neurons in the thalamus were plotted with the aid of Neurolucida ™ (MicroBrighField, Inc, Colchester, VT, USA) in the ipsilateral hemisphere. The observation of the labelling was performed on histological section taken at 1 mm intervals. Images of plotted sections were processed using Corel Draw 14.0 software and labelled neurons were then superimposed on photomicrographs obtained from the corresponding Nissl and or SMI-32 stained section group. The borders of thalamic nuclei were primarily defined according to the cytoarchitectonic criteria and the nomenclature previously described (Molinari et al., 1987; Raussela and Jones, 1991; Jones, 1998a, 2001, 2004, 2007). In addition to the classical Nissl staining, we have introduced here SMI-32 staining as an additional marker to better recognize the thalamic nuclei more specifically involved in this study (Figures 2A and 2B). The distribution of labelled thalamocortical neurons as a result of BDA injection in PM was analyzed by assembling the numerous thalamic nuclei into 5 main groups, as listed in Table 2 and shown in Figure 2 with color contours delineating the thalamic groups:

- Anterior group: thalamic nuclei AM, AV, AD and LD.
- Ventral group: thalamic nuclei VA, VL, VM and VP.
- Lateral group: thalamic nuclei LP and PU.
- Medial group: thalamic nucleus MD
- Intralaminar group: thalamic nuclei PC, CL and CM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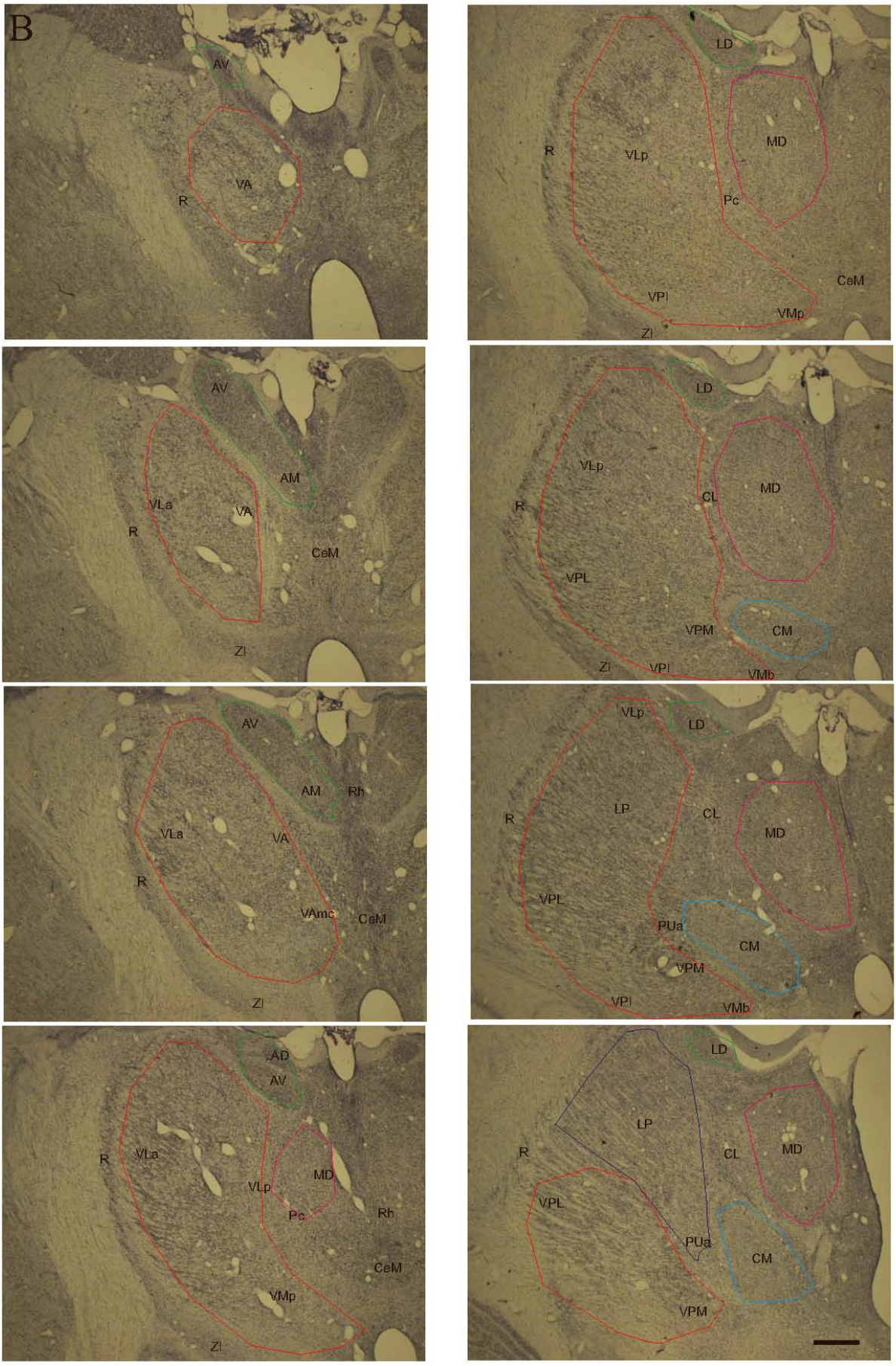


<u>Figure 2</u>: Parcellation of the thalamus as derived from SMI-32 staining (A) and Nissl staining (B). Scale bar: 1mm. See list of abbreviations on pages 7 and 8. The color contours delineate the 5 thalamic groups.

Results

1. Parcellation of the thalamus based on SMI-32 staining

Figure 2A shows a rostro-caudal sequence of SMI-32 frontal sections taken from a macaque monkey thalamus. For comparison with the parcellation of the thalamus based on SMI-32 (Figure 2A), Figure 2B shows a corresponding series of Nissl stained sections illustrating the delineation of the thalamus in macaque monkeys, as derived from previous studies (Molinari et al., 1987; Raussell and Jones, 1991; Jones, 1998a, 2001, 2004, 2007).

The SMI-32 staining, representing an original contribution of the present work, offers a further marker to delineate the thalamic nuclei, complementary to the standard Nissl staining. The border of some thalamic nuclei, such as VA, VPL, CL, MD for instance appear more clearly than in Nissl staining (Fig. 2A and B). In the present work, both stainings (SMI-32 and Nissl) were used to identify the thalamic nuclei, which were then distributed into 5 main thalamic groups.

2. Thalamocortical projections to the premotor cortex (PM) in the intact monkeys

The reconstruction of BDA labeled thalamocortical neurons in intact monkeys was performed in three animals, as a result of injection of BDA in the premotor cortex (PM). The sites of BDA injections are illustrated on a lateral view of the left hemisphere of the 3 intact monkeys in Figure 1. Typical injection sites in PM and retrogradely BDA labeled neurons in the thalamus are shown in Figure 3.

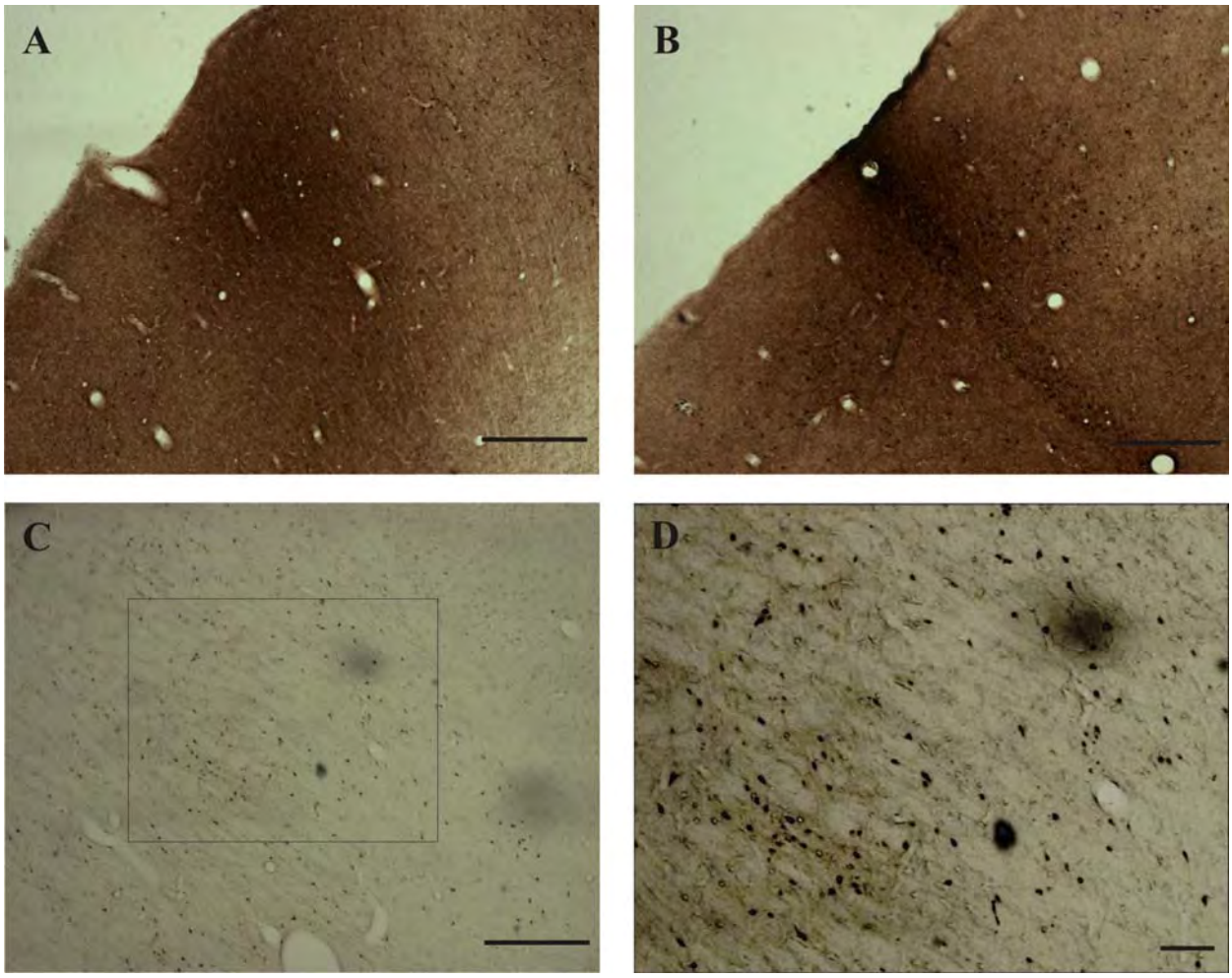
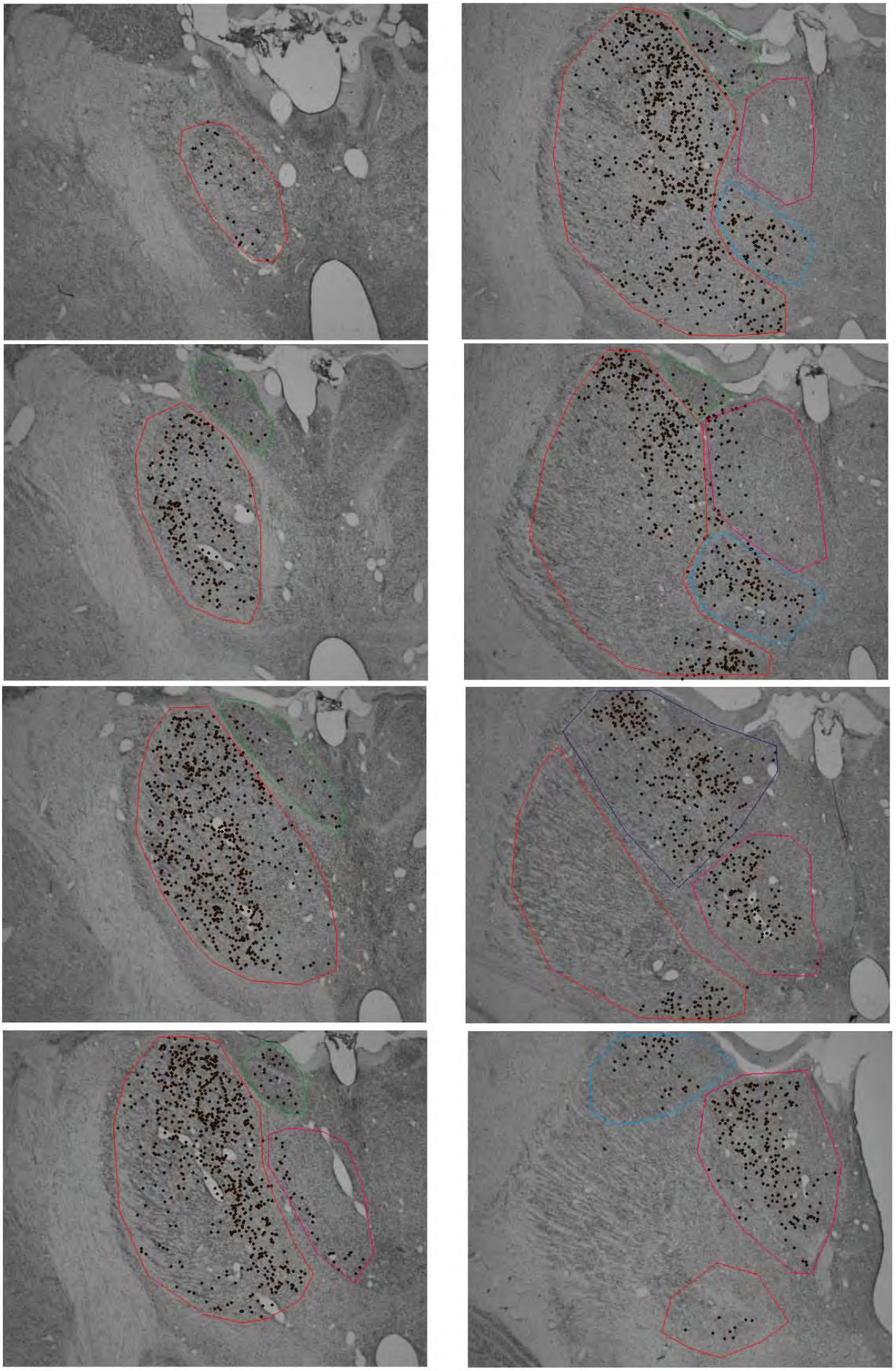


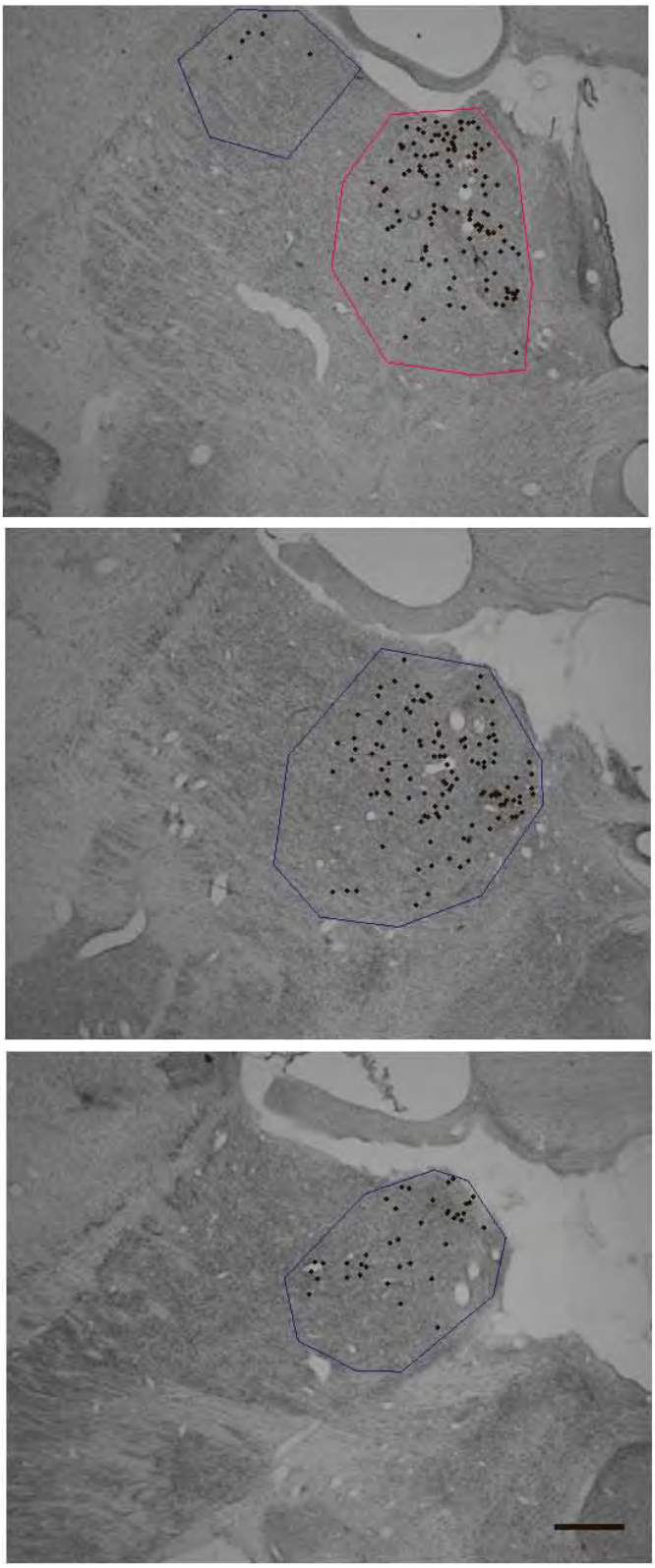
Figure 3: Photomicrographs showing the sites of BDA injection in PMd (A) and PMv (B) in monkey Mk-CH. Panel C represents the photomicrograph of retrogradely labelled neurons in the ventral group of the thalamus, with a higher magnification in panel D (of the rectangle zone in panel C). Scale bar: 500 µm.

In the thalamus of the intact monkey Mk-CH, most labeled neurons were located in the ventral group (69% of the total number of BDA stained neurons in the thalamus, mainly in VA and VL), together with additional labelled neurons in the medial group (13%), whereas fewer labeled neurons were seen in the anterior, lateral and intralaminar groups (Fig. 4; Table 2). In the ventral nuclei, labeled neurons were located predominantly in the nuclei VA (25%) and VL (36%), whereas few labeled neurons were observed in the nuclei VM and VP. In the anterior group, most labeled neurons were located in the nuclei AM and AV (Table 2), although fewer neurons were also present in the nuclei AD and LD. In the lateral group, there was a roughly comparable proportion of labeled neurons in the nuclei LP and PU (Table 2). In the intralaminar group, most of the labeled neurons were distributed in CL, representing about 4% of the total number of labelled thalamocortical neurons. Additionally, few labeled neurons were located in the nuclei CM and PC (Table 2).

Overall comparable results were obtained in the intact monkey Mk-R13 (Supplementary Figure 1, Table 2), where the major thalamocortical projections to PM were seen predominantly in the ventral group (76%) and in the medial group (14%). These labelled neurons were located mainly in VA (17%) and in VL (53%), with additional labelled neurons in VM (6%). Further thalamocortical projections to PM were observed in the lateral group (LP: 4%) and in the intralaminar group (CL and CM: see Table 2). Rare labelled neurons were also observed in the anterior group (1%).

In the case Mk-R12, BDA was injected only in PMd (Supplementary Figure 2, Table 2). A dense zone of labelled neurons was found in the ventral group (74%) and, but to a lesser extent, in the lateral group (12%) and in the medial group (11%). In the ventral group, labeled neurons were located mostly in VA (7%) and in VL (65%), although a few labeled neurons were observed in the nuclei VM and in VP. In the lateral group, most labeled neurons were located in LP (10%). Few labeled neurons were observed in the intralaminar group, representing 2% in the nucleus CL, whereas there was almost no neuron in the anterior group (0.05%).





<u>Figure 4</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the intact monkey MK-CH, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

Table 2: Percentage of labelled cells observed in the different thalamic nuclei.

		Intacts			Controls				Treated		
		Mk-CH	Mk-R13	Mk-R12	Mk-GE	Mk-RO	Mk-BI	Mk-CE	Mk-VA	Mk-MO	Mk-LA
Anterior group	AM	1.11	0.1	0	0	0	0	0	0.37	0.67	0
	AV	1.42	0.31	0	0	0	0	0.14	0.12	1.45	0
	AD	0.23	0	0	0	0	0	0.68	0	0	0
	LD	0.58	0.72	0.05	0	0.83	0.25	0.14	0.44	0	0.16
	Total	**3.34**	**1.13**	**0.05**	**0**	**0.83**	**0.25**	**0.96**	**0.93**	**2.12**	**0.16**
Ventral group	VA	24.53	16.91	6.73	2.93	1.24	4.04	10.75	3.68	15.2	3.4
	VL	36.28	52.66	64.93	73.04	78.51	79.42	52.11	58.55	60.98	76.86
	VM	6.99	5.92	0.16	3.35	0	5.81	2.58	5.12	5.35	1.78
	VP	1.44	0.43	2.5	6.15	0	2.27	6.39	9.11	0	2.75
	Total	**69.24**	**75.92**	**74.32**	**85.47**	**79.75**	**91.54**	**71.83**	**76.46**	**81.53**	**84.79**
Lateral group	LP	3.27	2.98	9.5	0.28	0	0	2.04	3.37	2.7	0
	PU	4.78	1.23	2.33	0	0	0	10.48	2.75	0	0
	Total	**8.05**	**4.21**	**11.83**	**0.28**	**0**	**0**	**12.52**	**6.12**	**2.7**	**0**
Medial group	MD	13.19	14.05	10.8	10.2	16.11	8.21	5.71	4.68	10.43	11.65
	Total	**13.19**	**14.05**	**10.8**	**10.2**	**16.11**	**8.21**	**5.71**	**4.68**	**10.43**	**11.65**
Intralaminar group	PC	0.51	0.46	0	1.12	0	0	0	0.19	0.93	0
	CL	4.33	2.74	2.08	0.56	2.48	0	6.94	3.37	1.5	1.94
	CM	1.34	1.49	0.92	2.37	0.83	0	2.04	8.24	0.78	1.46
	Total	**6.18**	**4.69**	**3**	**4.05**	**3.31**	**0**	**8.98**	**11.8**	**3.21**	**3.4**
Thalamus total (%)		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Absolute total number of labeled cells in the thalamus		**3950**	**4157**	**1842**	**716**	**242**	**792**	**735**	**1602**	**1927**	**618**

3. Thalamocortical projections to the premotor cortex in the untreated (control) monkeys subjected to M1 lesion

The distribution patterns of thalamocortical neurons in the untreated monkeys subjected to M1 lesion, that were retrogradely labeled after BDA injection into the ipsilesional PM, were studied in four animals (Table 1). In Mk-BI and Mk-GE, BDA was injected into both PMd and PMv. In the case of Mk-BI (Fig. 5, Table 2), large numbers of labeled neurons were widely distributed in the ventral group (92%), whereas additional labeling was located in the medial group (8%). Very rare neurons were labelled in the anterior group (0.25%) and no labelling was found in the lateral and intralaminar groups. In the ventral group, most labeled neurons were located in VL (79%), plus fewer labeled neurons in VA (4%), in VM (6%) and in VP (2%). In the anterior group, rare labeled neurons were located only in LD.

In the case of Mk-GE (Supplementary Figure 3, Table 2), the results were comparable to Mk-BI. BDA retrograde labelling from PM occurred mainly in the ventral group (85%) and in the medial group (10%). In the ventral group, many labeled neurons were seen in VL (73%), plus some labeled neurons located in VP (6%). The percentage of labeled neurons in VA and VM were almost the same. We observed also labeled neurons in the intralaminar group, with a main location in CM (2%), although few labeled were located in PC (1%). Very few labeled neurons were found in the lateral group (in LP). There were no labeled neurons in the anterior group.

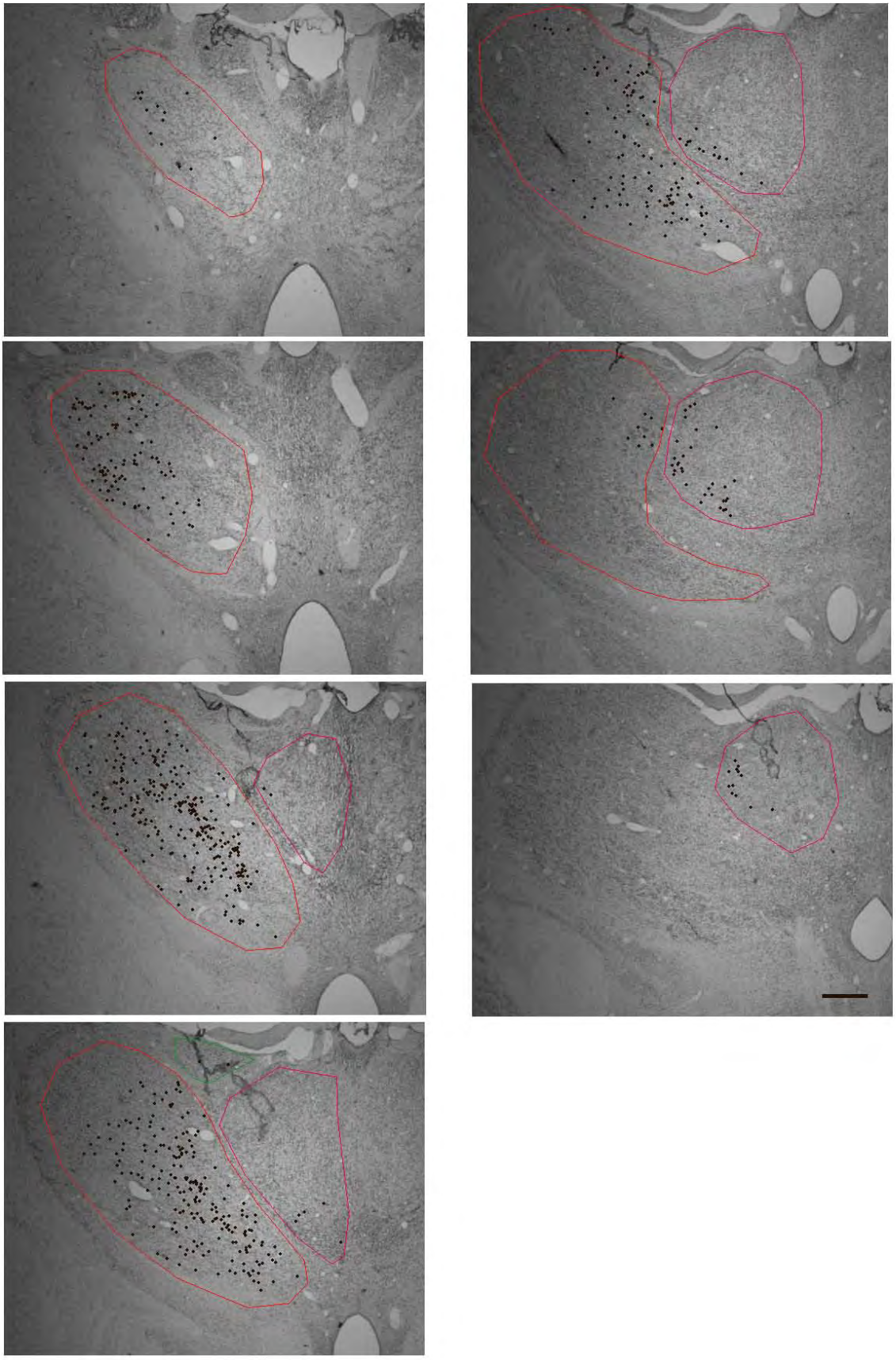
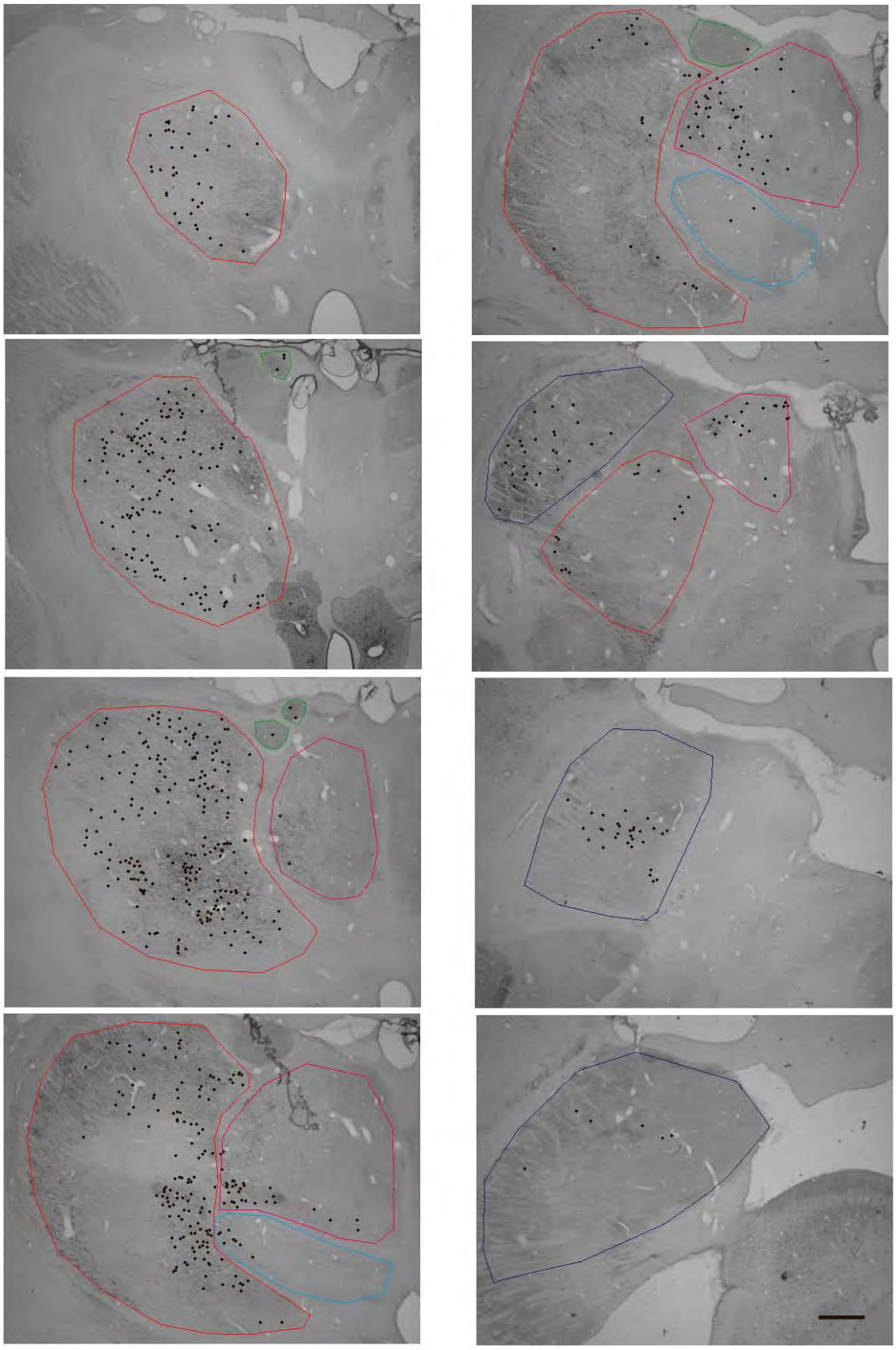


Figure 5: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the control monkey MK-BI, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

In contrast to the above 2 monkeys (Mk-BI and Mk-GE), BDA was injected only in PMd in the 2 monkeys Mk-CE and Mk-RO. In the case of Mk-CE (Fig 6, Table 2), the vast majority of labeled neurons were observed in the ventral group (72%). We found also labeled neurons in the lateral, intralaminar and medial groups. In the ventral group, most labeled neurons were located in VL (52%), but other labeled neurons were located in the nuclei VA (11%) and VP (6%). Few labeled neurons were seen in VM (3%). In the lateral group, the majority of labeled neurons were seen in PU (10%) and, to a lesser extent, in LP (2%). In the intralaminar group, significant labelling occurred in CL (7%) and less labeling was observed in CM (2%). Labeled neurons were also seen in the medial group (6%) and in the lateral group (13%). Very few (or almost none) labeled neurons were found in the anterior group (AD and LD). A comparable pattern of distribution for retrograde labelling in the thalamus was observed in Mk-RO (Supplementary Figure 4, Table 2). Labeled neurons were seen predominantly in the ventral group (80%), but also in the medial and intralaminar groups. In the ventral group, labeled neurons were located mainly in VL (79%), whereas very few labeled neurons were located in VA (1%). There was no labeled neuron in VM and VP. We observed several labeled neurons in the medial group (16%). In the intralaminar group, the main projections originated from CL (2%) and more rarely from CM (1%). Almost no labeled neurons were observed in the anterior group. There was no labeled neuron in the lateral group.



<u>Figure 6</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the control monkey MK-CE, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

4. Thalamocortical projections to the premotor cortex in the anti-Nogo-A treated monkeys subjected to M1 lesion

The distribution of BDA labelled neurons in the anti-Nogo-A antibody treated monkeys was assessed in three animals (Table 1). In the thalamus of Mk-VA (Fig. 7, Table 2), most labeled neurons were distributed the ventral group (76%), but labeled neurons were also seen in the intralaminar group (12%). Labeled neurons were also found in the lateral (6%) and in the medial (5%) groups. In the ventral group, labeled neurons were located predominantly in VL (59%), with fewer labeled neurons in VA (4%), VM (5%) and VP (9%). In the intralaminar group, the labeled neurons were located mainly in CM (8%), but also in CL (3%) and rarely in PC (0.19%). In the lateral group, labeled neurons were distributed roughly evenly in LP and Pl (Table 2). Rare labelled neurons were found in the anterior group, in the nuclei AM (0.4%) and LD (0.4%).

In the case of Mk-MO (Supplementary Figure 5, Table 2), the results were generally comparable to Mk-VA. The vast majority of labeled neurons were distributed in the ventral group (82%), accompanied by labeled neurons in the medial group (10%). Few labeled neurons were observed in the intralaminar, lateral and anterior groups (Table 2). In the ventral group, most labeled neurons were located in VL (61%), whereas other labeled neurons were located in VA (15%) and in VM (5%). No labeled neuron was seen in VP. In the intralaminar group, labeled neurons were located mainly in CL (2%), and more rarely in the nuclei PC (0.9%) and CM (0.8%). In the lateral group, labeled neurons were located only in LP (3%). In the anterior group, labeled neurons were located mainly in the nuclei AV (1%) and in AM (0.7%).

In the case of Mk-LA (Supplementary Figure 6, Table 2; treatment only during 2 weeks), the percentage distribution of labelled neurons in the different thalamic nuclei was as follows. Most labeled neurons were distributed in the ventral group (85%), plus significant labelling in the medial group (12%). Few labeled neurons were observed in the intralaminar group (3%) and very few or almost no neurons were present in the anterior group (0.2%). In the ventral group, most labeled neurons were located in VL (77%), plus other labeled neurons in VA (3%), in VP (3%), and in VM (2%). In intralaminar group, labeled neurons were located in CL (2%), and in CM (2%). In the anterior group, very few labeled neurons were seen only in LD (0.2%). No labelling was observed in the lateral group.

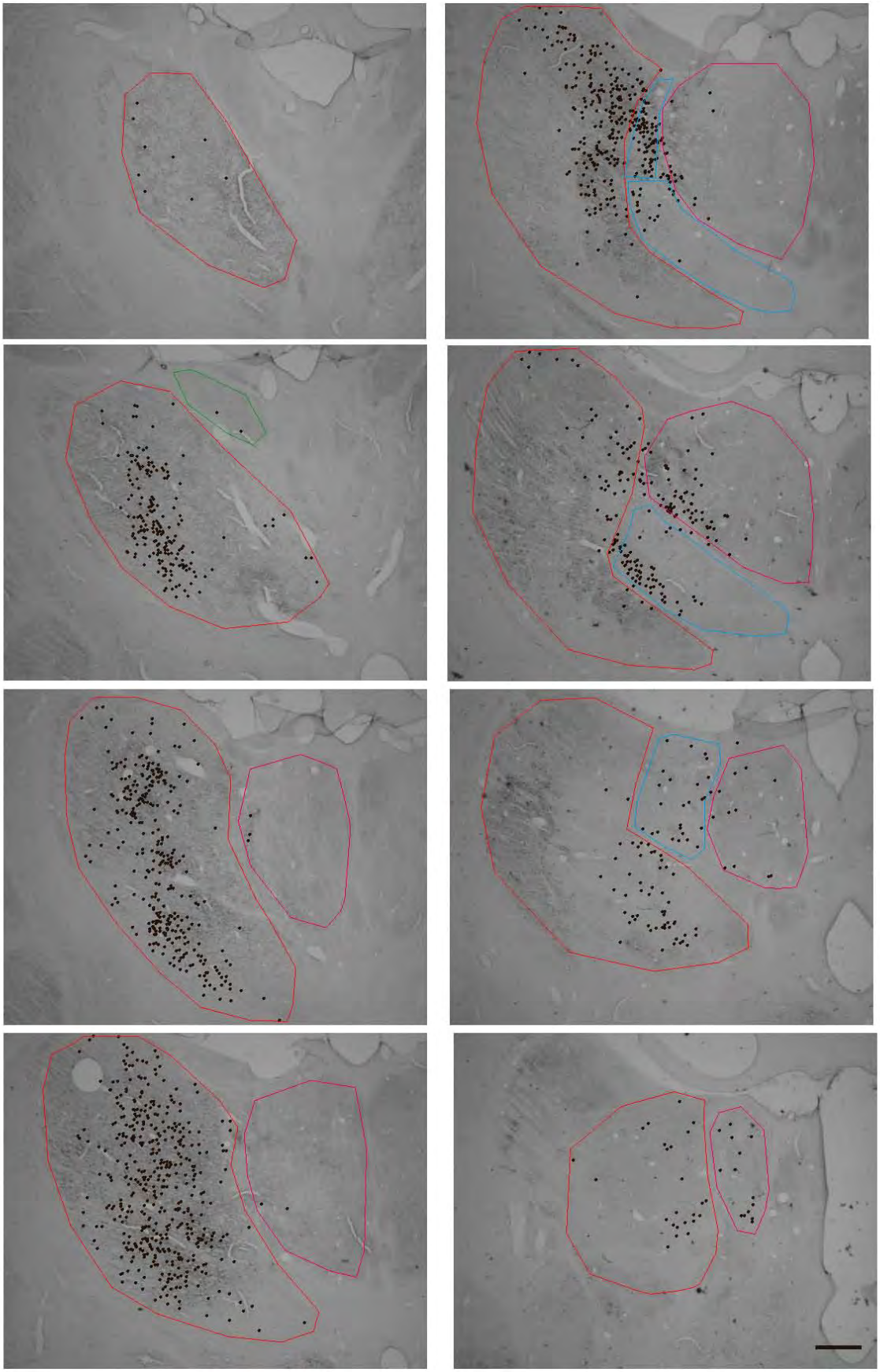


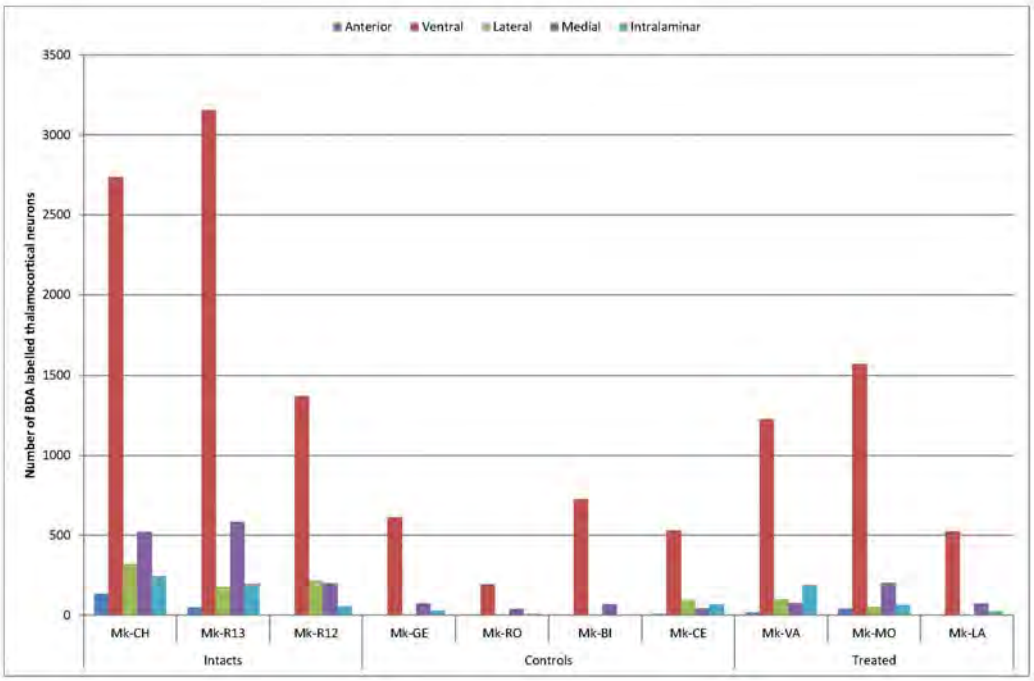
Figure 7: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the monkey MK-VA, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

5. Comparison of the number of BDA labelled thalamocortical neurons across the three groups of monkeys.

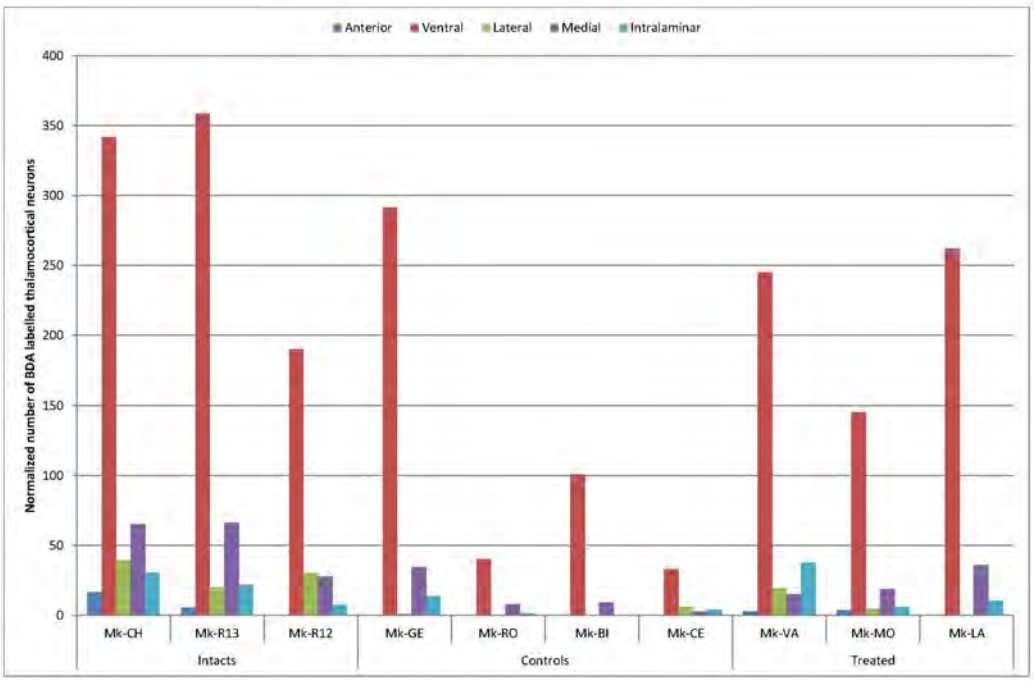
Figure 8 shows the absolute numbers of BDA labelled thalamocortical neurons in distinct regions of the thalamus across the ten monkeys. Due to variations of position of the BDA injection site, of the volume injected and of tracer uptake, the number of retrogradely labelled cells in the thalamus is quite variable across monkeys. Nevertheless, in all cases, the majority of labelled neurons was found in the ventral zone of the thalamus. Significant numbers of labelled neurons were observed in the medial thalamic region in most monkeys, whereas there were generally less neurons labelled in the other thalamic regions (anterior, lateral, intralaminar groups).

The same data are shown in Figure 9, but after normalization with respect to the size (volume in mm^3) of the injection site (core area), in order to tentatively compare the 3 groups of monkeys. There is a tendency towards fewer labelled thalamocortical neurons in the control groups, although this was true in 3 out of 4 monkeys (Mk-RO, Mk-BI, Mk-CE), but not in the fourth control monkey (Mk-GE), which exhibit numbers comparable to the other 2 groups of monkeys. There is overlap in the distribution of labelled neurons between the intact monkeys and the anti-Nogo-A treated monkeys.

To test the effect of different parameters taken for normalization, Supplementary Figure 7 shows the number of labelled thalamocortical neurons normalized with respect to the volume of BDA injected (in µl), instead of the size of the injection site. A major change with respect to Figure 9 is the decrease of the normalized number of labelled thalamocortical cells in the group of intact monkeys. Nevertheless, due to the variability among individual monkeys, it can be concluded for Figure 9 and Supplementary Figure 7 that there is no clear difference of normalized numbers of labelled cells across the 3 groups of monkeys.



<u>Figure 8</u>: Absolute number of BDA labelled thalamocortical neurons across the ten monkeys



<u>Figure 9:</u> Normalized number of BDA labelled thalamocortical neurons with respect to the size (volume in mm^3) of the injection site (core area).

Discussion

Thalamocortical projections to motor cortical areas (including PM) in primates have been studied extensively in intact adult macaque monkeys (Schell and Strick, 1984; Strick, 1985; 1986; Wiesendanger and Wiesendanger, 1985; Ghosh et al., 1987; Jones, 1987; Matelli et al., 1989; Darian-Smith et al., 1990a,b; Nakano et al., 1992; 1993; Inase and Tanji, 1994; Kurata, 1994; Rouiller et al., 1994; Shindo et al., 1995; Matelli and Luppino, 1996; Rouiller et al., 1999; Kurata, 2005; Morel et al., 2005; Cappe et al., 2007). The patterns of thalamocortical projections to the premotor areas have been also reported in galagos (Fang et al., 2006). To the best of our knowledge, no information on the thalamic projections to PM after M1 lesion was available so far, thus representing an original contribution of the present study.

In the present experiments, we established the pattern of the thalamocortical projections to PM following unilateral lesion of the ipsilateral primary motor cortex (M1) in adult macaque monkeys, as well as the possible influence of anti-Nogo-A antibody treatment based on the retrograde tracer BDA injected in the dorsal (PMd) and ventral (PMv) premotor areas in macaque monkeys, after these areas were identified by intracortical microstimulation (ICMS).

In the intact monkeys, both PMd and PMv receive the strong projections from the ventral group of thalamus such as VL, VA and less dense projections from VM and VP. Others projections were also present in the medial group and in the lateral group (LP and Pl). In the intralaminar group, the main projections were observed in CL and few projections in CM. PM receives very few projections from AM and AV. These results are in line with those of previous studies (Morel et al., 2005; Stepniewska et al., 2007). When a permanent lesion was performed in M1, the distribution of retrogradely labelled neurons in the thalamus after BDA injection in PM across thalamic nuclei was generally comparable to that observed in intact monkeys. These results suggest that M1 lesion does not modify the general pattern of organization of the thalamic projections. In the monkeys also subjected to a lesion of M1, but treated with the anti-Nogo-A antibody, a similar general distribution of labelled neurons in the thalamus was observed, suggesting that the anti-Nogo-A antibody treatment does not reshape the thalamocortical projections. The latter conclusion is based only on very preliminary data as there are only 2 monkeys in the treated groups, considering that Mk-LA should be excluded (very small lesion and treatement limited to 2 weeks instead of 4). Clearly, more animals are needed in the control group and in the treated group.

In spite of the limited number of monkeys included in the present study, the general conclusion is that a unilateral lesion of M1 did not modify substantially the thalamocortical projection directed to the homolateral PM. This observation thus contrasts with the clearly more prominent reorganization of the callosal projection reaching PM, after lesion of the homolateral M1. With that respect, the present results are largely consistent with the well established notion in sensory and motor systems that, after a lesion (at periphery, at thalamic level or at cortical level), the thalamocortical projection is much less re-arranged than corticocortical connections (e.g. Sharp and Gonzalet, 1986; Miller et al., 1991; Darian-Smith and Gilbert, 1995; Calford et al., 2003; Croquelois et al., 2005).

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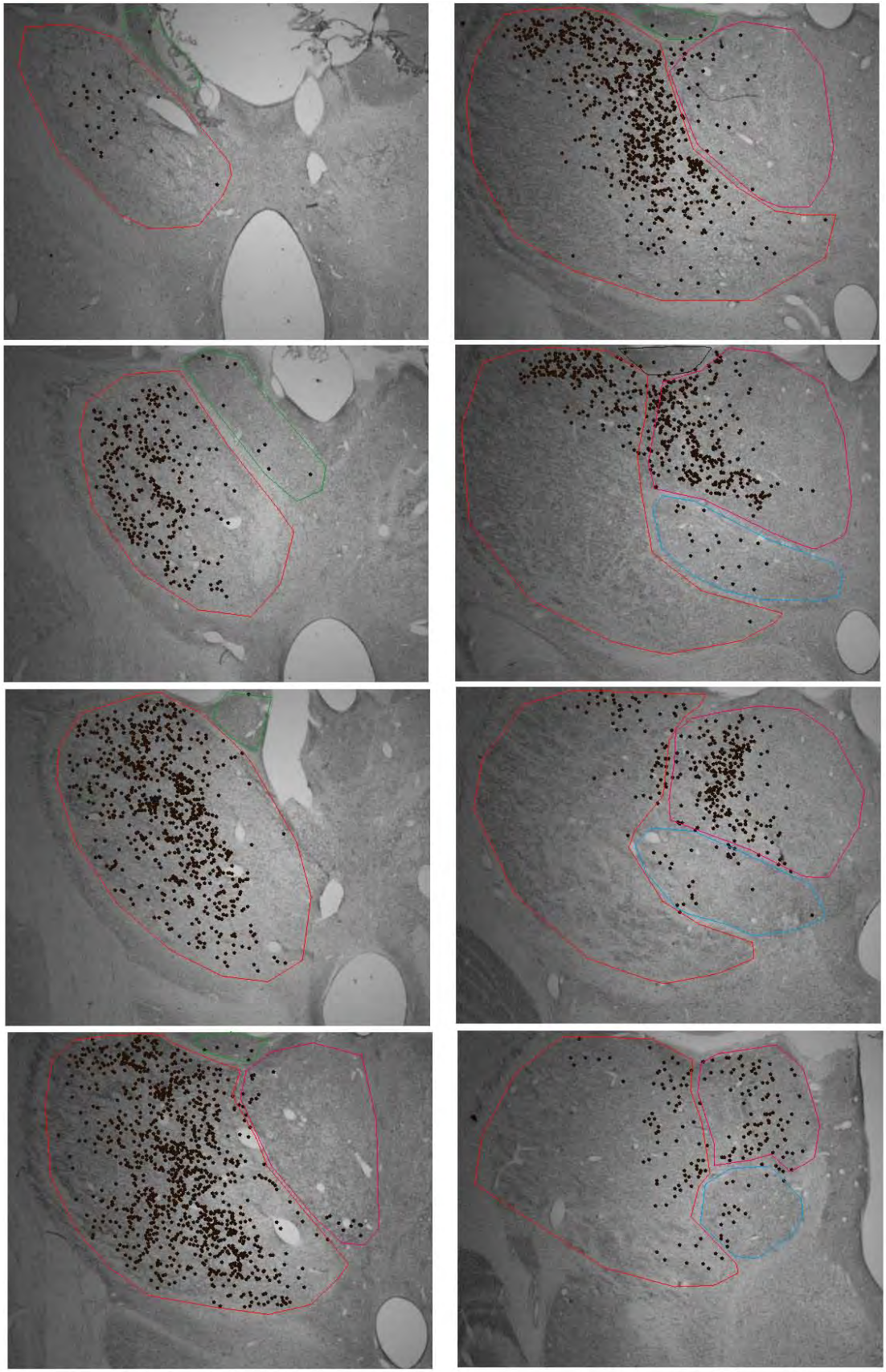
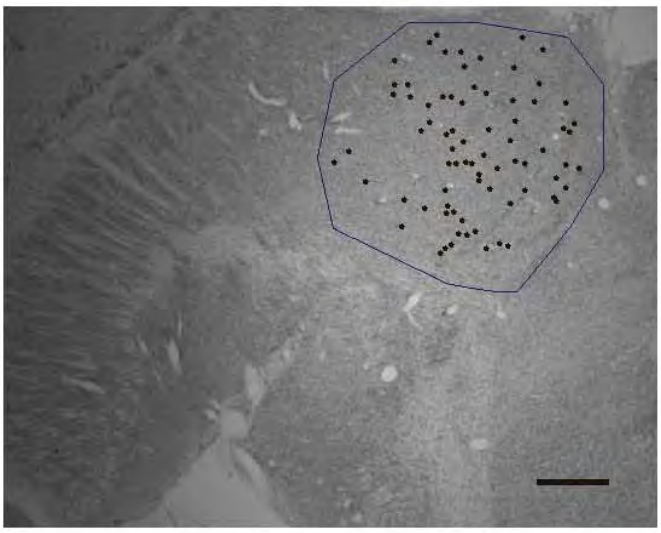
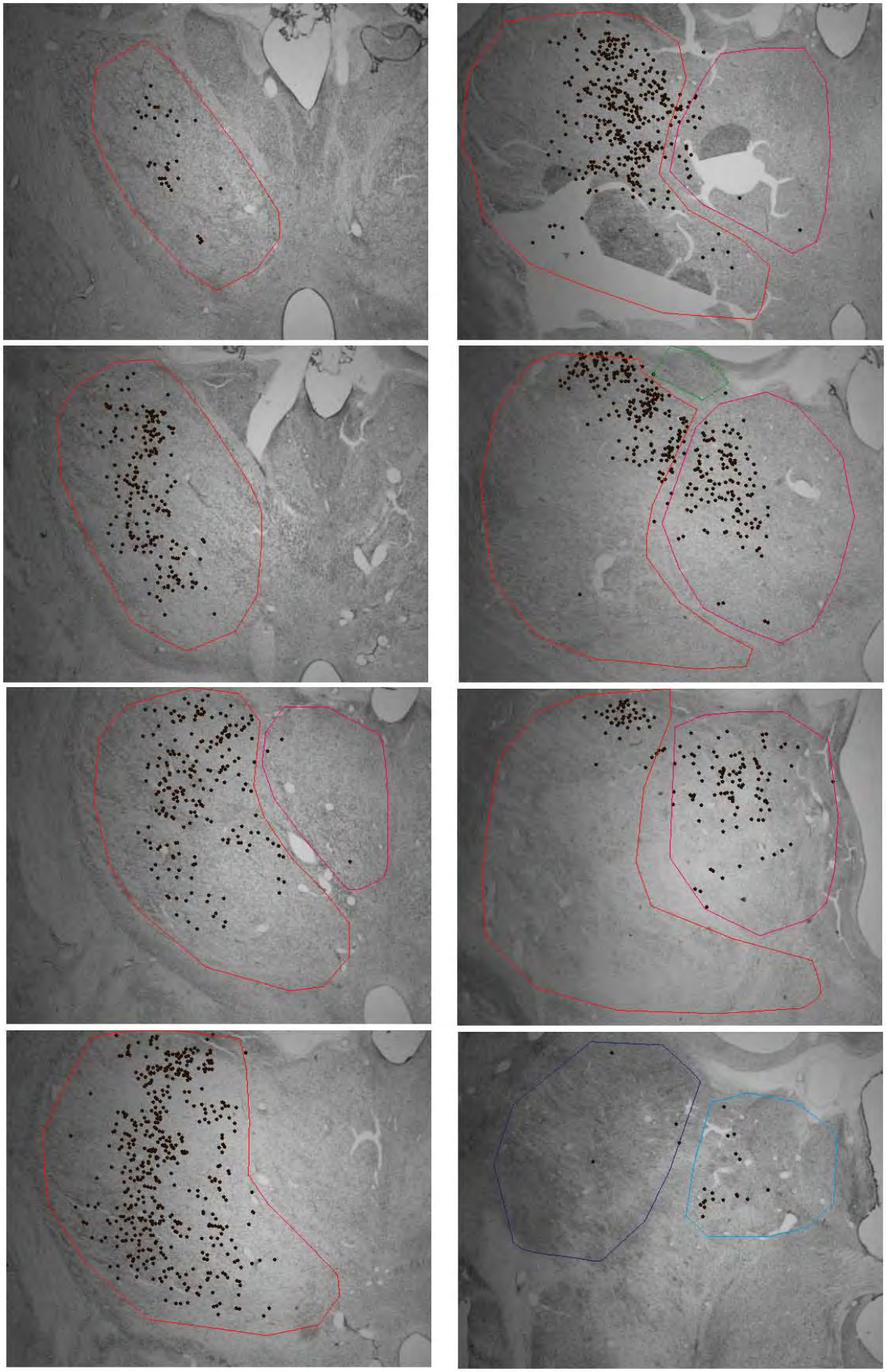
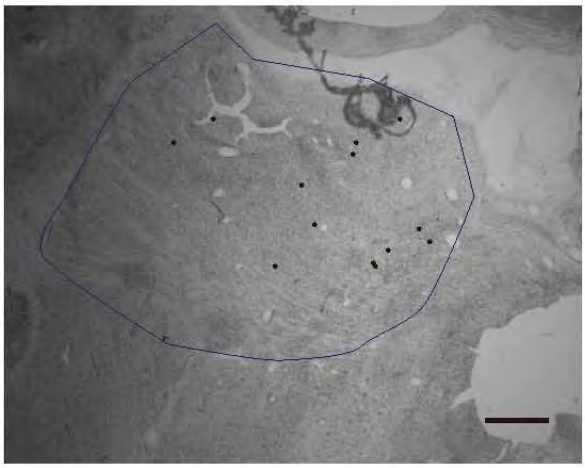
Supplementary figures



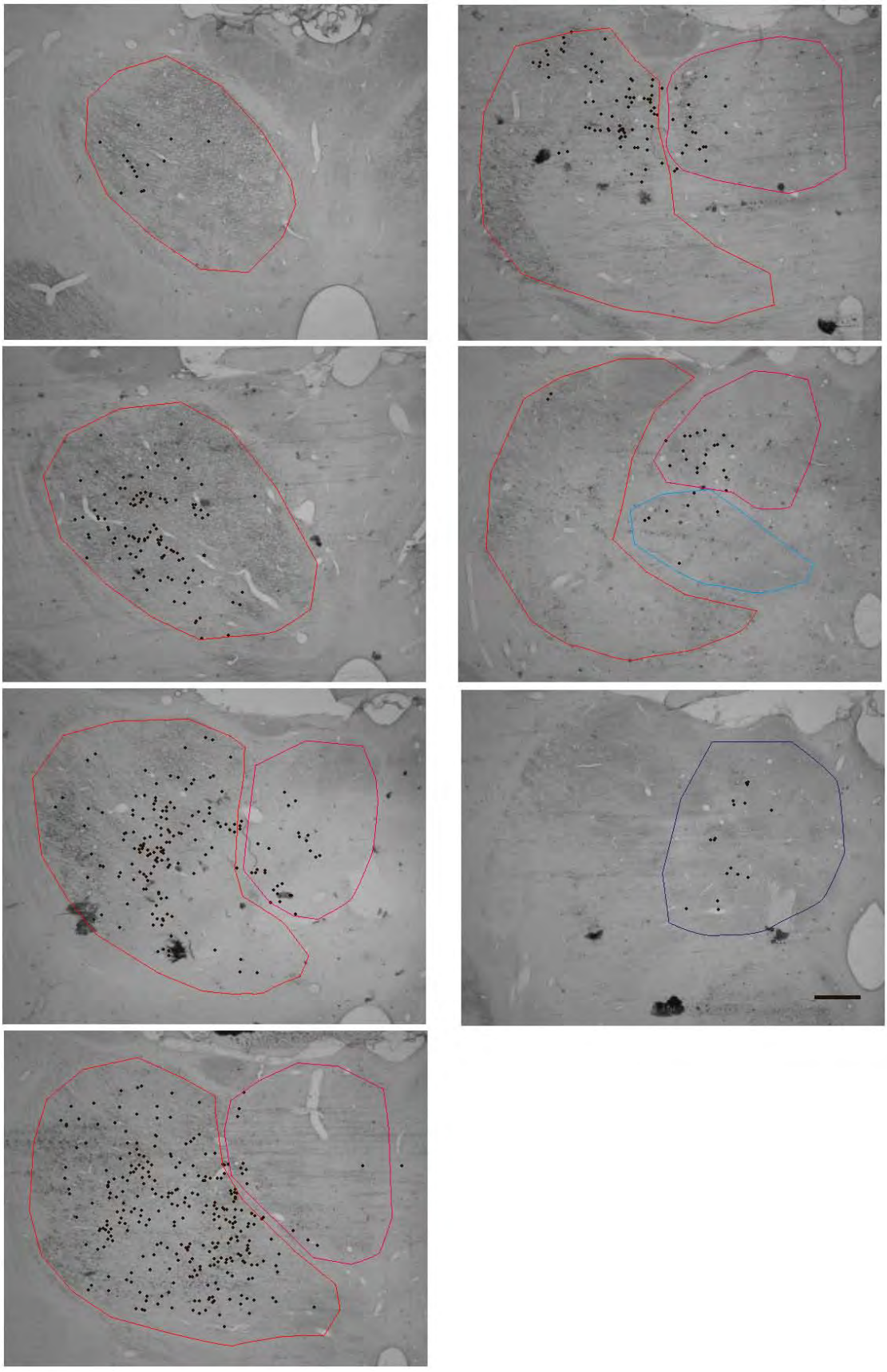


<u>Supplementary Figure 1</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the intact monkey MK-R13, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

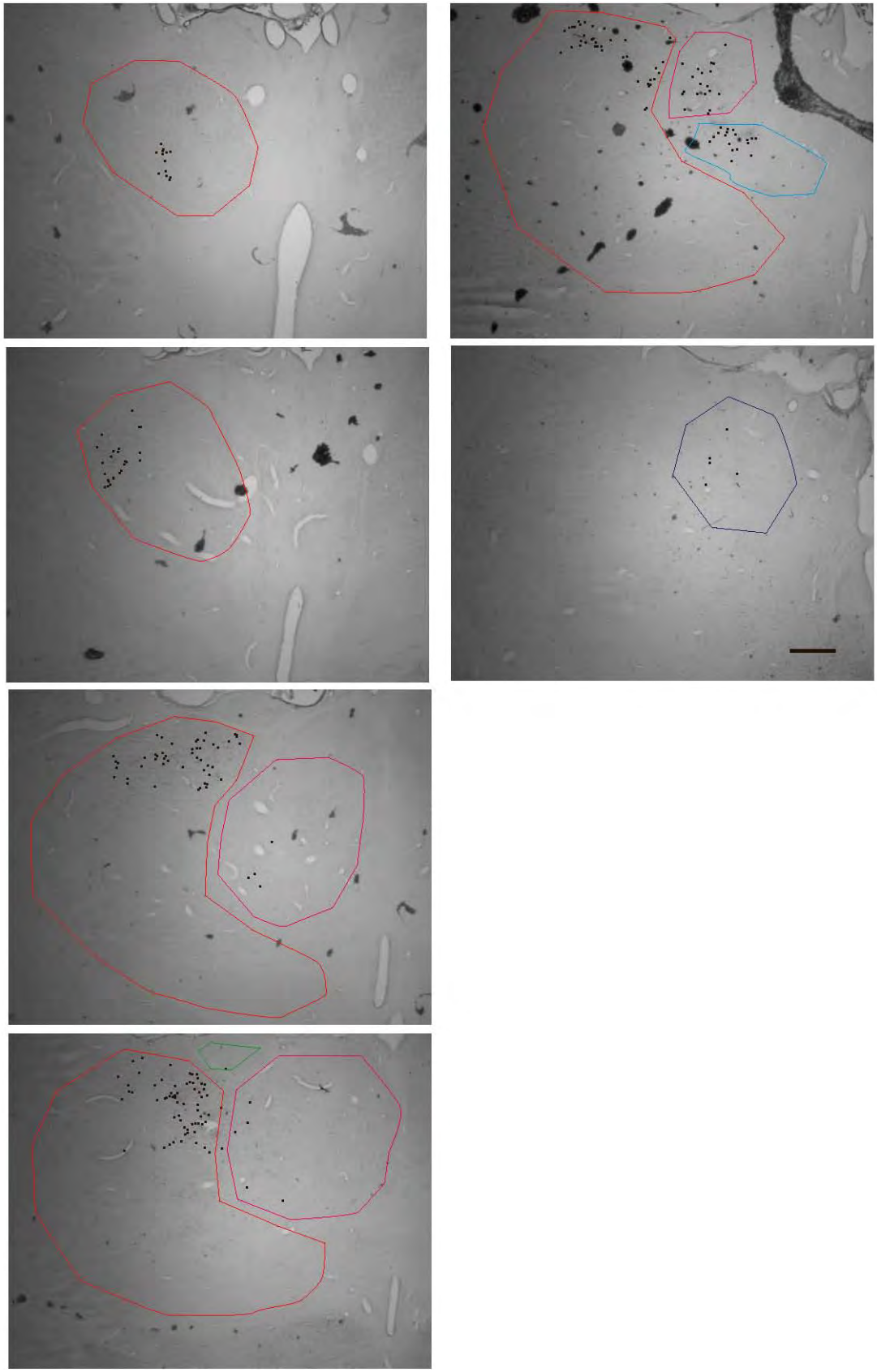




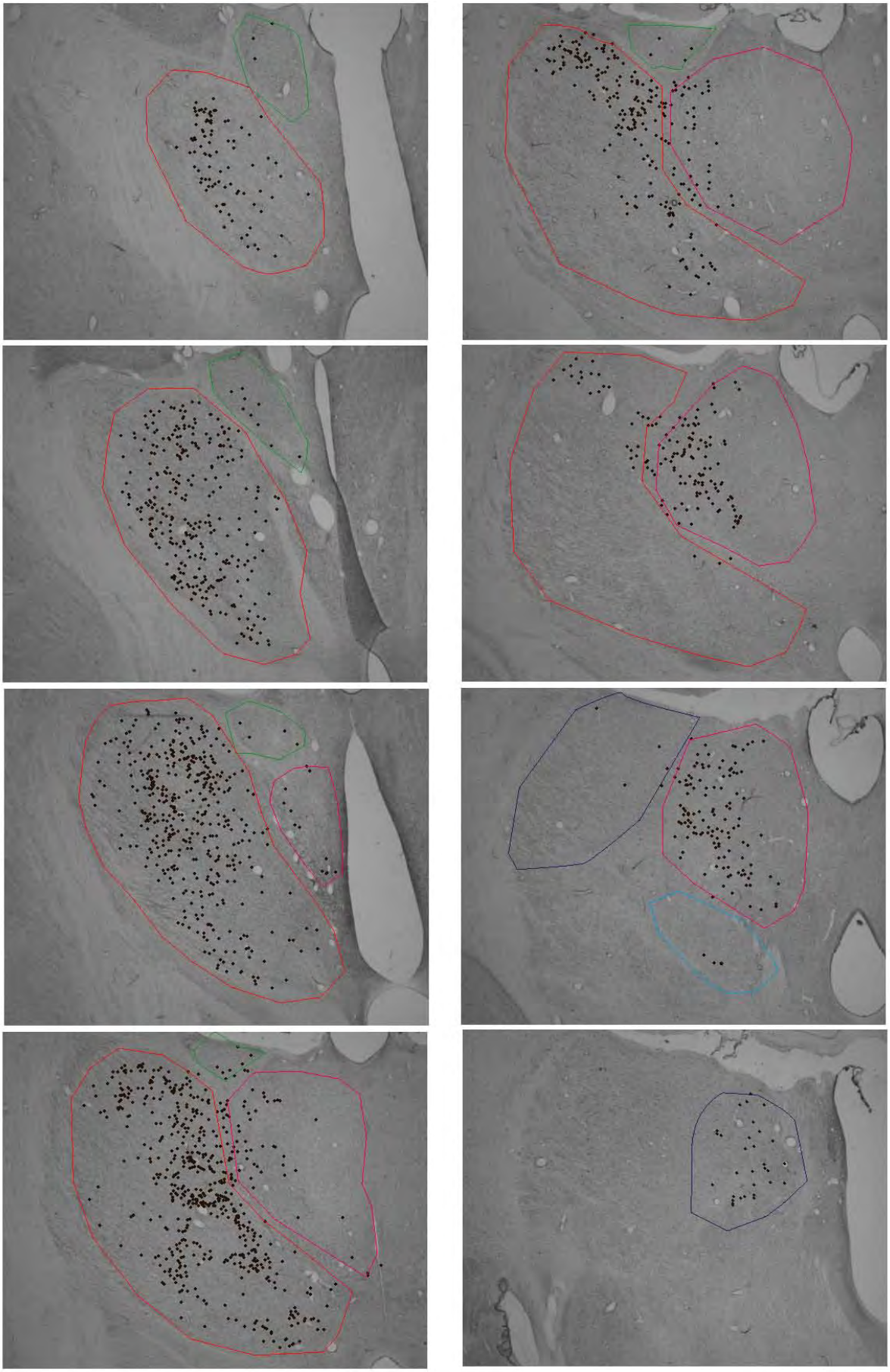
<u>Supplementary Figure 2</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the intact monkey MK-R12, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

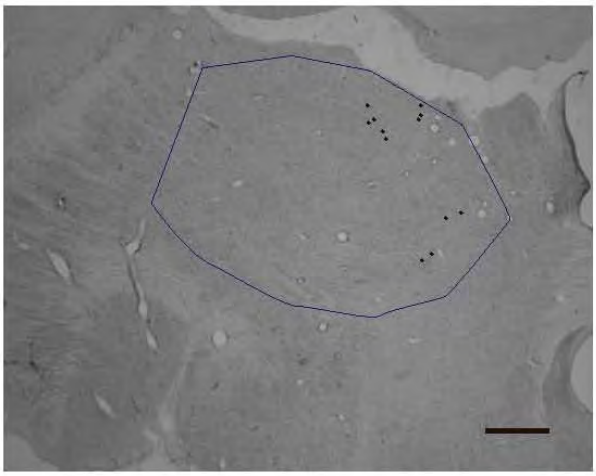


<u>Supplementary Figure 3</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the control monkey MK-GE, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

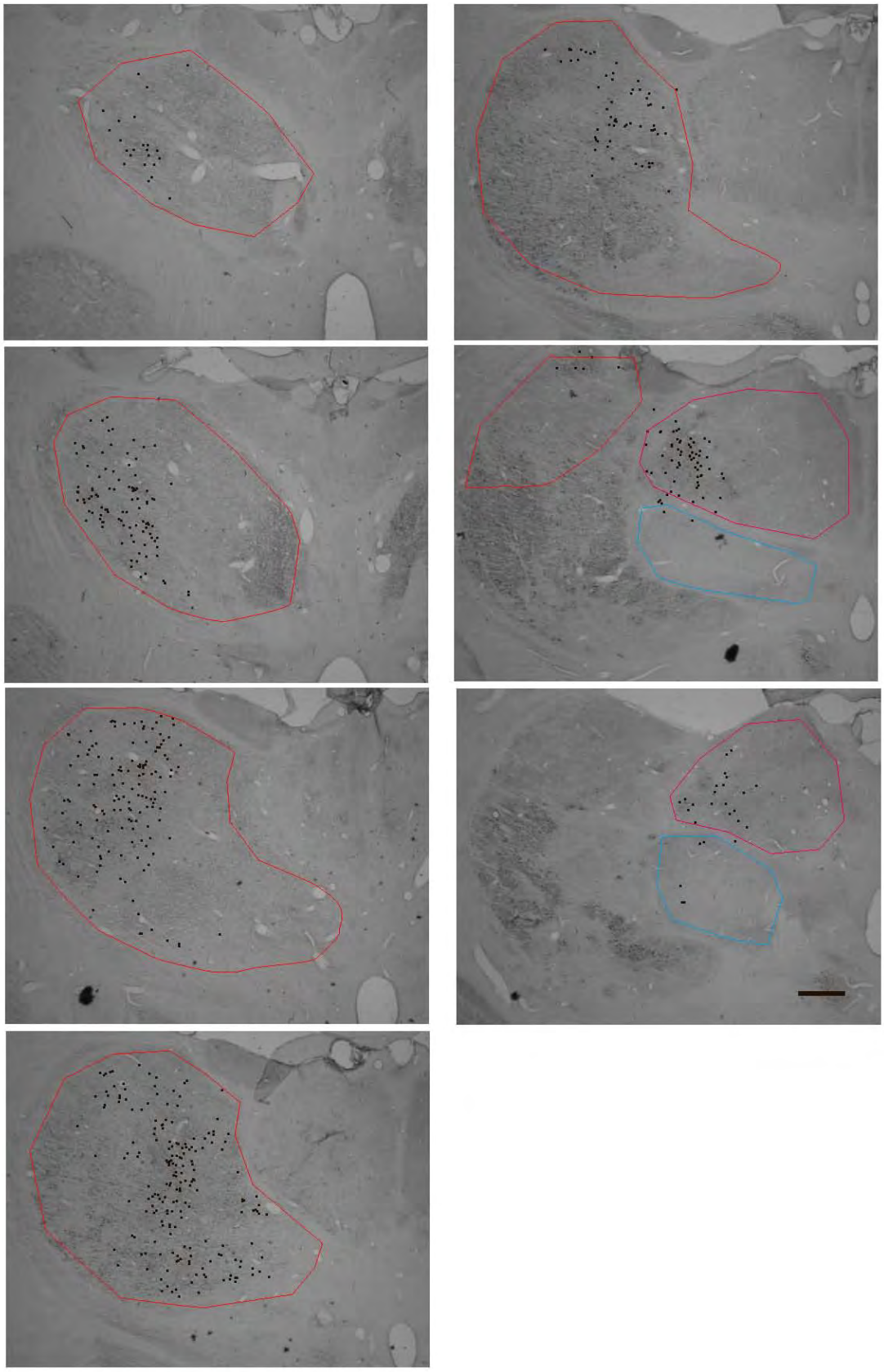


<u>Supplementary Figure 4</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the control monkey MK-RO, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.

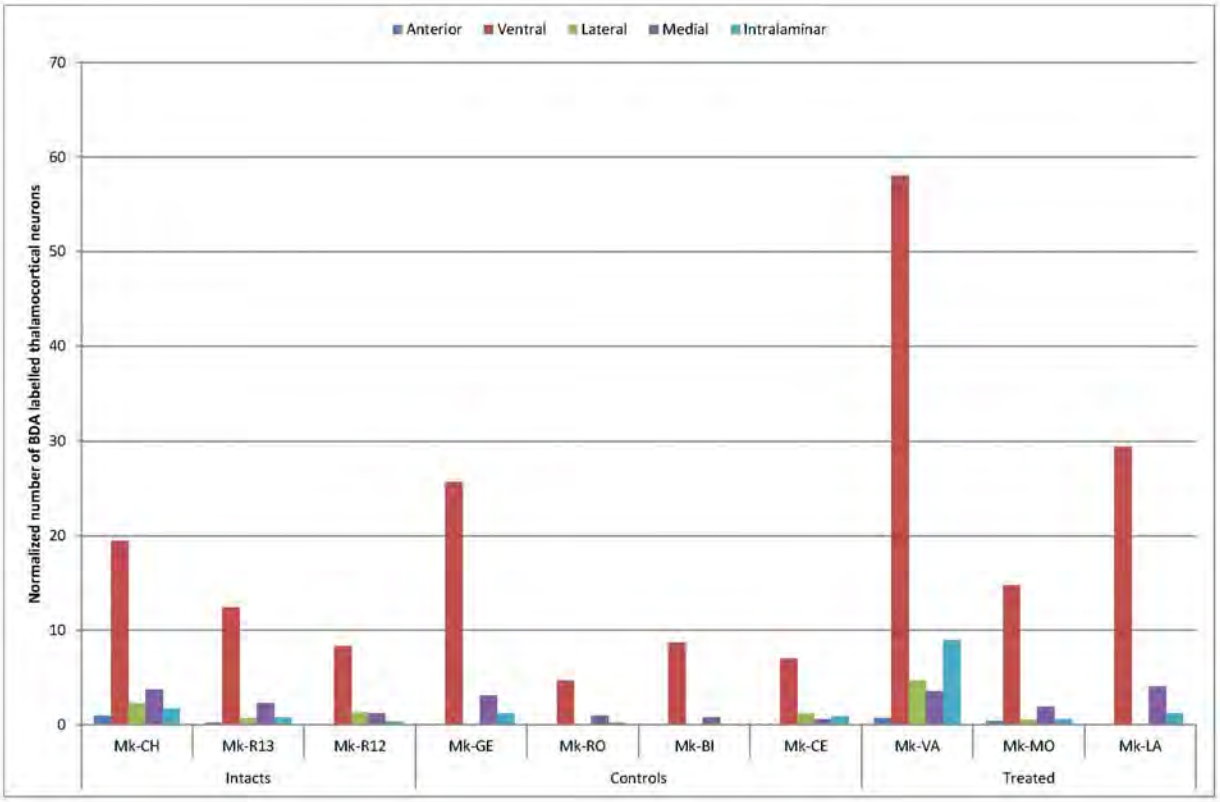




<u>Supplementary Figure 5</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the treated monkey MK-MO, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.



<u>Supplementary Figure 6</u>: Distribution of retrogradely labeled neurons in the thalamus after injection of BDA into PM in the treated monkey MK-LA, superimposed to photomicrographs obtained from Nissl stained sections. Scale bar: 1 mm. The color contours delineate the 5 main thalamic groups.



<u>Supplementary Figure 7</u>: Normalized number of BDA labelled thalamocortical neurons with respect to the volume of BDA injected (in µl).

4 Discussion générale et conclusion

Préambule

Le présent travail fait suite aux travaux antérieurs et pilotes effectués dans le laboratoire du Professeur Rouiller, portant sur les mécanismes de récupération des fonctions motrices suite à une lésion unilatérale du cortex moteur primaire chez les primates non-humains (Liu et Rouiller, 1999), qui a permis de décrire le déficit induit par une lésion de M1, ainsi que le décours temporel et l'étendue de la récupération fonctionnelle spontanée. La seconde phase de ce travail, qui englobe la présente thèse, vise à augmenter le nombre d'animaux lésés et de tester en première approche la pertinence d'un traitement à l'anticorps anti-Nogo-A, ou encore de l'implantation de cellules progénitrices adultes. Notre but était, d'une part, de comprendre les mécanismes de récupération des fonctions motrices suite à une lésion de M1 chez les macaques adultes par des études comportementales et électrophysiologiques. D'autre part, de voir les effets du traitement avec l'anticorps anti-Nogo-A, qui est connu pour améliorer la récupération fonctionnelle après lésion du cortex moteur chez les rats (Papadopoulos et al., 2002; Emerick et al., 2003; Emerick and Kartje, 2004; Seymour et al., 2005; Tsai et al., 2007, 2011; Cheatwood et al., 2008). Enfin, il s'agissait de déterminer la réorganisation de connexions dirigées vers le cortex prémoteur ipsilésionnel et provenant de différentes aires corticales de l'hémisphère intact, ainsi que l'influence du traitement avec l'anticorps anti-Nogo-A sur cette réorganisation. La compréhension de ces mécanismes de récupération des fonctions motrices chez les primates non-humains (singes macaques) revêt une importance capitale dans le développement de la recherche sur les lésions du système nerveux chez les humains et la mise sur pied de meilleurs traitements des lésions cérébrales.

Chez les primates, le système de projection corticospinal joue un rôle très important dans le control de la dextérité manuelle effectuée avec la main controlatérale (voir Lemon, 2008). Ces axones corticospinaux, qui décussent, se terminent sur les motoneurones qui controlent la musculature distale (Rouiller et al., 1999; Lacroix et al., 2004; Rosenzweig et al., 2009), tandis que ceux qui ne décussent pas contrôlent les mouvements des membres du coté ipsilatéral exécutés par la musculature proximale (Ganguly et al., 2009; Bradnam et al., 2010). Plusieurs études ont été effectués sur l'effet de la lésion du cortex moteur primaire sur la main contralatérale chez les primates non-humains (Passingham et al., 1983; Friel and Nudo 1998; Liu and Rouiller 1999; Frost et al., 2003; Plautz et al., 2003; Pizzimenti et al., 2007; Eisner-Janowicz et al., 2008; Murata et al., 2008; Darling et al., 2009; Mc Neal et al., 2010). Mais la

contribution du cortex moteur au contrôle de la main du coté ipsilatéral demeure sujet à controverse. Afin d'évaluer l'effet de la lésion de M1 sur la dextérité manuelle, plusieurs tests comportementaux ont été réalisés (voir le site web http://www.unifr.ch/neuro/rouiller/research/motorcontcadre.php) chez les animaux non traités et ceux traités à l'anticorps anti-Nogo-A.

Effet de la lésion unilatérale de M1 sur la main ipsilésionnelle (chapitre 1)

Dans le premier chapitre de cette thèse, nous avons évalué les effets à **court terme** de la lésion du cortex moteur primaire sur la dextérité de la main ipsilésionnelle chez les macaques adultes non traités ou ayant été traités à l'anticorps anti-Nogo-A ou par thérapie cellulaire après injection des cellules progénitrices autologues adultes prélevées dans le cortex préfrontal (Brunet et al., 2005; Kaeser et al., 2011). Deux paramètres ont été mesurés afin d'évaluer l'effet de la lésion sur la dextérité manuelle des macaques: 1) le score établi en 30 secondes, correspondant au nombre de récompenses récupérées avec succès en 30 secondes dans les deux types de puits (respectivement horizontal et vertical); 2) le temps de contact, défini comme l'intervalle de temps entre le moment où le doigt (souvent l'index) de l'animal entre dans le puit et le moment où l'animal sort du puit avec la récompense (Kaeser et al., 2010). Il ressort de ces travaux (chapitre 1 de la thèse) qu'une lésion unilatérale permanente de la région de la main du cortex moteur primaire n'induit pas systématiquement un déficit du contrôle moteur pour la main ipsilatérale, au contraire du déficit dramatique observé pour la main contralatérale. En effet, le déficit de la performance manuelle pour la main ipsilésionnelle reste plutôt modeste, uniquement durant quelques jours après la lésion. Ces résultats comportementaux ont fait l'objet d'une évaluation statistique pour chaque animal en définissant à partir de la période pré-lesionnelle un domaine de variabilité de la performance de dextérité manuelle normale sur l'animal intact (domaine défini par la moyenne +- 2 SDs). Pour qu'un changement de performance manuelle post-lésionnel soit considéré comme statistiquement significatif, il faut que le score, ou temps de contact, ou encore le temps total de préhension du jour testé sortent de ce domaine de variabilité de référence (voir Figs. 2, 3 et 4 du chapitre 1). C'est ainsi qu'il a été possible de montrer que les effets à court terme de la lésion de M1 sur la main ipsilésionnelle sont en général modestes, épisodiques (chez certains singes et pas chez d'autres) et limités à une période courte qui suit la lésion (quelques jours).. Il est important de noter ici que la présence d'un effet (modeste) ou non de la lésion sur la main ipsilésionnelle n'est pas corrélée avec la taille de la lésion de M1 (voir Tables 1 et 2 du chapitre 1). Il n'y a pas non-plus de corrélation avec la présence d'une extension de la lésion

dans la matière blanche (voir Table 1 du chapitre 1). Par contre, si l'on en croit les évidences pour des territoires spécifiques et très restreints dans M1 qui contrôlent la main ipsilatérale (voir Aizawa et al., 1990), il se pourrait toutefois que la taille de la lésion ne soit pas l'élément déterminant, mais plus cruciale serait la position précise de la lésion selon qu'elle touche ou non ces territoires spécifiques et restreints. Une hypothèse que nous n'avons pas pu tester, car contrairement à Aizawa et al. (1990), nous n'avons pas observés de sites ICMS qui produisent des mouvements de la main ipsilatérale. On ne peut ainsi pas déterminer si de tels territoires ont été lésés dans certains singes et pas dans d'autres. Il n'en reste pas moins que, si de tels territoires restreints existent, ils auraient dû être affectés par la lésion de M1 avec une plus grande probabilité chez les singes ayant une grande lésion (Mk-CE, Mk-JU) que chez les singes ayant une petite lésion (Mk-RO). Or, cette probabilité n'a pas été observée, ce qui met en doute, du moins à nos yeux, l'existence de ces territoires spécifiques et restreints. Pour expliquer ces résultats (épisodiques et modestes), on pourrait invoquer différentes (inter-individuelles) capacités et cinétiques d'adaptation à la lésion pour la main ipsilésionnelle.

La présente étude montre qu'une lésion unilatérale du cortex moteur a seulement des effets modestes sur la performance de la main ipsilésionelle. Ce résultat suggère ainsi que les activités neuronales observées dans M1 chez les singes exécutant des mouvements de la main ipsilatérale (Matsunami and Hamada 1981; Aizawa et al. 1990; Chen et al. 1991; Donchin et al. 1998, 2002; Kermadi et al. 1998, 2000; Kazennikov et al. 1999; Cisek et al. 2003) sont probablement liés à une activation des muscles proximaux (pour l'ajustement de la posture) et/ou aux activités visant à prévenir les mouvements simultanés de la main opposée.

Electrophysiologie (ICMS): changement de la représentation de la main (chapitre 2)

Les études électrophysiologiques basées sur les microstimulations intracorticales (ICMS) permettent de voir comment les aires corticales (principalement M1 dans le cas présent) sont modifiées suite à une lésion unilatérale de la représentation de la région de la main. Il a été ainsi possible d'établir des cartes corticales avant et après la lésion de M1, lorsque les animaux ont atteint un niveau stable de performance manuelle (plateau comportemental pré- et post-lésionnel). Le but de la stimulation post-lésionnelle était d'examiner comment la zone de la représentation de la main pré-lésionnelle dans M1 a été modifiée suite à la lésion provoquée par injection d'acide iboténique et de tenter de corréler ces éventuels changements avec la récupération de la dextérité manuelle. Plusieurs mois après la lésion, les territoires qui ont été lésionnés deviennent grandement non excitables, mais certains sites restent néanmoins excitables. On observe un réarrangement des cartes corticales

qui est variable d'un singe à l'autre tant à l'intérieur qu'aux alentours des territoires lésés, mais sans relation claire avec le degré de récupération fonctionnelle de la dextérité manuelle pour la main contralésionnelle. Les données comportementales dérivées des puits horizontaux, et non (ou à un degré moindre) des puits verticaux (voir Fig. 8 du chapitre 2), montrent un effet bénéfique du traitement avec l'anticorps anti-Nogo-A. Ceci par le fait que les puits horizontaux sont plus « challenging » (exigeants) et qu'il faut donc un déficit suffisant induit par la lésion pour mettre en évidence un effet bénéfique du traitement. Si la tâche comportementale n'est pas assez exigeante, alors la récupération spontanée est trop marquée et il n'est dès pas possible de discerner l'effet du traitement.

Inactivations réversibles (chapitre 3)

Les travaux sur l'inactivation réversible en infusant du muscimol ont permis aussi de déterminer quelles aires corticales contribuent à la récupération fonctionnelle de la dextérité manuelle après la lésion permanente du cortex moteur primaire et aussi d'évaluer l'éventuelle influence de l'anticorps anti-Nogo-A dans le processus de restitution des fonctions motrices. Il en résulte que M1, légèrement plus que PMd, lui-même plus que PMv contribuent à divers dégrés à la récupération fonctionnelle, sans toutefois de différence entre les singes traités et non-traités.

Sur un plan méthodologique, il faut bien réaliser que ces expériences d'infusions de muscimol à l'aide de plusieurs pénétrations avec des seringues Hamilton sont potentiellement dommageables pour le cortex et donc elles ont été en général limitées à une seule session par aire corticale dans l'hémisphère lésé (M1, PMd ou PMv). Pour cette raison, lorsque l'on mesure le score, il s'agit d'une observation unique qui ne permet pas de faire une analyse statistique pour comparer le score pre-infusion de muscimol avec le score post-infusion (à différents temps). En revanche, une analyse statistique a pu être appliquée sur les données du temps de contact, vu que ce dernier est mesuré pour 5 puits pour chaque orientation (puits verticaux et puits horizontaux). Toutefois, l'interprétation de la statistique est à prendre avec une certaine prudence vue le nombre faible de valeurs à chaque test (5 puits), surtout si l'on considère que le singe n'a pas été toujours capable de vider 5 puits dans les jours qui ont suivi la lésion à cause de sa capacité diminuée post-lésion à récupérer les granulés et le manque de motivation. Ainsi donc, un temps de contact grand se traduit par un score en 30 secondes qui est plus petit.

Etudes de traçage anatomique (chapitres 4 et 5)

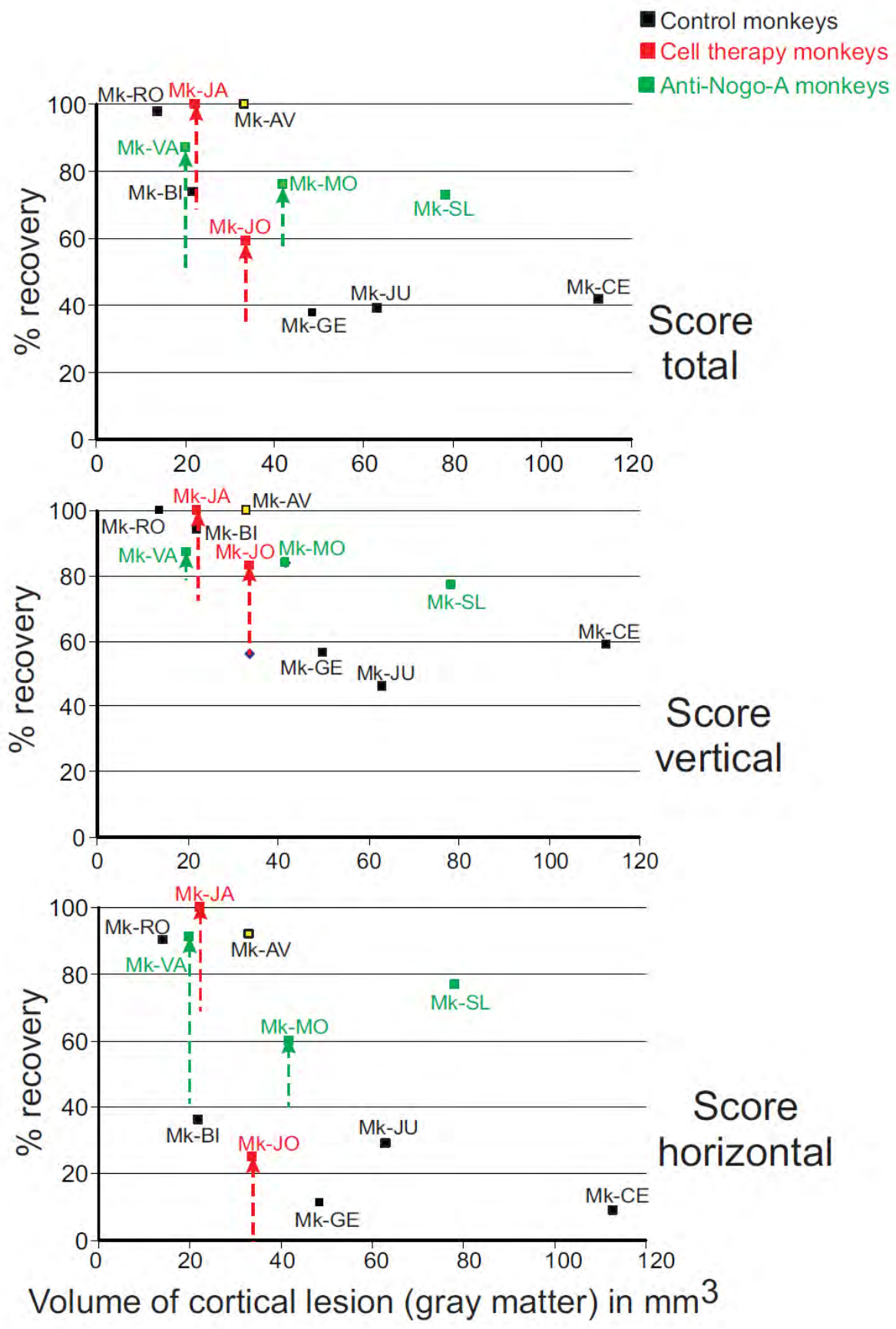
Par la suite, nous avons fait des études anatomiques sur la réorganisation des connexions callosales sur le cortex prémoteur ipsilésionnel suite à une lésion unilatérale de M1. Les singes traités avec l'anticorps anti-Nogo-A montrent une augmentation significative des connexions callosales vers le cortex prémoteur ipsilésionnel en provenance de l'hémisphère intact, sur la base du nombre de neurones marqués retrogradement, comparé aux singes non traités. L'analyse de l'origine des projections du thalamus au cortex prémoteur a été aussi effectuée suite aux mêmes injections de traceur retrograde (BDA) dans le cortex prémoteur. Chez les singes intacts, PM (PMd et PMv) reçoit des projections importantes à partir de la partie ventrale du thalamus et des projections moins importantes provenant de la partie médiale et latérale. Chez les singes traités, par contre, on note une augmentation des projections dans la partie antérieure et intralaminaire du thalamus.

Du fait de la variabilité entre les singes due à la position et à la taille des sites d'injection du traceur, du temps de survie du traceur (accumulation, dégradation), le nombre de neurones marqués par le BDA a été normalisé sur la base du volume du site d'injection de BDA en divisant ce nombre de neurones marqués dans chaque aire corticale par le volume du site d'injection en mm^3.

Pour des études futures, il serait bien entendu pertinent d'injecter BDA dans PM de l'hémisphère intact pour tenter de quantifier le marquage antérograde dans l'hémisphère lésé, et voir s'il y a une augmentation des champs terminaux dans PM ipsilésionnel, et quelques re-directions des projections, telles que celles observées par Dancause et collaborateurs (2005), de PM vers le cortex somesthésique primaire. Toutefois, on peut s'attendre à certaines difficultés pour détecter de telles modifications si elles sont mineures sur un plan quantitatif.

Status présent des 2 traitements considérés dans la thèse. Perspectives futures.

D'une manière générale, les présents résultats illustrent quelques facettes des conséquences d'une lésion unilatérale de M1, tant sur la main contralésionnelle que ipsilésionnelle, y compris le décours temporel et l'étendue de la récupération fonctionnelle. Certains mécanismes de récupération ont été en partie élucidés (voir plus bas). Pour ce qui est d'améliorer cette récupération par l'un (anti-Nogo-A) ou l'autre (transplantation des cellules autologues progénitrices adultes) traitement, nos études en sont à un stade pilote, avec toutefois de premières indications d'un effet favorable lié au traitement (voir Figure à la page suivante).



Légende page suivante.

Légende pour la figure de la page précédente:

Pour les 11 singes impliqués à ce jour dans l'étude en cours dans le laboratoire, le percentage de récupération fonctionnelle (dexterité manuelle mesurée par le score dans le test de la planche de Brinkman modifiée) suite à la lésion du cortex moteur est reporté en fonction du volume de la lésion corticale (matière grise). Le pourcentage de récupération fonctionnelle a été reporté pour le score total (puits verticaux et horizontaux cumulés) dans le graphe du haut et, séparément, pour les puits verticaux (graphe du milieu) et horizontaux (graphe du bas). Les animaux ont été séparés en 3 groupes: «*contrôles*» (lésion du cortex moteur et aucun traitement), «anti-Nogo-A antibody treated» (lésion du cortex moteur et traitement anti-Nogo-A), «thérapie cellulaire» (lésion du cortex moteur et implantation autologue de cellules progénitrices adultes: voir Kaeser et al., 2011 en annexe). Le singe Mk-AV est un «outlier» (indiqué par le carré en jaune) car il est un contrôle, mais la lésion est localisée plus dans le cortex prémoteur que dans M1; il en résulte que le déficit post-lésionnel était modeste et la récupération parfaite (ce singe n'a pas été considéré plus avant dans l'analyse). Pour les 2 groupes traités, les flèches verticales en traitillé indiquent que la récupération s'est faite en 2 phases, correspondant à deux plateaux post-lésionnels (voir chapitre 2 pour les singes anti-Nogo-A et Kaser et al., 2011 en annexe pour les singes ayant reçu la thérapie cellulaire).

La figure de la page précédente montre en effet quelques premières tendances en relation avec les traitements. Tout d'abord, chez les 5 animaux contrôles (symboles en noir) on observe une absence de récupération en 2 phases (double plateau), au contraire de 4 animaux traités sur 5. Ensuite, chez les animaux contrôles, comme on pouvait s'y attendre, il y a une forte diminution de la recupération fonctionnelle spontanée lorsque la taille de la lésion augmente, cela dans les 3 graphes. Pour ce qui est des animaux traités (symboles rouges ou verts), nous voyons pour le score total (puits verticaux et horizontaux cumulés dans le graphe du haut) que, à taille de lésion similaire, les 2 singes traités Mk-JA et Mk-VA récupèrent mieux que le singe contrôle Mk-BI, cela grâce au second plateau de récupération observé chez les 2 singes traités. De même, pour des lésions plus grandes, mais relativement comparables, les 2 singes anti-Nogo-A Mk-MO et Mk-SL récupèrent clairement mieux que les 2 singes contrôles Mk-GE et Mk-JU. A noter que Mk-MO montre aussi le 2ème plateau de récupération. Cela n'est pas le cas pour Mk-SL (seul singe traité sans 2ème plateau), mais il faut remarquer que cet animal atteint un niveau de récupération impressionnant compte tenu de la taille de la lésion (voir chapitre 2). Un effet de plafonnement pourrait avoir empêché l'observation du 2ème plateau. Le dernier singe traité Mk-JO va dans le sens d'une assez bonne récupération, mais pas remarquable compte tenu de la taille relativement petite de la lésion (voir aussi plus bas).

Concernant les puits verticaux (graphe du milieu), on a des tendances en général assez comparables au score total, avec toutefois moins d'écart entre les singes traités et les singes contrôles que pour le score total, ce qui s'explique par le fait, en principe, que les singes ont moins de difficultés à prélever les pellets dans les puits verticaux que dans les puits horizontaux. Le plus intéressant, surtout en ce qui concerne le traitement anti-Nogo-A, sont les résultats de récupération fonctionnelle obtenus pour les puits horizontaux (graphe du bas dans la Figure plus haut). Du fait de la plus grande difficulté de la tâche (que les puits verticaux), la récupération est clairement modeste pour les animaux contrôle (sauf Mk-RO avec la plus petite lésion). Quatre (Mk-VA, Mk-JA, Mk-MO et Mk-SL) des 5 animaux traités montrent une récupération clairement plus marquée que les animaux contrôles. La seule exception est le singe MK-JO, chez qui la thérapie cellulaire n'a pas apporté de grand bénéfice pour les puits horizontaux (au contraire des puits verticaux).

La séparation entre puits verticaux et horizontaux est donc importante. En effet, une tâche pas trop difficile (comme les puits verticaux) est accompagnée après léesion du cortex moteur d'une récupération spontanée assez élevée, ce qui laisse peu de marge pour une amélioration éventuellement apportée par un traitement. Par contre, pour une tâche plus difficile (puits horizontaux), la marge de progression est plus grande pour mettre en évidence un effet d'un traitement. Pour cette raison, dans ce type d'étude, comme il n'est pas toujours possible de prédire à l'avance cette marge de progression pour une tâche donnée, il n'est pas inutile de concevoir une tâche comme la planche de Brinkman modifiée avec 2 niveaux de difficulté (puits verticaux et puits horizontaux) mais aussi d'introduire une plus vaste palette de tâches avec des degrés de difficultés différentes et aussi testant différents paramètres du contrôle moteur. C'était le but d'ailleurs de l'étude récemment publiée par notre laboratoire sur ces aspects méthodologiques du comportement moteur (voir Schmidlin et al., 2011 en annexe), travail auquel le présent auteur de la thèse a aussi contribué.

Ces premiers résultats sur les 2 traitements sont donc relativement encourageants mais, néanmoins, il est essentiel d'augmenter le nombre d'animaux afin de confirmer, ou non, ces premiers résultats. En effet, un plus grand nombre d'animaux (environ 6 par groupe) est nécessaire pour appliquer une statistique de groupe bi-variée et non-paramétrique (voir Freund et al., 2009 dans le cas des lésions spinales).

Mécanismes de récupération fonctionnelle suite à une lésion unilaterale de M1

Le but de ces expériences multiples et pluridisciplinaires rapportées dans la présente thèse était de collecter des évidences en faveur ou contre différents mécanismes possibles de récupération de la dextérité manuelle par la main contralésionnelle suite à la lésion unilatérale de M1. En vue de tirer des conclusions générales, il est nécessaire de procéder à une confrontation et une synthèse des résultats présentés dans les différents chapitres. Quels mécanismes de récupération pouvait-on envisager (avant la réalisation des expériences) ? **Quatre** mécanismes possibles ont été ainsi énumérés (liste non-exhaustive):

i) **la préservation /réparation de sous-territoires corticaux à l'intérieur de la zone lésée, qui peuvent contribuer à la récupération fonctionnelle**. Les expériences ICMS (chapitre 2) démontrent qu'après la lésion et la récupération, on trouve bien dans la région de M1 sujette à la lésion permanente des territoires qui restent (ou re-deviennent) micro-excitables, pouvant provoquer des mouvements distaux au niveau du membre antérieur contralésionel (par exemple chez les singes Mk-BI, Mk-RO, Mk-MO et, à un degré moindre, Mk-VA et Mk-SL; voir Figs. 1, 2 et 3 du chapitre 2). Par contre, de tels territoires microexcitables post-lésion sont pratiquement absents chez les singes Mk-GE, Mk-CE et Mk-JU (voir Figs. 2 et 3 du chapitre 2). On peut en conclure que le mécanisme proposé ici peut contribuer à la récupération fonctionnelle, mais pas de manière systématique et, si présent, à des degrés divers. Ce mécanisme, présent chez certains singes, est aussi en accord avec nos observations d'une perte de la dextértié manuelle récupérée suite à une inactivation reversible de M1 par infusion de muscimol (dans la même zone que celle lésée avec l'acide iboténique; voir chapitre 3).

ii) **la substitution par territoires de M1 immédiatement adjacents à la lésion**. L'analyse détaillée des cartes ICMS post-lésion (chapitre 2) montre que cela n'est généralement pas le cas. En effet, on voit peu ou pas de territoires qui correspondaient avant la lésion à une représentation plus proximale (coude, épaule) ou de la face et qui se sont transformés post-lésion en des territoires dans lesquels la main est représentée (réponses ICMS à bas seuil ; voir Figs. 1-3 dans le chapitre 2). Toutefois, il faut mentionner que les cartes ICMS sont représentatives pour les seuils les plus bas uniquement et qu'il ne faut pas exclure des effets sur la main, mais à des intensités de stimulation plus élevées appliquées dans des régions du coude, de l'épaule ou encore de la face. Par contre, un tel mécanisme a été démontré suite à une lésion de M1 néonatale (quelques semaines après la naissance) chez les singes macaques (Rouiller et al., 1998): plus tard, à l'état adulte, ces mêmes singes montrent une représentation de la main dans une zone normalement dévolue au coude et à

l'épaule, des territoires eux-mêmes repoussés plus médialement. Une telle différence entre une lésion néonatale et lésion adulte reflète une plasticité supérieure chez le jeune, accompagnée par une récupération fonctionnelle meilleure.

iii) **le rôle des aires corticales de l'hémisphère intact via la projection non-croisée et/ou indirectement via le corps calleux.** Ce mécanisme est peu peut probable sur des aniamux non-tratés, selon Liu et Rouiller (1999), à partir des expériences pilotes réalisées sur 2 singes (Mk-CE et Mk-JU), lors d'une inactivation reversible au muscimol. En effet, une inactivation des aires corticales motrices de l'hémisphère intact n'a pas influencé les scores post-lésion, chez ces 2 singes (Liu et Rouiller, 1999). On ne peut pas exclure qu'il en serait autrement si l'on considère le temps de contact. Un tel mécanisme impliquerait une extension du contrôle moteur non-croisé à partir des motoneurones des muscles proximaux (cible sur l'animal intact) vers les motoneurones des muscles distaux. Un tel scénario pourrait être favorisé par un traitement de type anti-Nogo-A, grâce au bourgeonnement ainsi stimulé des axones corticospinaux. L'expérience de l'inativation de l'hémisphère intact chez des singes lésés dans M1 et traités avec l'anticorps anti-Nogo-A reste à faire dans le futur. Pour ce qui concerne une influence indirecte, interhémisphérique via le corps calleux, les résultats du chapitre 4 indiquent une augmentation de la projection de PM contralésionnel (hémisphère intact) sur PM ipsilésionnel en relation avec le traitement anti-Nogo-A, ce qui suggère bien un rôle indirect de l'hémisphère intact.

iv) **le rôle d'aires corticales motrices non-primaires ipsilésionnelles.** Ce mécanisme a été montré initialement sur le même modèle, chez 2 singes lésés dans M1 et non-traités (Liu et Rouiller, 1999), sous la forme d'une contribution de PM ipsilésionnel. En effet, comme confirmé sur d'autres singes dans la présente thèse (chapitre 3), une inactivation reversible de PM entraîne une baisse de la dextérité manuelle récupérée suite à la lésion permanente de M1. De la même manière, il a été démontré par les travaux de McNeal et ses collaborateurs (2010) que SMA ipsilésionnel peut aussi contribuer à la récupération. En effet, suite à une lésion de M1 et PM, il y avait une augmentation (sprouting) de la partie terminale dans la moelle cervicale de la projection corticospinale provenant de SMA ipsilésionnel, qui est en partie le support de la récupération fonctionnelle. Cela est démontré par l'observation qu'une lésion subséquente de SMA provoque une réapparition du déficit initialement observé après lésion de M1 et PM. D'autres aires ipsilésonnielles que PM peuvent contribuer aussi à la récupération fonctionnelle comme PM et SMA. C'est le cas de S1 (voir Sasaki and Gemba, 1984), qui pourrait jouer un rôle dans la récupération, un mécanisme également cohérent avec les données anatomiques de Dancause et al. (2005), qui montrent après une lésion de M1 une

redirection des projections de PM sur M1 vers S1. On peut en conclure dans ce contexte que les différentes aires motrices corticales (et S1) constituent ensemble un réseau fonctionnel de contrôle moteur de la dextérité manuelle, avec une certaine redondance de manière à permettre une reprise en charge partielle par un des partenaires du réseau lors d'une lésion d'un autre partenaire (principe de vicarité). Ceci va de paire avec une augmentation du nombre d'aires corticales pour une modalité donnée lors de la philogenèse.

Limites et spécificités des études sur le primate non-humain

Ce travail de thèse met en évidence de nombreuses difficultés inhérentes à ce type d'étude sur le modèle du primate non-humain. Premièrement, la durée énorme que prend le protocole expérimental sur chaque animal si l'on songe que plus de 10 ans se sont écoulés entre les premières études pilotes (Liu et Rouiller, 1999) et les résultats présentés ici sur environ 8 autres singes. Une telle durée s'explique par le fait que le protocole expérimental n'a pas été élaboré pour uniquement aborder la question de l'efficacité d'un traitement, mais plutôt de tenter d'élucider des mécanismes (voir paragraphe précédent). Le prix à payer est une forte augmentation de la complexité du protocole, avec des interventions supplémentaires pouvant influencer le résultat purement lié au traitement (par exemple ICMS). Sur une telle durée, il est inévitable que les protocoles soient légèrement ajustés, en fonction des résultats obtenus sur les singes précédents. Cela a pour résultat d'augmenter la variabilité entre singes. Certains changements de protocole sont introduits pour des raisons légales et/ou éthiques, tel que par exemple l'injection d'acide iboténique dans l'état vigile sur les premiers singes, puis sur les suivants sous anesthésie au propofol. Or, une telle anesthésie amortit l'effet de l'acide iboténique, ce qui a créé une situation instable quant à la taille voulue pour la lésion de M1.

Dans un tel contexte, il est compréhensible qu'un travail de thèse tel que celui-ci ne puisse contribuer qu'à une partie de toute l'étude, et cela dans le cadre d'un travail d'équipe. En effet, au mieux, un expérimentateur individuel ne peut s'occuper que de 2 singes en parallèle, cela sur une période de 2 ans. Il est dès lors indispensable de «pooler» des animaux provenant de différentes études sur une longue durée. Comme dit plus haut, les prochaines études auront pour but d'augmenter le nombre d'animaux afin de, si possible, valider la pertinence de l'un ou l'autre des traitements. Sur le plan des mécanismes, nous sommes d'avis que l'approche ICMS est limitée, car invasive et restreinte à une petite zone du cortex. C'est pourquoi, dans le futur, l'approche EEG est envisagée car non-invasive et susceptible de donner des informations sur des ré-organisations corticales à plus grande distance de la lésion de M1.

On peut reprocher à ce type d'étude la grande variabilité inter-individuelle. C'est correct, mais cependant cette variabilité sur ce modèle reflète en fait la variabilité encore plus grande chez les patients humains, ce qui est aussi un avantage de ce modèle dans un but translationel. Cependant, comme démontré dans l'annexe 1 (Kaeser et al., 2010), par rapport à la situation clinique, le présent modèle sur le primate non-humain présente l'avantage sur le plan de la puissance statistique, de comparer pour le même individu, la performance post-lésionnelle à la performance pré-lésionnelle, ce qui est bien entendu exclu chez les patients. Cela permet de réduire ainsi le nombre de singes, ce qui est heureux sur le plan éthique. Cependant, la présente étude montre toutefois qu'un nombre minimum de singes est requis, probablement aux alentours de 6-10 par groupe de traitement.

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5 Annexes

Dans le cadre des travaux qui ont été effectués dans le laboratoire du professeur Rouiller sur l'effet d'une lésion de M1 sur la dextérité manuelle chez les primates adultes, ainsi que les mécanismes de récupération fonctionnelle (en absence ou en présence de traitement), j'ai également participé comme co-auteur à plusieurs études. Les articles correspondants ont été publiés et placés en annexe de ce présent travail. Le premier article (Kaeser et al., 2010) traite de l'effet de la lésion unilatérale du cortex moteur sur la préhension de la main ipsilésionnelle et la performance en relation avec la récupération de la main contralésionnelle. Le second article (Kaeser et al., 2011) est une étude pilote qui traite de l'effet de la transplantation de cellules corticales autologues sur la récupération fonctionnelle suite toujours à une lésion unilatérale du cortex moteur. Un 3ème article (Peuser et al., 2011) traite du suivi de l'activité corticale et de sa sctructure par l'utilisation de laser et d'imagerie par résonnance magnétique. Enfin, le dernier article (Schmidlin et al., 2011) montre tous les tests de dextérité manuelle éffectués dans le laboratoire du Professeur Rouiller.

Annexe 5.1

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

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

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

INTRODUCTION

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

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

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

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

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

METHODS

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

Treatments

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

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

	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 to 0.5 yr)	4.5	5.0	5.0	4.0	5.5	5.5	5.5	3.5	3.5	4.0
Weight at time of lesion, Kg	3.8	3.6	2.8	3.2	4.9	4.6	5.6	4.3	3.4	4.3
Time window for prelesion assessment of manual dexterity, days	99	128	70	29	119	77	29	20	29	60
Time window for assessment of long-term deficit of ipsilesional hand post-lesion, days	150–287	154–264	78–115	48–77	153–225	99–128	70–93	100–166	140–164	260–308
Volume of ibotenic acid injected, μL	40	40	13	18	15.5	18	20	15	15	38*
Number of ICMS sites injected with ibotenic acid	21	21	13	12	11	11	20	10	10	38
Total volume of lesion (in mm^3)	112.8	63.01	48.7	14	20	78.2	41.8	33.2	33.6	22.2
Gray matter (motor cortex + postcentral gyrus)										
Volume of lesion in postcentral gyrus	10.1	0.0	7.6	0.0	5.8	1.8	0.0	0.0	3.8	2.5
Long-term performance of ipsilesional hand in the modified Brinkman board task "Score" (percentage of prelesion score)	93.9%	92%	100%	124%	119.2%	100%	109.1%	138.5%	105.7%	128.3%

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

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

Behavioral assessment of manual performance

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

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

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

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

Surgery

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

Electrophysiology: intracortical microstimulation

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

slightly rostral to the central sulcus, ICMS effects were obtained along a distance of ≤10–12 mm, along the rostral bank of the central sulcus, forming a band of gray matter perpendicular to the cortical surface (see red dashed line in Fig. 1*B*). When the electrode penetration was located more rostral, the cortical layers were oriented parallel to the cortical surface and the distance along which ICMS effects were present was shorter (≤4–5 mm; see green dashed line in Fig. 1*B*). Depths of the ICMS sites were determined with the zero corresponding to the surface of the dura mater, which became progressively thicker with time (it was regularly scratched to facilitate the electrode penetrations every 3–4 wk). The effect of ICMS was assessed by visual inspection and/or palpation of the body part (articulation) where a movement was elicited. The minimal current (ICMS threshold) producing this movement was determined at each stimulation site. The repeated ICMS electrode penetrations were performed for several weeks prelesion. The ICMS map as seen from the surface was finally represented in the form of an unfolded map of M1 (Supplemental Fig. S1),[1] as previously reported (Park et al. 2001, 2004) and served as the basis to guide injections of ibotenic acid to produce a permanent lesion of the motor cortex, targeting the hand area of M1 (see following text).

[1] The online version of this article contains supplemental data.

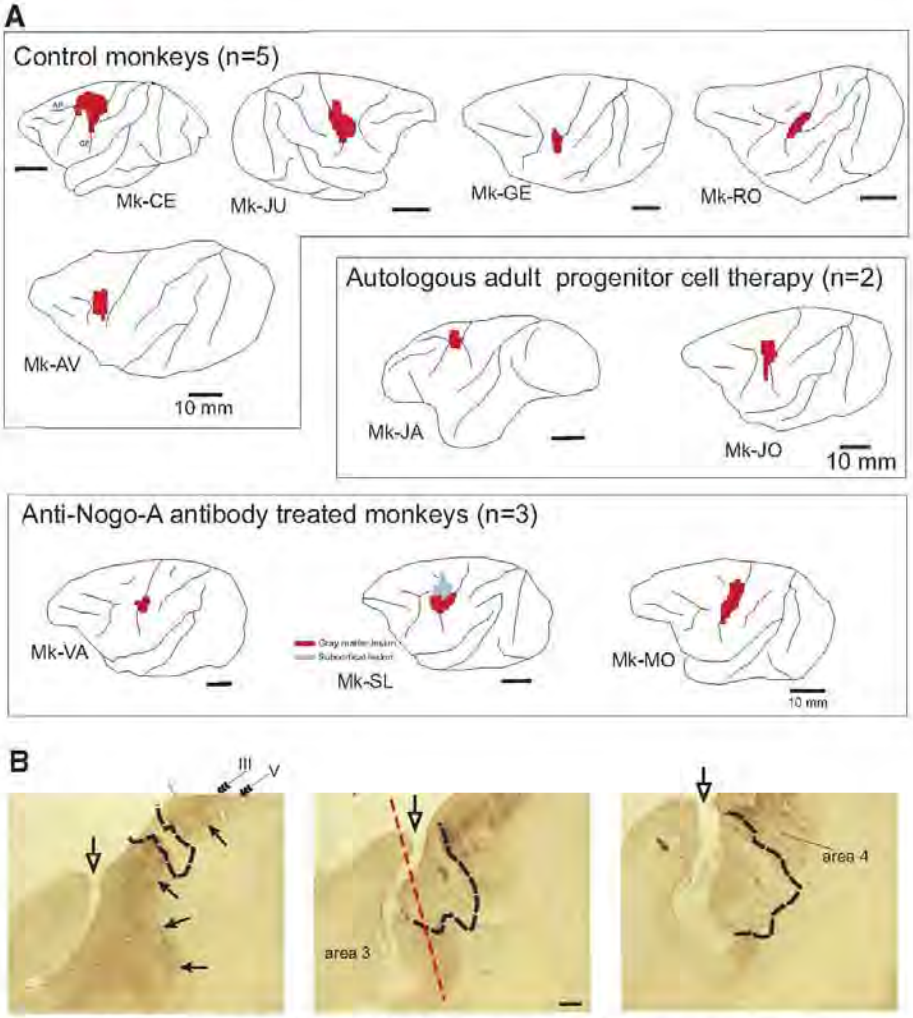


FIG. 1. *A*: location and extent of the permanent unilateral lesion of the hand representation in the motor cortex, as seen on corresponding lateral views of the brain for the 10 monkeys included in the present study (see Table 1). The lesion area, represented in red, was determined from the lesioned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

Permanent lesion of M1 hand representation with ibotenic acid

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

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

Data analysis

Within the pre- and postlesion time frame of behavioral analysis defined for each monkey (see Table 1), the pellet retrieval score was plotted as a function of time in days (e.g., Fig. 2*A*). The prelesion period was used to establish the reach and grasp performance of reference, indicated by the median value (red horizontal lines in Fig. 2*A*). Postlesion, behavioral sessions were conducted for several months (e.g., Fig. 2*A*). 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. 2*A*). Finally, the comparison of the two median values (prelesion vs. long-term postlesion) allowed a quantitative assessment of the effect of the lesion several months thereafter. The behavioral data were analyzed statistically using an unpaired nonparametric Mann–Whitney test. A similar analysis was conducted on the second behavioral parameter, the contact time. The prelesion data used to address the issue of hand dominance (see *Hand dominance for the modified Brinkman board?* in RESULTS) were analyzed using a paired comparison of daily scores obtained for the left hand and the right hand (paired *t*-test or Wilcoxon test). The statistical analysis and related graphs were obtained using the software SigmaStat 3.5 and SigmaPlot 10.0.

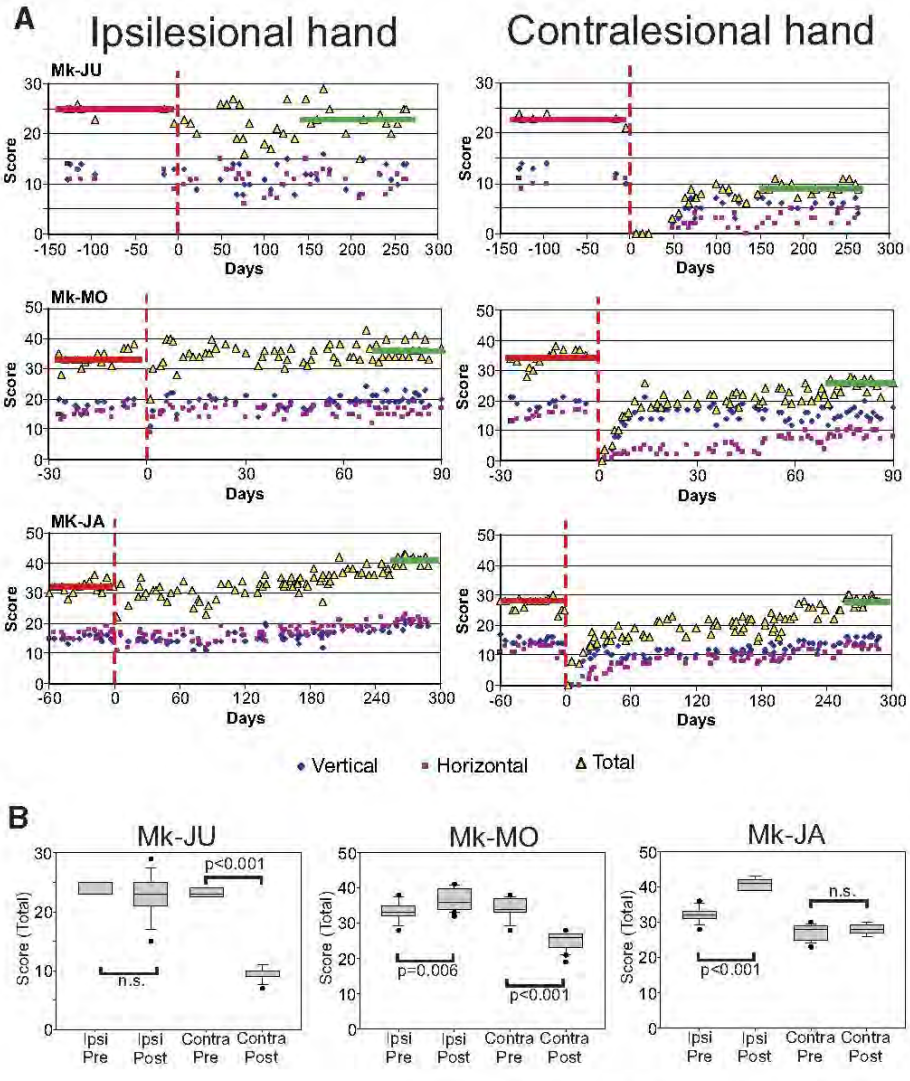


FIG. 2. *A*: number of pellets retrieved in 30 s as a function of time (days) in the modified Brinkman board task for 3 monkeys (Mk-JU, *top*; Mk- MO, *middle*; and Mk-JA, *bottom*). The numbers of retrieved pellets (scores) are indicated separately for the vertical wells (blue diamonds) and the horizontal wells (red squares); the yellow triangles represent the total score determined by summing the vertical and horizontal scores. The vertical red dashed line at *time 0* is the day of the unilateral motor cortex lesion (prelesion days are negative and postlesion days are positive). Prelesion, the median total score is given by the red horizontal line (plateau); postlesion, the median long-term total score is given by the green horizontal line (plateau). Prelesion, sessions took place earlier than the first day indicated on the abscissa, but the animals' performance had not yet reach a stable plateau. Because Mk-JU was generally slower than the other 2 monkeys (fewer pellets collected in 30 s), note that the maximal value of the ordinate for Mk-JU was 30 (instead of 50), to preserve resolution of the individual data points. *B*: for the same 3 monkeys as in *A*, the total score is represented in the form of box and whisker plots for the ipsilesional hand (2 *left boxes*) and the contralesional hand (2 *right boxes*), allowing comparison of the pre- and postlesion performances for each hand. In the box and whisker plot, the boundary of the box closest to zero corresponds to the 25th percentile, the line within the box is for the median value, and the boundary of the box farthest from zero is for the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Black dots are for outlying points. The result of the statistical comparison pre- vs. postlesion (Mann–Whitney test) is indicated with the corresponding *P* value (n.s., not statistically significant at $P > 0.05$).

At the end of the experiments, the animals were sacrificed with an intraperitoneal overdose of pentobarbital sodium (90 mg/kg body weight). Transcardiac perfusion with 0.9% saline (500 ml) was followed by fixative (4,000 ml of 4% phosphate-buffered paraformaldehyde). The brains were placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection for 3–5 days. Frontal sections (50 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 (Beaud et al. 2008; Liu et al. 2002; Wannier et al. 2005). The epitope recognized by the SMI-32 antibody lies on nonphosphorylated regions of neurofilament protein and is expressed only by specific categories of neurons (Campbell and Morrison 1989; Tsang et al. 2006). The two series of sections were then used to reconstruct on consecutive sections the position and extent of the permanent lesion in the cerebral cortex, especially using the SMI-32 stained sections (Fig. 1*B*), 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. 1*A*). Using an ad hoc function of the Neurolucida software (based on the Cavalieri method; see, e.g., Pizzimenti et al. 2007), the volume of the cortical lesion (in mm^3) affecting the cortical gray matter was extrapolated from the reconstructions of the lesion on consecutive histological sections of the brain (see Table 1).

RESULTS

Unilateral lesion of the motor cortex

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

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

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

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

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

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

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

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

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

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

Overall (Figs. 2 and 3), long-term postlesion enhancement of reach and grasp performance in the ipsilesional hand was found in six of ten monkeys, as assessed by the total retrieval score in the modified Brinkman board task. In these six monkeys, this enhancement was associated with relatively good recovery of the contralesional hand. To better analyze the dependence between the two hands, the long-term postlesion manual performance in the ipsilesional hand (expressed in % of prelesion score) was plotted as a function of the percentage of recovery of the contralesional hand (Fig. 4). There is a strong correlation between these two parameters, with a coefficient of correlation $r = 0.932$ ($P < 0.001$), consistent with the notion that, after unilateral lesion of the motor cortex, a good recovery with the contralesional hand is associated over the long term with an enhancement of manual performance in the ipsilesional hand.

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

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

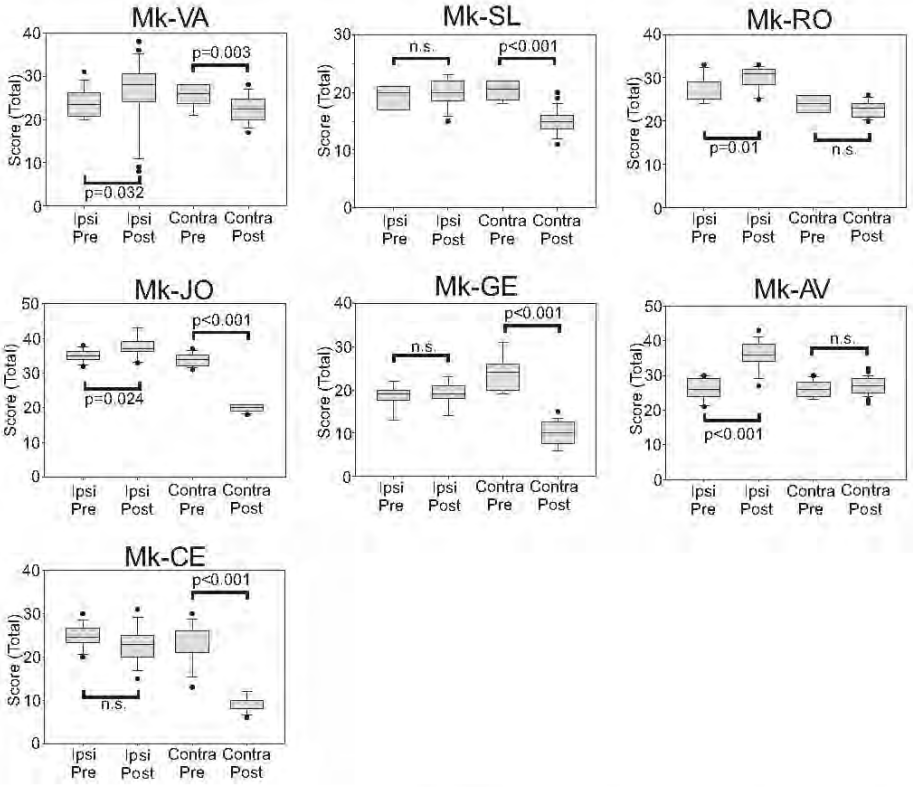


FIG. 3. Box and whisker plots (as in Fig. 2*B*) for the other 7 monkeys included in the study, showing the total score in the ipsilesional hand (2 *left boxes*) and in the contralesional hand (2 *right boxes*) for comparison of the pre- and postlesion scores for each hand. As in Fig. 2, note that the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

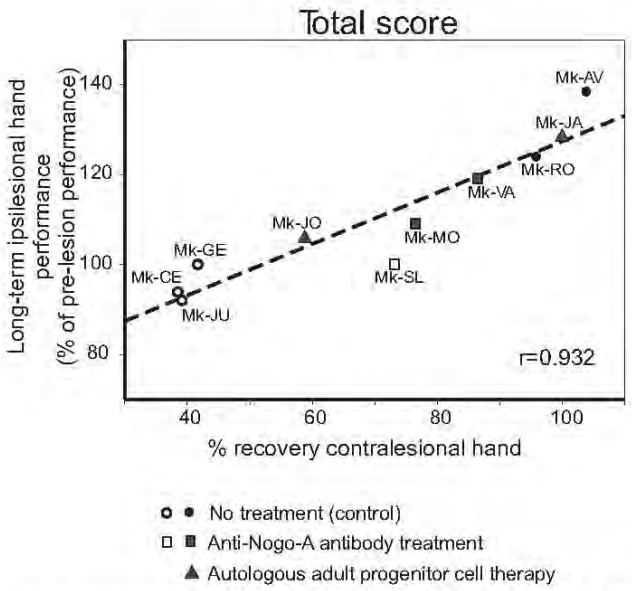


FIG. 4. Long-term ipsilesional hand performance (expressed in % of the prelesion total score in the modified Brinkman board task) was plotted as a function of the percentage of recovery in the contralesional hand (compared with the prelesion total retrieval performance). The dashed line is the regression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

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

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

The cited data indicate that the long-term enhancement of manual performance in the ipsilesional hand is strongly correlated with the degree of recovery for the contralesional hand (Figs. 4 and 6). One may wonder whether the same parameter is correlated with the extent of the motor cortex lesion. Manual performance in the ipsilesional hand assessed over the long term (expressed as percentage of the prelesion score) was plotted as a function of the volume of the lesion, expressed in cubic millimeters, encompassing primarily the gray matter in M1 and, to a lesser extent, gray matter in S1 in some monkeys (see also Table 1). As shown in Fig. 7 (*top*), there is a significant inverse correlation ($r = -0.735$; $P < 0.01$) between the long-term performance in the ipsilesional hand and the volume of the lesion (in the motor cortex and S1). Clearly, the six monkeys with a significant long-term enhancement of manual performance of the ipsilesional hand (filled symbols in Fig. 7, *top*) had a smaller lesion than that of the other four monkeys. For a more comprehensive description of the relationship between the three relevant parameters, the *bottom panel* of Fig. 7 shows a three-dimensional plot of the long-term performance in the ipsilesional hand versus the percentage of recovery of the contralesional hand and the volume of the cortical lesion.

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

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

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

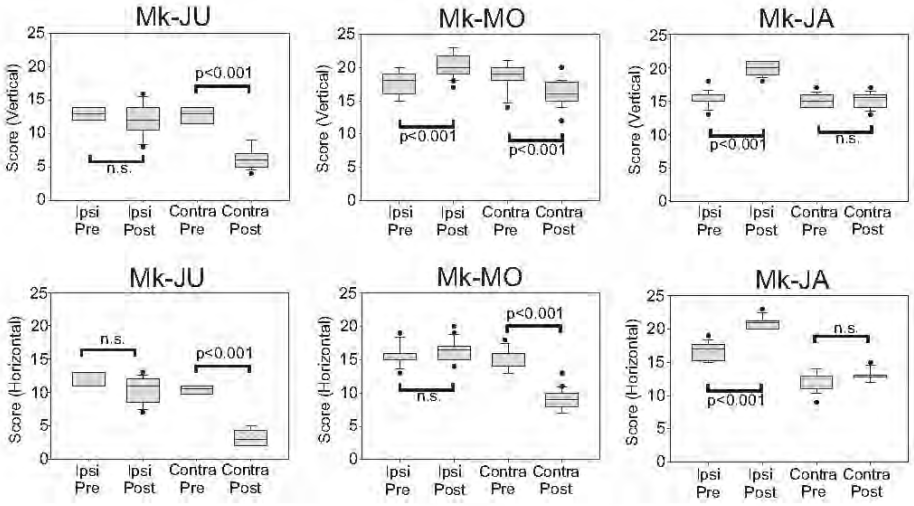


FIG. 5. Same data as in Fig. 2*B* (Mk-JU, Mk-MO, and Mk-JA), but for the 2 slot orientations (vertical and horizontal) analyzed separately.

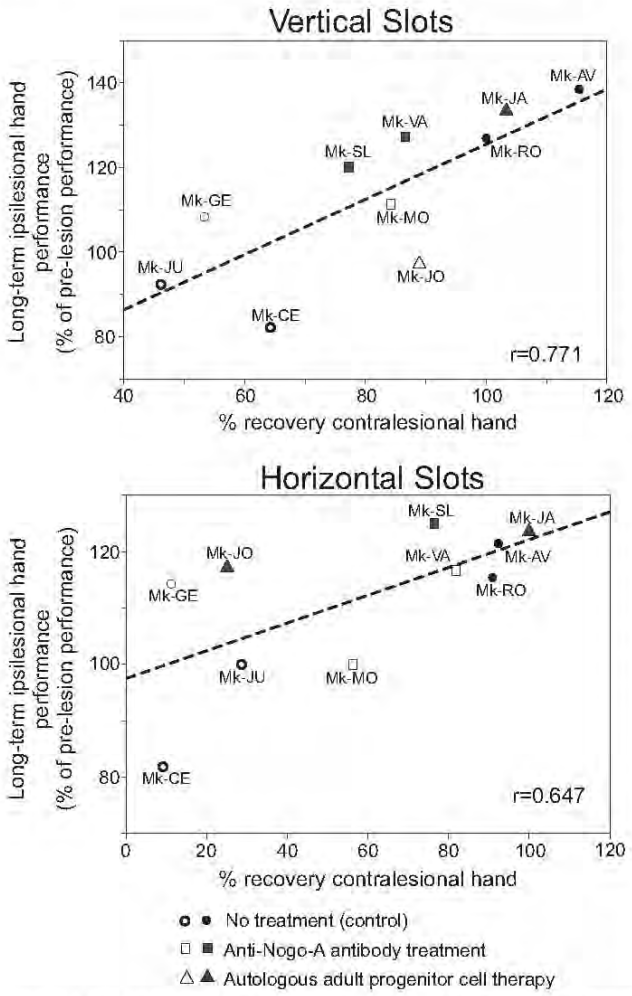


FIG. 6. Same data as in Fig. 4 (correlation between long-term postlesional ipsilesional hand performance and percentage recovery of the contralesional hand) for the 2 slot orientations (vertical and horizontal) analyzed separately. Filled symbols are for the monkeys exhibiting significant long-term enhancement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

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

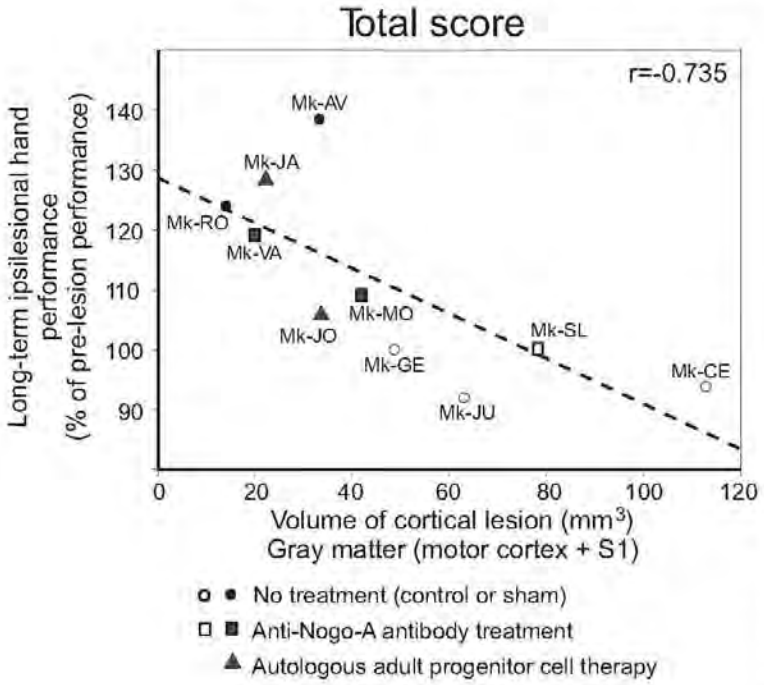
With respect to the ipsilesional hand, the contact time data showed less difference between the prelesion and the long-term postlesion periods than did the retrieval score data. An enhancement of postlesion ipsilesional hand manual dexterity over the long term, evidenced by a decrease in contact time,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

Differences with clinical studies

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

Hand dominance for the modified Brinkman board?

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



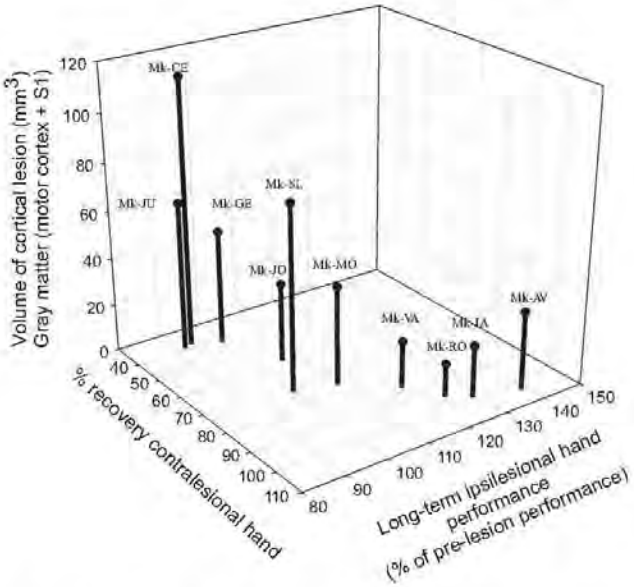


FIG. 7. *Top*: the postlesion long-term ipsilesional hand performance (compared with prelesion) is plotted as a function of the volume of the motor cortex lesion (corresponding to the volume of gray matter affected by the lesion in the motor cortex and in the primary somatosensory cortex; see Table 1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

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

DISCUSSION

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

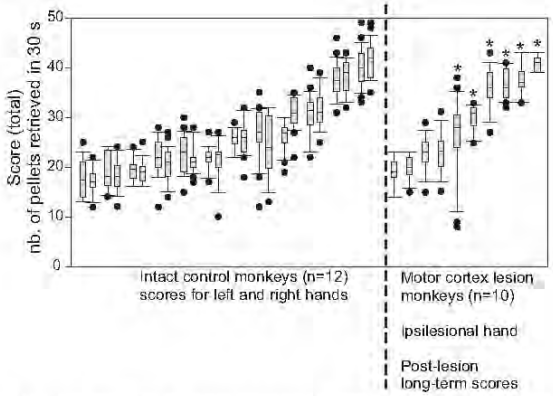


FIG. 8. Box and whisker plots showing the total number of pellets retrieved (score) in the modified Brinkman board task for 2 groups of monkeys. On the *left*, the performance is shown for a group of intact control monkeys (in fact prelesion score of monkeys subjected later on to spinal cord injury [SCI];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

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

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

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

Limitations of interpretation

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

Spread of the lesion to cortical areas adjacent to M1

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

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

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

Comparison of score and contact time data

The contact time data specifically reflect the grasping function by measuring the time of manipulation of the pellet with the fingers before successful retrieval. The retrieval score data also reflect this manipulation but, in addition, comprise other facets of the task, such as arm reaching, arm withdrawal, and transport of the pellet to the mouth. The observation of long-term enhancement of manual performance in the ipsilesional hand after unilateral motor cortex lesion in six monkeys comes largely from score data (Figs. 4 and 6), whereas the contact time data showed only a trend in that direction (Supplemental Fig. S6). How can it be explained that contact time data are not fully corroborating with the retrieval score data? To address this question, the strategy used by the monkey to perform the modified Brinkman board was investigated. To assess one facet of the strategy, the cumulative distance between consecutive slots was determined, both prelesion and postlesion. If monkeys visit the slots in a systematic manner (e.g., starting at a given extremity of the board and then moving progressively toward the other extremity of the board), then the cumulative distance is smaller than that in the case of random spatial choice of the slots. For each monkey, the difference of cumulative distance between consecutive slots (postlesion minus prelesion) was calculated. For the ipsilesional hand, there was a significant inverse correlation between the difference of cumulative distance and the long-term manual performance (not shown). In other words, the monkeys that did not exhibit enhancement of manual performance in the ipsilesional hand had a postlesion strategy in which they visited slots more randomly. In contrast, monkeys with long-term enhancement of the ipsilesional hand visited the slots in a more ordered sequence, both prelesion and postlesion. When visiting the slots randomly, subjects exhibited some hesitation before moving to the next slot, resulting in fewer pellets retrieved in 30 s. It can be tentatively concluded that the enhancement of manual performance reflects more an improvement of strategy than a better manual dexterity per se. As a consequence, the correlation with the contact time was weaker than that with the retrieval score, which includes the entire temporal course of the trial, including some strategic aspects. Finally, there was some disparity across monkeys, ranging from a reliable correlation between retrieval score and contact time to an absence of correlation between the two parameters.

Comparison with functional recovery in human subjects

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

Potential mechanisms: cortical contribution

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

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

Potential mechanisms: subcortical contribution

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

Hand dominance and pertinence of the nonhuman primate model

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

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Annexe 5.2

Autologous Adult Cortical Cell Transplantation Enhances Functional Recovery Following Unilateral Lesion of Motor Cortex in Primates: A Pilot Study

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BACKGROUND: Although cell therapy is a promising approach after cerebral cortex lesion, few studies assess quantitatively its behavioral gain in nonhuman primates. Furthermore, implantations of fetal grafts of exogenous stem cells are limited by safety and ethical issues.

OBJECTIVE: To test in nonhuman primates the transplantation of autologous adult neural progenitor cortical cells with assessment of functional outcome.

METHODS: Seven adult macaque monkeys were trained to perform a manual dexterity task, before the hand representation in motor cortex was chemically lesioned unilaterally. Five monkeys were used as control, compared with 2 monkeys subjected to different autologous cells transplantation protocols performed at different time intervals.

RESULTS: After lesion, there was a complete loss of manual dexterity in the contralesional hand. The 5 "control" monkeys recovered progressively and spontaneously part of their manual dexterity, reaching a unique and definitive plateau of recovery, ranging from 38% to 98% of prelesion score after 10 to 120 days. The 2 "treated" monkeys reached a first spontaneous recovery plateau at about 25 and 40 days postlesion, representing 35% and 61% of the prelesion performance, respectively. In contrast to the controls, a second recovery plateau took place 2 to 3 months after cell transplantation, corresponding to an additional enhancement of functional recovery, representing 24% and 37% improvement, respectively.

CONCLUSIONS: These pilot data, derived from 2 monkeys treated differently, suggest that, in the present experimental conditions, autologous adult brain progenitor cell transplantation in a nonhuman primate is safe and promotes enhancement of functional recovery.

KEY WORDS: Adult, Autologous, Lesion, Monkey, Motor cortex, Neurotransplantation, Recovery

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Brain lesions in the adult have dramatic consequences, because the spontaneous capacity of the brain to functionally recover is limited. Besides existing rehabilitative therapeutic approaches (eg, physiotherapy), several lines of research aim at developing treatments to promote and refine brain plasticity to enhance functional recovery following brain injury. Stem cell research, which represents a promising strategy to treat several nervous diseases, segregates into 2 main potential lines of treatment: implantation of exogenous cells or use of endogenous cells. The implantation of exogenous neural stem cells, either of embryonic or fetal origin, represents an encouraging approach, although there are serious limitations on the ethical, scientific, and clinical viewpoint.[1-12] Another strategy aims at stimulating and/or recruiting endogenous stem cells or precursor cells available in the adult central nervous system. These cells are located principally in the forebrain subventricular zone (SVZ) and in the subgranular zone (SGZ) of the dentate gyrus of

ABBREVIATIONS: M1, primary motor cortex; **PM**, premotor cortex; **SGZ**, subgranular zone; **SVZ**, subventricular zone

the hippocampus.[12] These cells, however, migrate either only to a specific target (olfactory bulb from the SVZ) or only locally in the dentate gyrus (from the SGZ). The possibility to repair postlesion parts of the central nervous system (cerebral cortex, basal ganglia, spinal cord) remote from these 2 neurogenic niches is therefore limited. Attempts to experimentally enhance the migration of stem/precursors from the neurogenic niches to distant zones of the brain or to use resident progenitors led to a modest compensatory replacement of lost cells, without clinically relevant functional recovery.[12] Stem/precursors taken from the SVZ or SGZ and transplanted into another brain area may not develop their full potential of cell replacement in a different environment.[12] Stem/precursors residing in the cerebral cortex or basal ganglia may be too few to successfully replace lost cells after a lesion. To bypass these 2 limitations, the aim of the present pilot study was to extend preliminary experiments to fully test the feasibility and functional relevance of an original endogenous strategy in a nonhuman primate model of cerebral (motor) cortex lesion: first, to collect adult neural progenitor cells resident in the cerebral cortex of the lesioned subject itself[13]; second, to put the progenitor cells in culture to increase their number[14,15]; and third, to transplant them into the cerebral cortex, in and/or nearby an experimental lesion and assess the functional outcome.

A crucial step toward feasible, safe, and efficient clinical application requires the most appropriate animal model for the corresponding neural pathology, closely mimicking the characteristics of human diseases. The nonhuman primate model is often mandatory to address safety issues and scientific concerns, especially to include exquisite neural functions present only in primates.[16,17] In a previous study, we established in 3 monkeys the basis for feasible autologous cell reimplantation, from the point of view of safety and the fate of the implanted cells.[15] As a next step, the present pilot study aimed at providing, as proof of principle, first quantitative behavioral evidence in macaque monkeys for a beneficial outcome of cell therapy following injury of the cerebral cortex. To this aim, we used a previously established model of cerebral cortex lesion in nonhuman primates,[18] introduced originally to assess the extent and time course of "spontaneous" functional recovery.[19,20]

MATERIALS AND METHODS

Subjects

Data were collected from 7 adult macaque monkeys (*Macaca fascicularis*; 6 males and 1 female) weighing between 3 and 6 kg, ranging from 2.5 to 5 years old at onset of motor training sessions. The monkeys originated from the breeding colony in our own animal facility or were purchased from a certified supplier (Bioprim; 31450 Baziège; France), with the authorization to import delivered by the Federal Veterinary Office (BVET, Bern, Switzerland). All procedures were conducted in accordance to the Guide for Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by local veterinary authorities. Separate data from monkeys included in the present study were reported in previous articles or abstracts on other parameters.[18,21,22]

Seven adult macaque monkeys were subjected to unilateral lesion of the hand representation in motor cortex, producing a selective paresis of the contralesional hand.[18] Postlesion, 2 of these monkeys were "treated" (autologous adult brain progenitor cells were reimplanted) for comparison of their functional recovery of manual dexterity with 5 lesioned, untreated ("control") monkeys.

Monkeys were housed in our animal facilities in rooms of 12 m^3, in which usually 2 to 4 monkeys were free to move and interact with each other (a new swiss regulation has been introduced in September 2010 requesting a new volume of 45 m^3 at least to be given to a group of 2-5 macaque monkeys). Before behavioral testing in the morning, the animal caretaker placed the monkeys in temporary cages for subsequent transfer to a primate chair. The monkeys had free access to water and were not food deprived. The rewards (pellets) obtained during the behavioral tests represented the first daily access to food. After the tests, the monkeys received additional food (fruits, cereals). The body weight of the animals was monitored before every behavioral session. In case the body weight dropped by 10% or more, the experiment was interrupted until the monkey regained the lost weight (this criterion for interruption was not met in the course of the present experiments).

Behavioral Task

The seven monkeys were trained to perform the modified (static) Brinkman board task, aimed at assessing manual dexterity.[18-25] The subjects had to grasp food pellets from small slots oriented either vertically (n = 25) or horizontally (n = 25) by using precision grip (opposition of thumb and index finger), as described earlier in detail.[23] Each monkey was its own control, as the dexterity scores were compared pre- vs postlesion. The slot dimension was 15 mm long, 8 mm wide, and 6 mm deep. The monkeys performed the task daily with each hand until they reached a plateau reflecting a stable prelesion performance. The duration of the daily behavioral session was about 1 hour. After the motor cortex lesion, the behavioral sessions were pursued for a period ranging from 80 to 310 days. The main analysis was focused on the number of pellets retrieved in 30 seconds (retrieval score) from the vertical and the horizontal slots, respectively. The total retrieval score was also documented (the sum of the pellets retrieved in 30 seconds from both slot orientations). Besides the retrieval score, reflecting the entire motor sequence (reaching, grasping, and withdrawal of the hand), a second parameter was measured, namely the contact time, as recently reported.[22,23,26] The contact time is defined as the time of contact (in seconds) between the fingers and the pellet, representing the time interval between the first contact with the pellet in the slot (usually with the index finger) and the precise time of successful grasping when the pellet is removed from the slot. For each session, the contact time was determined separately for each of the first 5 vertical slots and each of the first 5 horizontal slots visited by the monkey. In general, the contact time was longer for the horizontal than the vertical slots, in line with the notion that grasping is somewhat more difficult for the horizontal slots, thus requiring a longer manipulation of the pellet before successive grasping.[23]

In addition to the modified (static) Brinkman board task, another task was introduced to test the ability to grasp moving objects (pellets), presented on a circular Brinkman board rotating at a speed of 10 rotations per minute. The monkey had to anticipate the rotation of the Brinkman board, turning either clockwise or counterclockwise. In a given behavioral session, the monkeys performed, one hand after the other, the rotating Brinkman board task once for each direction of rotation, corresponding each to the prehension of 32 pellets in slots distributed in 4 concentric circles. The contact time was measured as

described above in the modified Brinkman board, for the first 10 slots aimed by the monkey. The 2 grasping tasks (modified Brinkman board and rotating Brinkman board) can be seen on the following web page: http://www.unifr.ch/neuro/rouiller/research/motorcontcadre.htm.

Animal Care for Surgery

After completion of prelesion training, the animals were subjected to the following surgical procedures. The monkeys were first tranquilized with ketamine (Ketalar; Parke-Davis, 5 mg/kg, intramuscularly); atropine was injected (0.05 mg/kg, intramuscularly) to reduce bronchial secretions. Before surgery, the animals were treated with the analgesic carprofen (Rymadil, 4 mg/kg, subcutaneously) and the antibiotic Albipen (ampicillin 10%, 30 mg/kg, subcutaneously). Subsequently, they were anesthetized with intravenous perfusion of 1% propofol (Fresenius) mixed with a 5% glucose solution (1 volume of propofol and 2 volumes of glucose solution); ketamine was added to the perfusion solution (65 mg/100 mL). To prevent edema, methylprednisolone (Solu-medrol, Pfizer) was added to the propofol/glucose solution (1 mg/mL). The level of anesthesia was kept at an optimal level with a perfusion rate of the propofol/glucose mixture of 0.1 mL·min $^{-1}$·kg $^{-1}$. All surgeries were performed under sterile conditions. Heart rate, respiration rate, expired CO_2, arterial O_2 saturation, and rectal temperature were monitored throughout the surgery. Each monkey was chronically implanted over the left forelimb area in the primary motor cortex (M1) with a stainless steel recording chamber (22 × 17 × 15 mm) allowing intracortical microstimulation sessions to map the primary motor cortex and locate the hand representation, as previously described.[18,27] After surgery, the monkeys received carprofen (pills of Rymadil mixed with food) daily and Albipen (subcutaneously) every 2 days for 1 to 2 weeks.

Biopsy of Prefrontal Cortical Tissue and Cell Preparation

In the 2 treated monkeys (Mk-JO and Mk-JA; during prelesion phase), following the same surgery protocol as described above, a squared bony sector of 8 × 8 mm was opened above the right dorsolateral prefrontal cortex. The dura mater was incised and an approximate volume of 8 to 20 mm^3 of cortical tissue was extracted using a surgical blade (no. 11, Paragon) and placed into sterile cold culture medium.[15] As previously described,[15,28] a few hours after the biopsy was performed, the cortical tissue collected was dissected to obtain gray matter cells, placed first in RPMI 1640 medium with fetal bovine serum and an antibiotic/antimycotic cocktail under horizontal agitation, and then kept in culture medium without serum, in suspension and under slow agitation. There was no specific cell sorting other than a selection imposed by the medium. Before reimplantation, a batch of cells were stained for subsequent tracking with fluorescent viable membrane dyes, PKH26 (red) for the cells reimplanted into the lesion site (Mk-JO and Mk-JA) and with PKH67 (green) for another batch of cells, reimplanted near the lesion site in the intact cortex (only Mk-JA). For the reimplantation (see below), the cells were cryopreserved, as previously reported.[14]

After the biopsy specimen was extracted, the bony sector was put back in place on the dura mater and sewed to the skull. The muscle and skin were then sutured. The extraction of brain tissue biopsy from the prefrontal cortex did not affect the motor capacity to grasp the pellets from the Brinkman board tasks.

Primary Motor Cortex Lesion

Several weeks after the biopsy, the hand area in the left M1 was permanently lesioned in the 7 monkeys by multiple injections of ibotenic acid (either Fluka 99% or Sigma 95%), as previously reported.[18,22] A volume of 1 to 1.5 μL of ibotenic acid solution (10 μg/μL in phosphate-buffered saline) was injected at each site by using a Hamilton microsyringe positioned at relevant sites previously defined by intracortical microstimulation. The total volume of ibotenic acid injected in each monkey ranged from 15 to 40 μL. More relevant than the volume of ibotenic acid injected is the volume of the actual lesion (volume of gray matter in motor cortex affected by the lesion), as determined for each monkey based on reconstruction of the lesion from consecutive histological sections stained for SMI-32. A typical lesion produced by infusion of ibotenic acid in M1 is illustrated in a recent report.[22]

Cell Reimplantation

Following the same surgical protocol as described above, cells contained in culture medium were reimplanted stereotaxically with a Hamilton microsyringe. For one monkey (Mk-JO), the reimplantation was performed into the lesion site 15 days after the motor cortex lesion. A total volume of 20 μL with PKH26 labeled cells containing 12 500 cells/μL was injected. For the other monkey (Mk-JA), the first reimplantation was performed into the lesion site (PKH26-labeled cells) and near the lesion site in the intact cortex (PKH67-labeled cells), once the monkey had reached a spontaneous functional recovery plateau. A second reimplantation was performed in the intact cortex, near the lesion site 49 days later (PKH67-labeled cells). The total volume (concentration of 3000 cells/μL) injected was 100 μL with PKH26 cells and 100 μL with PKH67-labeled cells (first implantation) and 49 μL with PKH67-labeled cells (second implantation), respectively. The 2 distinct viable membrane markers aimed at distinguishing, in the histological analysis, cells implanted in the lesion site from cells implanted in the intact cortex adjacent to the lesion (in Mk-JA).

Necropsy and Histology

At the end of the experiment, the monkeys were sacrificed as previously described,[27,29] for histological analysis of the lesion and of the implanted cells' fate. Frozen sections of the brain were cut in the frontal plane at a thickness of 50 μm, collected in 8 series for further processing to visualize different markers, such as SMI-32, glial fibrillary acidic protein, nestin, MAP2, and doublecortin, as previously reported.[15,29] Standard light, fluorescent, and confocal microscope analyses were conducted. Sectors of the brain sections where PKH26- and PKH67-labeled cells had been detected were selected and anatomically analyzed further.

RESULTS

Modified Brinkman Board

The description of the results is focused on the total score (sum of vertical and horizontal slots). Before lesion, the monkeys exhibited stable scores of manual performance (Figure 1; see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequences 1, 5, and 9 for Mk-JO, Mk-JA, and Mk-GE, respectively). As a result of unilateral lesion of motor cortex, a total loss of the contralesional hand performance was observed, as reflected by a grasping score dropping to zero in the modified Brinkman board task, both in control and treated monkeys (Figure 1A and B; see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequences 2, 6, and 10 for Mk-JO, Mk-JA, and Mk-GE,

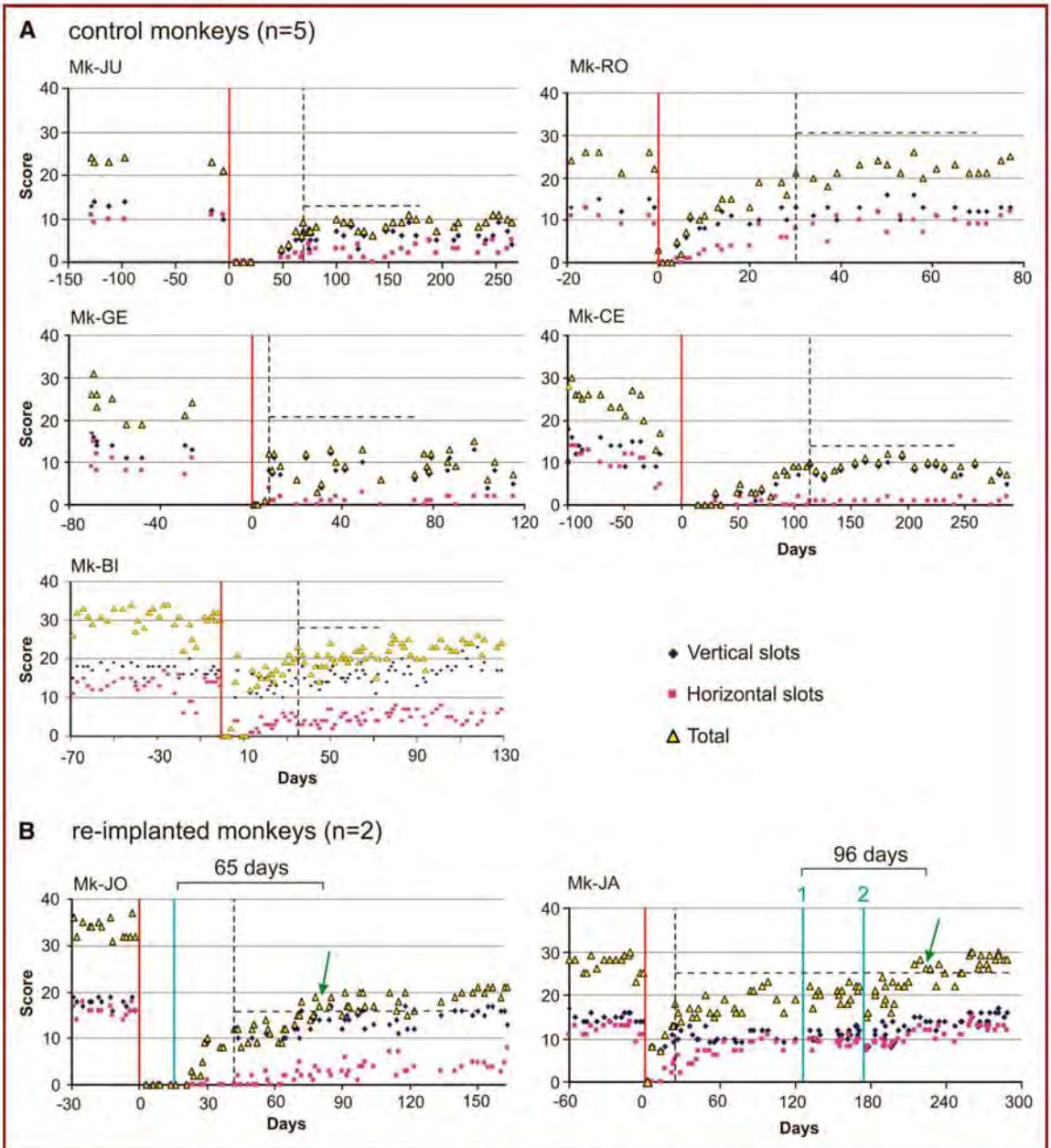

FIGURE 1. *Behavioral data (retrieval score). A and B, time course in days of manual dexterity performance (score), pre- and postlesion, as derived from the modified Brinkman board task in 5 control monkeys (A) and in 2 treated monkeys subjected to cell transplantation (B). The score is given by the number of pellets successively retrieved by the monkeys during 30 seconds. Yellow triangles represent the total score, blue diamonds the score for vertical slots, and pink squares the score for horizontal slots. The vertical red line indicates the time of the lesion (day zero). In A, the vertical dashed line shows the time point at which a spontaneous plateau of recovery was reached (see text for criterion to define the plateau). In B, the vertical dashed line represents the first (spontaneous) plateau of recovery. In the 2 treated monkeys (B), the vertical blue lines show the time of autologous cell implantations. The green arrows point out to the onset of a second behavioral plateau, reached following a rebound in the recovery curve (see text), occurring after a delay after cell implantation given in days above the graph. The horizontal dashed line represents a level of score defined by the median score calculated at the plateau of spontaneous recovery plus twice the difference between the highest value and the median score. In the control monkeys, the length of the horizontal dashed line represents the time window in which the spontaneous recovery was assessed quantitatively at plateau. The score level represented by the horizontal dashed line was taken as the criterion to be met to define a second plateau, representing an enhancement of functional recovery, observed in the 2 treated monkeys but not in the 5 control monkeys.*

respectively). The 5 control monkeys (Figure 1A) then exhibited a progressive, spontaneous functional recovery starting after 5 to 50 days, reaching a postlesion plateau 10 to 120 days postlesion, depending on the monkey. In the recovery curve approaching saturation, the onset of the plateau was defined as the first individual data point (total score) for which, among the next 3 individual data points, none exhibits a higher score (vertical dashed line in Figure 1A). This spontaneous recovery plateau ranged across the 5 control monkeys from 38 to 98% of the prelesion score of manual performance (Figure 2, top), thus

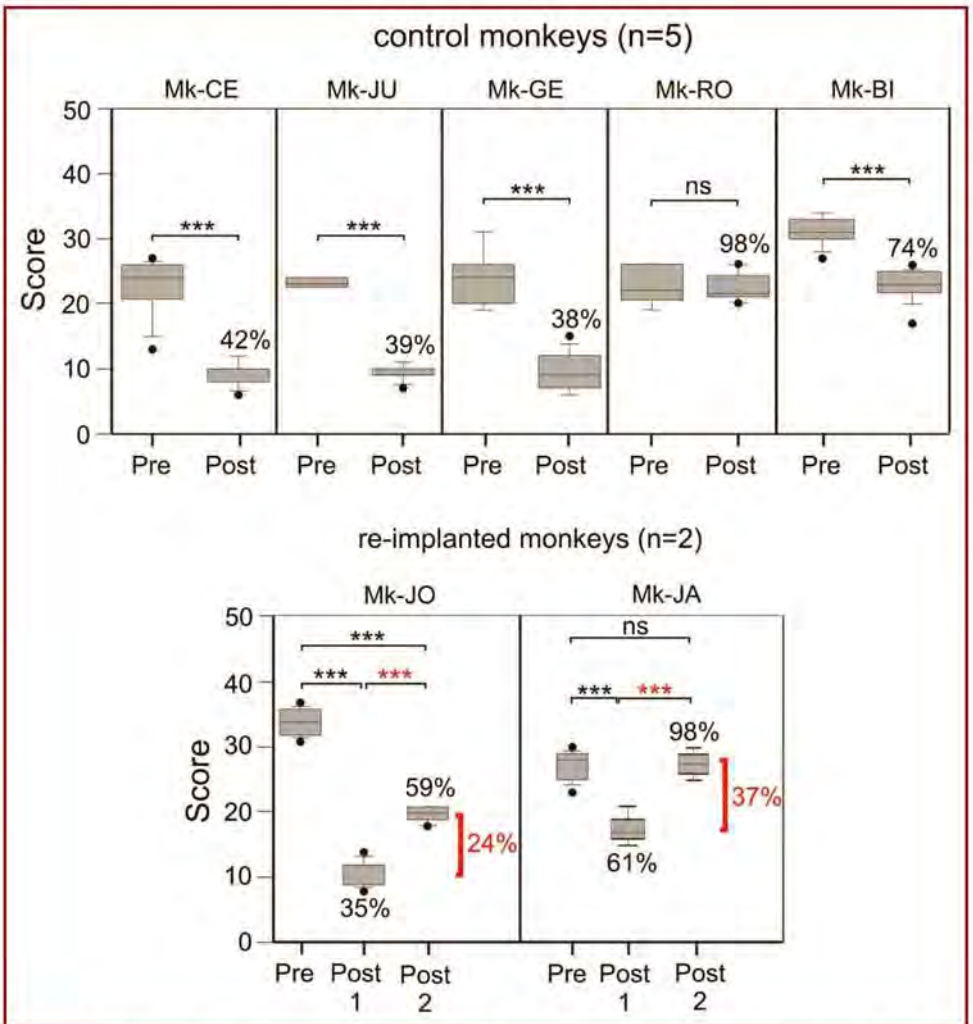

FIGURE 2. *Extent of functional recovery (retrieval score). The behavioral data are represented by box-and-whisker plots, comparing the prelesion and postlesion retrieval scores at plateau. In the 5 control monkeys (top), there was only one postlesion plateau, whereas, in the 2 treated (reimplanted) monkeys (bottom), in addition to the first plateau of spontaneous recovery (Post 1) there was a rebound in the recovery curve leading to a second plateau (Post 2) of manual dexterity scores. The different plateau scores were statistically compared (Mann-Whitney test, as previously reported[23]) with* $P < .001$ *(***) whereas "ns" is for* $P > .05$*. The percentage values in black next to each postlesion plateau represent the percentage of spontaneous functional recovery compared with the prelesion median score. The percentage values in red are for the magnitude of the behavioral rebounds in the 2 treated monkeys, compared with the first plateau of spontaneous recovery. In box-and-whisker plots, the horizontal line in the box corresponds to the median value and the top and bottom of the box are for the 75th and 25th percentile values, respectively. The top extremity of the whisker above the box is the largest data point included in the range going from the 75th percentile up to a level defined as the sum of the 75th percentile plus 1.5 times the interquartile distance. The bottom extremity of the whisker below the box is the smallest data point included in the range going from the 25th percentile down to a level defined as the 25th percentile minus 1.5 times the interquartile distance.*

corresponding to an incomplete functional recovery. In the control monkey exhibiting the fastest recovery (Mk-GE), it turned out to be mainly limited to the vertical slots with a poor long-lasting recovery for horizontal slots (Figure 1A). A poor recovery for the horizontal slots was also observed in Mk-CE. The control monkey with the best recovery (Mk-RO, 98%) had the smallest lesion. In Mk-RO, because the spontaneous recovery was nearly complete already after a couple of months, the experiment was interrupted after 80 days, in contrast to much longer periods of observation in the other monkeys. A statistical comparison of pre- and postlesion plateaux is presented in Figure 2 (top) for the 5 control monkeys, showing that the spontaneous recovery remained dramatically incomplete (38% to 42%) in 3 of 5 monkeys. In the other 2 control monkeys, the spontaneous recovery was more prominent (74% to 98%), as expected for a smaller lesion of the motor cortex. The postlesion plateau then remained largely stable for several months in the control monkeys (Figure 1A).

For the 2 treated monkeys (Figure 1B), after complete loss of manual performance lasting 25 days in Mk-JO and 5 days in Mk-JA, the same progressive spontaneous recovery as seen in control monkeys took place, reaching a first plateau 40 days after lesion in Mk-JO and 25 days after lesion in Mk-JA (vertical dashed lines in Figure 1B). In Mk-JO, this first plateau represented 35% of prelesion score and lasted about 30 days (see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequence 3). Meanwhile, 15 days after lesion, the autologous adult brain cell transplantation was performed. Starting around 70 days postlesion, there was a rebound of recovery, as reflected by a "second plateau" 80 days after the lesion (green arrow in Figure 1B), respectively, 65 days after the autologous transplantation. The second plateau was defined by data points in the recovery curve standing out above a level (horizontal dashed line in Figure 1B) calculated as the median score at first plateau to which twice the difference between the highest value and the median value at plateau 1 was added. The median score and the difference between the highest value and median were calculated from 15 data points taken from the beginning of the first plateau. The time onset of the second plateau was arbitrarily defined as the third data point (green arrow) greater than the limit (horizontal dashed line) defined above. This second behavioral plateau represented 59% of the prelesion score in Mk-JO. The difference between the first and second postlesion plateau (24%) was statistically significant ($P < .001$; see red stars in Figure 2; see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequence 4 and compare with video sequence 3 at first plateau).

In the treated Mk-JO described above, the autologous cell transplantation was performed into the lesioned territory in the motor cortex, 15 days after the lesion during the first recovery phase. In this monkey, as shown by the histological analysis, the reimplanted cells remained mainly at a precursor stage (positive for the markers nestin, vimentin, and doublecortin), surrounded by endogenous astrocytes (glial fibrillary acidic protein reactive gliosis), although some cells were found to be either engaged in a neuronal differentiation process (positive for the marker β-tubulin III) or differentiated (positive for the marker SMI-32). Because the transplanted cells were found to be agglomerated, they did not migrate at all to occupy the whole lesioned territory. In an earlier feasibility study without behavioral assessment,[15] transplantation of autologous neural cells was performed in 3 monkeys not only in the lesion itself, but also in the adjacent intact tissue, near the lesioned territory, at a later time point after the lesion (40 days). The reimplanted cells showed in these 3 monkeys an ability to massively migrate from the reimplantation site toward the damaged area, as well as to differentiate massively (positive for the neuronal marker MAP-2). Based on these data (feasibility study and Mk-JO of the present pilot study), in the second monkey subjected here to the transplantation of autologous adult brain cells (Mk-JA), the reimplantation was performed both in and near the lesioned territory in M1, as well as at a later time point postlesion than in Mk-JO, to investigate the impact of these 2 parameters on the cell migration and differentiation ability.

In Mk-JA, the reimplantation of the autologous cells was performed in 2 steps, 125 days and 175 days postlesion. The first, spontaneous behavioral plateau represented 61% of the prelesion score and lasted more than 150 days (Figure 1; see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequence 7). Then, as in the other treated monkey, a rebound of recovery took place as reflected by a second plateau (data points above the horizontal dashed line in the right panel of Figure 1B) at about 96 days after the first implantation. Moreover, this second plateau exhibited further improvement at postlesion day 260, possibly in relation to the second implantation of autologous cells (corresponding to a delay of about 85 days). The second plateau corresponded to a nearly complete recovery (98%): the enhancement of recovery presumably related to the cell transplantation was 37% (see red stars in Figure 2 for statistical comparison of plateaux 1 and 2; see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequence 8 and compare with video sequence 7 at first plateau).

In the 2 treated monkeys, a rebound of manual performance score was present, as reflected by the second plateau of functional recovery. In contrast, in the 5 control monkeys, when the first plateau of recovery was reached, variations of the score across daily sessions never reached the criterion defined by the median score plus twice the difference between the highest value and the median score (horizontal dashed lines in Figure 1A). Among the 5 control monkeys, one animal (Mk-BI) exhibited some improvement after the plateau of recovery was reached, but it did not fulfill the criterion of surpassing the median score plus twice the difference between the highest value and the median score; the final level of recovery reached by Mk-BI was separated from the criterion level (horizontal dashed line in Figure 1A) by a score gap of half the difference between the median score and the highest value at plateau. The enhancement of manual performance observed in the 2 treated monkeys, corresponding to the second plateau, is illustrated qualitatively by video sequences (see http://www.unifr.ch/neuro/rouiller/ACCI/videos.htmcompare sequences 3 and 4 for Mk-JO as well as sequences 7 and 8 for Mk-JA), showing that the 2 monkeys achieved a better manual performance at the level of the second plateau than

at the level of the first plateau. For comparison, video sequences taken at 2 remote time points along the unique plateau of spontaneous recovery in Mk-GE show that there was no enhancement of manual dexterity (http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm, compare video sequences 11 and 12).

To further support the notion that the second plateau observed exclusively in the 2 treated monkeys corresponds to an enhancement of manual dexterity per se (better manipulation of pellets between the thumb and index finger), a further analysis was based on the contact time (see Materials and Methods). The contact time was determined in the sessions included in time windows corresponding to the first and second plateaux in Mk-JO (40-68 postlesion days and 9-164 postlesion days for plateau 1 and plateau 2, respectively) and in Mk-JA (80-180 postlesion days and 252-300 postlesion days for plateau 1 and plateau 2, respectively). The distribution of the contact times observed at plateau 1 and 2 is shown in the form of box-and-whisker plots in Figure 3A, separately for the vertical and horizontal slots, for each of the 2 treated monkeys. In Mk-JO, irrespective of the slot orientation, the contact times were significantly shorter at plateau 2 than at plateau 1, reflecting an improvement of manual dexterity when the second plateau was reached (Figure 3A). This was also the case in Mk-JA for the vertical slots, whereas there was no statistically significant difference for the horizontal slots, although the contact times were less variable at plateau 2 than at plateau 1 (Figure 3A). In agreement with the retrieval score data, the extent of decrease of median contact times going from plateau 1 to plateau 2 in Mk-JO (both slot orientations) and Mk-JA (vertical slots) ranged from 24 to 30%, representing an enhancement of manual dexterity comparable to the improvement of performance reflected by the retrieval score (24-37%; Figure 2).

Rotating Brinkman Board Task

A separate assessment was made of whether the 2 monkeys (Mk-JO; Mk-JA) subjected to transplantation of autologous adult cortical cells achieved a second level of recovery that reflected a significant improvement of manual dexterity over the first plateau. In this assessment, the contact times were measured in the rotating Brinkman board, comparing the sessions taken within the time windows corresponding to the 2 distinct plateaux (see above). As shown in Figure 3B, irrespective of the direction of rotation of the board, there was a statistically significant decrease of contact time going from postlesion plateau 1 to postlesion plateau 2, indicative of an enhanced manual dexterity. Comparing the median values, there was a decrease of contact time from plateau 1 to plateau 2 ranging from 42% to 57%, thus even more pronounced than for the modified (static) Brinkman board task.

Relationship Between Lesion Size and Recovery

There was a substantial variation of postlesion recovery across monkeys (Figures 1 and 2). To assess the dependence on lesion size (see Figure 4B), the degree of functional recovery in the modified Brinkman board (total score) in percent was plotted as a function of lesion volume, corresponding to the extent of gray matter in motor cortex affected by the lesion (Figure 4A). As expected, a small lesion in a control monkey (Mk-RO) was followed by a nearly complete spontaneous functional recovery (98%). A somewhat larger lesion in a second control monkey (Mk-BI) was followed by a reduced, though still prominent (74%), spontaneous recovery. In contrast, still in control monkeys, above a lesion volume of about 40 mm^3 and up to a volume of about 100 mm^3, the extent of spontaneous recovery remained fairly constant near 40% (Figure 4A). The extent of spontaneous recovery across the control monkeys is quite variable and the functional recovery observed in the 2 treated monkeys is in the same range. Nevertheless, a difference between the 2 subgroups of monkeys is the presence of a rebound in the postlesion recovery curve in the 2 treated monkeys (second plateau of an extent represented by the vertical dashed line in Figure 4A), a phenomenon absent in the 5 control monkeys (see also Figures 1 and 2).

The 2 treated monkeys of the present pilot behavioral study, as well as the 3 monkeys of our previous report also subjected to autologous cell transplantation,[15] did not exhibit any sign of pain or discomfort related to the autologous cell transplantation, indicating that this procedure does not produce undesired, secondary effect.

Cell Analysis

When looking at SMI-32-stained brain sections in motor cortex in an intact territory, there were darkly stained SMI-32 positive neurons in layer III and layer V, both in control and treated monkeys (Figure 4C, panel c1). In contrast, in the lesion territory, there was no SMI-32 staining in the control monkeys as expected (Figure 4C, panel c2), whereas lightly stained SMI-32 positive neurons were present in the lesion territory of the treated monkeys (Figure 4C, panel c3). When considering more closely these SMI-32-positive cells located in the lesion site of the treated monkey (Figure 4C, panel c4), it appeared that they sometimes corresponded to PKH26 (red, designed by yellow arrows) and PKH67 (green, designed by white arrows) positive cells that were transplanted in the monkey's brain (Figure 4C, panels c5–c7). In a treated monkey, a 3D reconstruction of the motor cortical area shows the distribution of PKH26 (red)- and PKH67 (green)-labeled cells (Figure 4C, panels c8–c10), implanted respectively in the lesion and at the vicinity of the lesion. Finally, PKH67-positive cells were present in the lesion site, indicating that they migrated from the intact cortex toward the lesion territory. The proportion of surviving transplanted cells amounted to about one third on average, a proportion most likely consistent with a clinical effect. The histological observations did not reveal the presence of teratoma or tumor formation.

DISCUSSION

Survey of the Data

To the best of our knowledge, the present behavioral pilot study is the first report in nonhuman primates suggesting that transplantation of autologous adult brain progenitor cells leads to

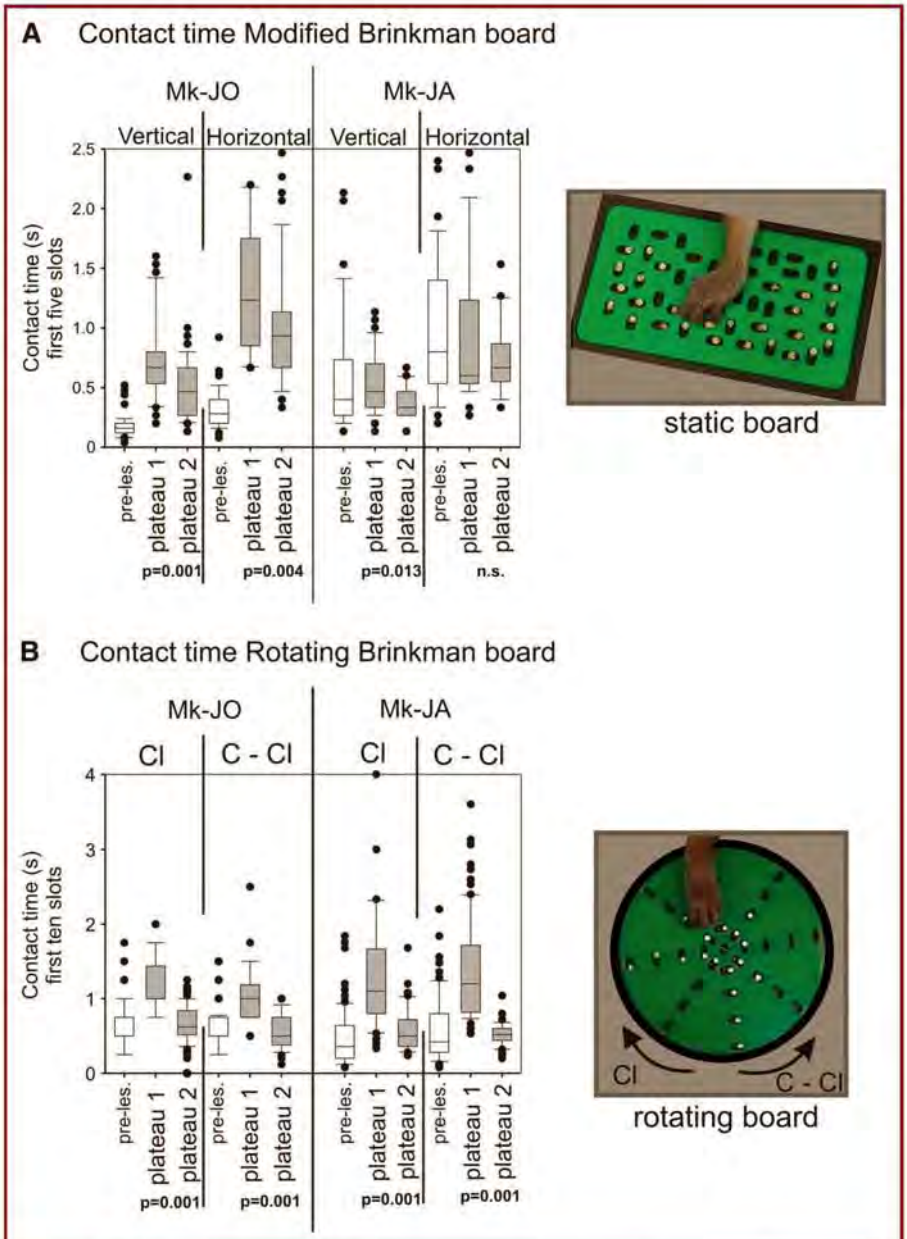

FIGURE 3. *Behavioral data (contact time). The contact times, measured separately during the postlesion plateaux 1 and 2 (gray boxes), are plotted in the form of box-and-whisker plots, for the modified (static) Brinkman board task (A) and the rotating Brinkman board task (B). The shorter contact time the better is the manual dexterity because less time of contact between the pellets and the fingers was needed to successfully grasp the pellet. In Mk-JA, data were collected from 9 behavioral sessions during the first plateau and from 4 sessions during the second plateau. In Mk-JO, data were collected from 6 behavioral sessions during the first plateau and from 12 sessions during the second plateau. n.s., nonsignificant difference between plateau 1 and plateau 2 ($P > .05$). The statistics refer to the comparison of contact times between the plateau 1 and the plateau 2 (Mann-Whitney test). For completeness of the information, the corresponding prelesion data are given by the white boxes, derived from 10 behavioral sessions. The conventions for the box-and-whisker plots are the same as in Figure 2. The insets on the right display the boards used for the 2 tests while a monkey is grasping a pellet. In the rotating Brinkman board, Cl is for clockwise rotation and C-Cl is for counterclockwise rotation.*

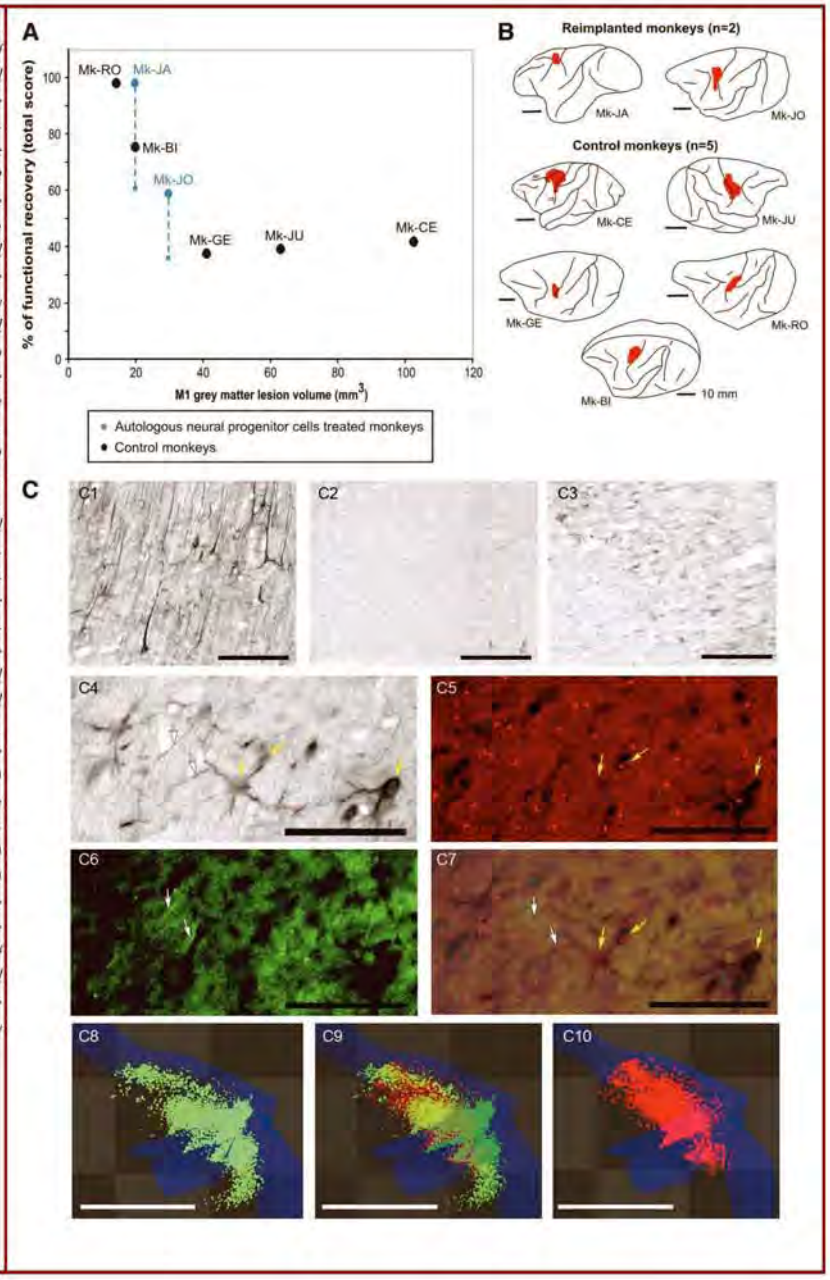

FIGURE 4. *Relationship with morphological data.* **A**, *plot of the percentage of postlesion functional recovery of the contralesional hand (total score in the modified Brinkman board) as a function of the volume of the primary motor cortical lesion. Black-filled circles represent the control monkeys and blue-filled circles represent the monkeys treated with autologous brain cells. For the 2 treated monkeys, the vertical dashed line represents the additional recovery observed (rebound), presumably in relation to the cell reimplantation (see text).* **B**, *lateral views of the projection on the cerebral cortex surface of the approximate location and extent of the left M1 gray matter lesion (red) as observed on frontal histological sections of the monkeys' brain. Theses reconstructions do not take the depth of the lesion into account. The precise extent of the lesion is given by its volume in* **A**. *At the bottom, the brain of the 4 control monkeys are shown, whereas the brain of the 2 monkeys reimplanted with autologous brain cells are represented at the top. AR, arcuate sulci; CE, central sulci. Scale bar = 10 mm.* **C**, *histological data in transplanted and control lesioned motor cortex area. (C1 to C3) Brain diaminobenzidine-immunohistology for SMI-32 neurons. In C1, SMI-32-positive neurons are as seen in the intact part of the motor cortex (at some distance from the lesion); SMI-32-positive neurons are present in layer III and layer V. The lesion territory is shown in C2 and C3 for a control monkey and a treated monkey, respectively; in the control monkey, no SMI-32 staining is visible (C2); in contrast, there are some positively stained cells in the lesion of the treated monkey (C3). Scale bar = 200 μm. (C4 to C7) High magnification of SMI-32-positive cells in the lesion site of the treated monkey (C4 and C7). These SMI-32-positive cells (yellow and white arrows in C4 and C7) correspond to PKH26-labeled cells (yellow arrows in C5) or to PKH67-labeled cells (white arrows in C6). Scale bar = 200 μm. (C8-C10) 3D reconstruction of the motor cortex lesion territory showing the distribution of PKH67-labeled cells (green dots in C8 and C9) and PKH26-labeled cells (red dots in C9 and C10). Note that the 2 sets of cells migrated in the same lesion territory in the overlay reconstruction (C9). Scale bar = 1 cm.*

an enhancement of functional recovery after brain injury. The enhancement of recovery is not directly reflected by a difference of postlesion behavioral plateau between groups of "treated" and "control" monkeys: the recovery was quite variable across monkeys because of interindividual variability of lesion volume and precise position of the lesion. Because of this high variability, an intergroup comparison would require larger numbers of monkeys in each group. The preliminary evidence for enhancement of functional recovery in relation to the treatment is rather based on the presence of a rebound (second plateau) in the postlesion recovery curve, present in the 2 treated monkeys and not in the 5 control monkeys (Figures 1, 2, and 4). The manual performance obtained at the second plateau was higher than the performance reflected by the first plateau of recovery (Figures 1,

2, and 3; see also http://www.unifr.ch/neuro/rouiller/ACCI/videos.htm video sequences). Furthermore, the rebounds observed in the treated monkeys turned out to occur within a fairly comparable time window in the 2 treated monkeys (about 2-3 months; Figure 1B). The enhanced functional recovery in the 2 treated monkeys (reflected by the second plateau), compared with the control monkeys, cannot be explained by a more frequent practice of the motor tasks postlesion in the monkeys subjected to the transplantation of autologous cells, because the frequency of behavioral sessions were overall comparable across the 2 groups of monkeys.

Limitations of Interpretation

The interpretation of the present pilot results is limited by the low number of monkeys, especially in the group of treated monkeys, limited to 2 cases. Nevertheless, the consistent data obtained in the 5 control monkeys in the sense of an absence of significant rebound of functional recovery (even after a long time postlesion; see Figure 1A) represents a reliable observation, considering that 5 monkeys constitute a decent group size for nonhuman primates, although a group of 6 to 7 animals would be optimal.[23] More preliminary is the observation of a rebound of functional recovery most likely related to the autologous cell therapy, because it is based on only 2 monkeys, who underwent somewhat different injection protocols. However, the enhancement of recovery occurred with a time course consistent with plausible scenarios (see below). As far as the safety of the present therapeutic procedure is concerned, the 2 treated monkeys confirm the data previously obtained on 3 monkeys in a feasibility study.[15]

Possible Mechanisms of Enhancement of Functional Recovery

The delay of 2 to 3 months between cell reimplantation and rebound of functional recovery may reflect 2 possible mechanisms: cell replacement and/or endogenous neurogenesis induced by factor delivery. First, the reimplanted cells may integrate the neuronal network after differentiation into mature neurons that express MAP2[15] and SMI-32 (Figure 4C). As shown previously,[15] 1 month after transplantation, the cells still expressed progenitor markers such as nestin and were differentiated into neurons at 3 months postreimplantation. Furthermore, Koch et al[30] demonstrated that human embryonic neural stem cells can functionally integrate with and receive synaptic input from host brain tissue after 18 to 24 weeks. Such integration may occur in our study, where the autologous strategy is expected to facilitate the migration and integration of the transplanted cells. In line with this ability to colonize the entire lesion, the re-implanted cells express doublecortin, a migrant neuroblast marker that participates in the regulation of microtubule dynamics and stability during neuronal morphogenesis and migration.[31] Second, the cells may have a bystander effect through factor delivery and influence the endogenous brain modeling.[32] Preliminary results (data not shown) indicate that, in vitro, these adult primate brain cells secrete neurotrophic factors, such as brain derived neurotrophic factor, leukemia inhibitory factor, and ciliary neurotrophic factor. Based on the posttreatment delay of 2 to 3 months, the cell replacement mechanism appears likely but the factor delivery effect cannot be excluded at that time, although it should occur more rapidly. It is known that, after lesion of the M1 in monkey, the ipsilesional premotor cortex (PM) plays a significant role in the "spontaneous" functional recovery,[18,33-35] although other cortical areas may contribute.[36] Among possible mechanisms, it was shown that the normal projection from PM to M1 is redirected to the postcentral gyrus (somatosensory cortex[37]). It may be that the transplanted autologous adult cortical cells secrete factors favorable for such redirection of relevant projections originating from intact adjacent motor cortical areas.

Both the absence of rebound in the recovery curve of the 5 control monkeys and the consistent delay of about 2 to 3 months between autologous cells implantation and the rebound leading to a second plateau argue in favor of a positive effect of the cell transplantation on the functional recovery of manual dexterity following lesion of the hand area in M1 in macaque monkeys. In line with such interpretation, the autologous cell implantation took place at different time points with respect to the lesion in the 2 treated monkeys. If the rebounds would be due to factors independent from the autologous cells' implantation, then a consistent delay of 2 to 3 months would most likely not have been observed in the 2 treated monkeys. Another important observation derived from Mk-JA is that the rebound in the postlesion recovery curve occurred even though the autologous cell implantation took place a long time after the lesion (more than 3 months). The delay between the lesion and the cell reimplantation may play an important role, and the optimal timing remains to be determined in future experiments. For instance, it may be favorable to wait for the completion of the whole perilesional inflammatory phenomenon to obtain an optimal cell migration and differentiation.

The magnitude of the rebound of the postlesion recovery curve in the 2 treated monkeys ranged from 24 to 37%, as assessed in the modified Brinkman board task, adding to the "spontaneous" postlesion recovery score (Figure 2). In the second behavioral test (rotating Brinkman board), the effect even reached about 50% improvement of manual performance (Figure 3). In the control monkeys, there was no sham treatment (for instance, infusion of vehicle only). However, it is unlikely that the absence of rebound is due to the lack of cortical penetration in the perilesional territory with a syringe. In the entire cohort of monkeys with M1 lesion (not Mk-JO and Mk-JA, but comprising monkeys not included in the present study, eg, treated with anti-Nogo-A antibody), postlesion intracortical microstimulation sessions conducted at the level of the plateau of recovery did not exhibit a second plateau within the next 2 to 3 months, indicating that a surgical insult in itself does not enhance functional recovery.

A similar rebound effect as in the 2 monkeys with cell transplantation was found in another group of monkeys subjected to a comparable lesion of M1, but treated with anti-Nogo-A

antibody,[21] a therapeutic strategy known to also enhance functional recovery after cervical cord lesion in monkeys.[23,38] The time course of rebound observed in the anti-Nogo-A antibody-treated monkeys subjected to lesion of M1 was comparable to the rebound delay of about 2 to 3 months observed here in the monkeys subjected to autologous transplantation. This observation suggests that both treatments, although based on different mechanisms, exert their effect following a comparable time course, which may represent a favorable situation to tentatively combine the 2 treatments in future experiments in monkeys, with the aim to obtain an additive, if not synergistic effect.

Comparison With Previous Studies

Following stroke, a majority of patients experience serious loss of hand function. The present monkey model focused on the recovery of hand function after cortical lesion thus represents a pertinent model to test therapeutic strategies. A direct comparison of the present data with previous animal studies is limited, because nearly all preclinical studies of cell therapy on animal models of cerebral infarct were conducted on rodents as the host animal.[10] One exception is a recent study,[39] in which human neural stem cells were transplanted in a monkey model of ischemic stroke. Evidence for cell replacement was found but the functional outcome was not tested. Considering another neurological disease (Parkinson disease), the transplantation of dopaminergic neurons generated from embryonic stem cells in monkeys into 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine monkeys was followed by an attenuation of neurological symptoms,[40] based on a neurological score testing a wide repertoire of functions, but less quantitative than the present assessment of manual dexterity. In a monkey model of Parkinson disease, using the same transplantation strategy of autologous adult progenitor cells, there is also evidence for a neuroprotective effect[28] and enhancement of functional recovery compared with control monkeys.[41] Taken together, the latter study on Parkinson disease and the present one on cortical lesion, both based on reimplantation of autologous adult progenitor cells, provide new and encouraging results, as suggested by an enhanced functional recovery possibly related to the treatment. However, these 2 studies are at a pilot step and therefore the conclusion of enhanced functional recovery prompted by autologous cell transplantation needs to be confirmed on a larger sample of monkeys and, preferably, in combination with other strategies, such as neutralization of Nogo-A, for instance.[23,38] Even at the present pilot stage, these studies provide evidence that the reimplanted autologous brain cells did not form teratoma or tumor over time.

Future Perspectives

Considering that the autologous strategy does not involve as complex ethical and/or immunosuppression concerns as in heterologous strategies, there is hope to successfully translate this approach into the clinics in the reasonably close future, for instance, in case of irreversible cerebral pathology, like brain trauma or stroke. The present animal model was aimed at reflecting mechanisms taking place after cerebral insult, such as acute lesions (vascular or trauma) leading to excitotoxicity provoking cell death (mimicked here by infusion of ibotenic acid). On a more general perspective, the present study provides additional evidence in favor of a strategy aimed at recruiting endogenous stem cells or progenitor cells, associated in synergy with exogenous stems cell actions, as recently advocated.[12]

Disclosure

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COMMENT

Stroke is the third leading cause of death in the United States and it is a leading cause of disability in adults. The armamentarium available for improvement of neurological function after stroke is currently limited to acute treatment in specialized stroke units, optimal therapy for medical complications, and intense physical, occupational, and speech rehabilitation. Despite many clinical trials, there is no effective intervention to improve neurological outcome following ischemic stroke. A growing number of studies have demonstrated stem cell-based therapy provides a potential approach to the restoration of lost brain function after stroke. Replacement of damaged cells and restoration of function may be accomplished by transplantation of many different cell types, such as embryonic, fetal, or adult stem cells, human fetal tissue, and genetically engineered cell lines.[1,2] Adult neural stem cells offer the advantage of avoiding the ethical problems associated with embryonic or fetal stem cells and can be harvested as autologous grafts from the individual patients. Current enthusiasm concerning the clinical applications of autologous adult stem cells in stroke is based on promising results obtained during experimental animal studies and initial clinical trials.

The authors describe a pilot study evaluating 7 monkeys that underwent a chemical lesion in their cortex. Five were followed as controls for spontaneous recovery and 2 were injected with autologous cortex progenitor cells. All 5 control monkeys achieved varying degrees of spontaneous recovery in a single phase. The 2 monkeys injected with progenitor cells achieved a spontaneous recovery in an initial phase, but also had a delayed recovery 2 to 3 months subsequent to the initial recovery. The small number of subjects studied makes it difficult to interpret the results, and with the 2 test subjects being injected differently with progenitor cells, there is no statistical power to the study. Furthermore, there is a lack of experimental consistency because each monkey tested did not undergo equal lesions, equal volume of injection, or equal locations of the injections. That being said the study does have some points that make it viable pilot research in which the authors learned enough to standardize the variables for a valid study. The authors put together an extensive control period, and most importantly, in a primate model with behavioral endpoints. It was demonstrated that there was a second boost of recovery that occurred in only the 2 monkeys injected with progenitor cells and not observed in the controls. When viewed as a pilot study it shows good initial insight into this potential treatment paradigm despite its apparent weaknesses. It will hopefully lead to a confirmation of the findings in a larger group with a more adequately designed fixed protocol.

At this point we are left to wonder: what is the plausible mechanism for this second phase of recovery? Spontaneous recovery of manual dexterity takes place to a highly variable extent across subjects and may depend on the recruitment of other motor areas.[3] Transplanted stem cells from multiple sources can generate neural cells that survive and form synaptic connections in the stroke-injured brain. More importantly, stem cells from multiple sources also exhibit neuroprotective properties that may ameliorate stroke deficits. In many cases, functional benefits observed are most likely independent of neural differentiation, although the mechanisms remain inadequately understood. A study of human umbilical cord cells showed improvement in neurobehavioral function

and the reduction of infarct volume related more to the neuroprotective effects rather than the formation of a new network between host neurons and the implanted cells.[4] Endogenous neurogenesis has been observed in response to ischemic injury, and can be enhanced via transplants or infusion of appropriate cytokines. Enriched housing and voluntary running exercise enhanced migration of transplanted stem cells toward the region of injury after stroke and trends to the increase of survival of stem cells.[5] Thus the ability of transplanted stem cells in promoting recovery can be augmented by environmental factors such as rehabilitation. For maximal functional recovery, regenerative therapy will need to incorporate multidisciplinary approaches, which may include cell replacement, trophic support, protection from oxidative stress, and enhanced endogenous neuronal stem cells response. The loss of appropriate guidance cues and supporting architecture in the infarct cavity will likely impede the restoration of lost circuitry. Biologically compatible nanofiber scaffolds may provide the structure needed,[6] but getting grafted neurons to survive and make functional synapses as replacements to lost neurons is still a dream.

Before embracing clinical applications, several essential precautions must be suitably addressed. For example, the regenerative potentials of adult stem cells decline with age, and stem cells isolated from aged patients may retain dysfunctional characteristics. Functional recovery from stroke reaches a maximum level by 3 to 6 months after onset, and no further recovery occurs beyond this time. The time course for therapeutic intervention still needs to be worked out. Are the available autologous cells appropriate for therapeutic application in stroke? What are the optimal injection routes and cell doses? Success in future clinical trials will depend on careful investigation at the experimental level, to allow us to understand not only the clinical practicalities of stem cell use, but also the underlying biological principles involved. Furthermore, stimulation of endogenous adult stem cell-mediated repair mechanisms in the brain might offer new avenues for stroke therapy without the necessity of transplantation. Thus, important scientific issues need to be addressed to advance our understanding of the molecular mechanisms underlying the critical steps in cell-based repair to allow the introduction of these experimental techniques into clinical practice.

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Annexe 5.3

Journal of Biomedical Optics

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Follow-up of cortical activity and structure after lesion with laser speckle imaging and magnetic resonance imaging in nonhuman primates

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Follow-up of cortical activity and structure after lesion with laser speckle imaging and magnetic resonance imaging in nonhuman primates

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Abstract. The nonhuman primate model is suitable to study mechanisms of functional recovery following lesion of the cerebral cortex (motor cortex), on which therapeutic strategies can be tested. To interpret behavioral data (time course and extent of functional recovery), it is crucial to monitor the properties of the experimental cortical lesion, induced by infusion of the excitotoxin ibotenic acid. In two adult macaque monkeys, ibotenic acid infusions produced a restricted, permanent lesion of the motor cortex. In one monkey, the lesion was monitored over 3.5 weeks, combining laser speckle imaging (LSI) as metabolic readout (cerebral blood flow) and anatomical assessment with magnetic resonance imaging (T2-weighted MRI). The cerebral blood flow, measured online during subsequent injections of the ibotenic acid in the motor cortex, exhibited a dramatic increase, still present after one week, in parallel to a MRI hypersignal. After 3.5 weeks, the cerebral blood flow was strongly reduced (below reference level) and the hypersignal disappeared from the MRI scan, although the lesion was permanent as histologically assessed post-mortem. The MRI data were similar in the second monkey. Our experiments suggest that LSI and MRI, although they reflect different features, vary in parallel during a few weeks following an excitotoxic cortical lesion. [DOI: 10.1117/1.3625287]

Keywords: monkey; motor cortex; ibotenic acid; cerebral blood flow; excitotoxic lesion.

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1 Introduction

The spontaneous capacity of the brain to functionally recover from a lesion is limited, as it is the case after stroke, for instance. A crucial step toward feasible, safe, and efficient clinical application of a therapeutic strategy requires basic investigations and proofs of principle in animal models. The nonhuman primate model is often mandatory to address safety issues and scientific concerns, especially to take into consideration exquisite neural functions present only in primates,[1,2] such as manual dexterity.[3] The nonhuman primate model of the macaque monkey was extensively used in our laboratory to assess the extent and mechanisms of spontaneous recovery from spinal cord or motor cortex lesion and to test strategies aimed at enhancing recovery.[4–12]

The nonhuman primate model is suitable to study the consequences of a lesion of the motor cortex, produced by various interventions (surgical lesion, block of a cerebral artery, chemical lesion),[11–17] and to establish possible mechanisms of recovery.[4,14] The interpretation of the behavioral data is strongly dependent on the properties of the lesion, such as its extent (volume) and its precise location. Furthermore, the dynamics of the lesion procedure is also likely to influence the magnitude of the deficit and the extent of recovery. In our nonhuman primate model of motor cortex lesion,[4,11,12] the hand representation in the primary motor cortex (M1) was first delineated with intracortical microstimulation and then damaged by infusion of the excitotoxic ibotenic acid at the most excitable sites, leading to a permanent damage of the corresponding brain area. The precise time course of the devastating effect of ibotenic acid on the cortical tissue is not known, as well as the follow-up of the appearance of the cortical lesion site produced by ibotenic acid infusion in M1 on magnetic resonance imaging (MRI) scans during the weeks post-lesion. To fill this gap, we have implemented in parallel a functional readout [a measure of cerebral blood flow based on laser speckle imaging (LSI) technique] and a structural readout (MRI scan) at consecutive time points post-lesion.

The LSI method[18,19] is relatively low-cost and easy to implement, and it was broadly used in biomedical studies on small animals over the last decade.[20–30] The main field of application consisted of creating maps of capillary and perfusion blood flow in tissue. A number of improvements with regard to signal processing and quantitative data analysis have been reported in the literature.[31–36] The LSI technique can be applied to the exposed cerebral cortex or, in some cases, to the intact (surgically thinned) skull,[33,36] albeit at reduced performance. Applications for larger animals or humans are impractical due to the limited coherent penetration of light into the skull, although

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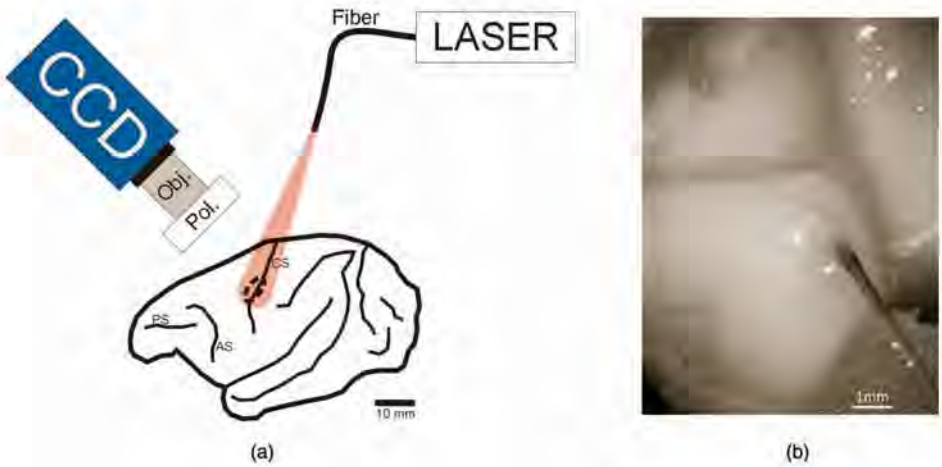

Fig. 1 (a) Setup of the LSI experiment, schematized over a standard lateral view of the left hemisphere of the macaque monkey. CS = central sulcus; AS = arcuate sulcus; PS = principal sulcus. (b) Time integrated image of the cortical surface, taken with the CCD camera. A vertically oriented blood vessel on the right follows the central sulcus, whereas another blood vessel of large diameter is horizontally oriented over the pre-central gyrus. The image was taken when the needle of an Hamilton syringue was inserted into the motor cortex, rostral to the central sulcus, to perform one of the six penetrations along which ibotenic acid was infused.

intraoperative use of LSI as a monitoring tool in neurosurgery has been reported.[37]

Despite the importance and widespread use of the LSI technique in neurosciences using rodents, a successful application in nonhuman primates is still missing, to our knowledge. The present study thus uses LSI to monitor, in nonhuman primate and over several weeks, the variation of cerebral blood flow of a cortical territory subjected to a permanent lesion induced by infusion of ibotenic acid and, in parallel, the changes of structural properties of the same territory assessed with MRI. A more specific goal was to measure, with LSI, changes of cerebral blood flow during surgery in real time when ibotenic acid was infused in the cerebral cortex to generate a permanent excitotoxic lesion. A further goal was to refine the LSI technique in order to improve the quality of the image by introducing a sliding window processing scheme, as well as to reduce the interference due to movements related to heart beat.

2 Materials and Methods

2.1 *Lesion of the Motor Cortex*

LSI and MRI data were collected in parallel from an adult macaque monkey (a male macaca fascicularis: Mk-JH), 7 years old and weighting 7 kg at the time of the cortical lesion. Additional MRI data were obtained from a second male macaque monkey (Mk-BI; 5 years old, 5 kg body weight). All procedures were conducted in accordance to the Guide for Care and Use of Laboratory Animals (ISBN 0–309-05377–3; 1996) and approved by local veterinary authorities. As previously reported,[11] the monkeys were housed in our animal facilities in rooms of 12 m^3, in which usually two to four monkeys were free to move and to interact among each other. (A new Swiss regulation was introduced in September 2010 requesting a volume of 45 m^3 at least to be given to a group of up to five macaque monkeys.) The monkeys had free access to water and were not food deprived.

To perform the lesion of M1 in both monkeys (Mk-JH and Mk-BI) and, subsequently, to measure cerebral blood flow with LSI in Mk-JH, the animals were subjected to the following surgical procedures. The monkeys were first tranquilized with ketamine (Ketalar®; Parke-Davis, 5 mg/kg, intramuscularly); atropine was injected (0.05 mg/kg, intramuscularly) in order to reduce bronchial secretions. Before surgery, the animals were treated with the analgesic Carprofen (Rimadyl®, 4 mg/kg, subcutaneously) and the antibiotic Albipen® (Ampiciline 10%, 30 mg/kg, subcutaneously). Subsequently, Mk-JH and Mk-BI were anaesthetized with intravenous perfusion of 1% propofol (Fresenius®) mixed with a 5% glucose solution (1 volume of propofol and 2 volumes of glucose solution); ketamine was added to the perfusion solution (65 mg/100 ml). To prevent edema, Methylprednisolone (Solu-medrol, Pfizer®) was added to the propofol/glucose solution (1 mg/ml). The level of anesthesia was kept at an optimal level with a perfusion rate of the propofol/glucose mixture of 0.1 ml/min/kg. All surgeries were performed under sterile conditions. Heart rate, respiration rate, expired CO_2, arterial O_2 saturation, and rectal temperature were monitored throughout the surgery. After surgery, the monkey received Carprofen (pills of Rimadyl® mixed with food) daily and Albipen® (subcutaneously; same dose as above) every two days during one to two weeks.

A squared osseous sector of about 30 × 30 mm was opened above the central sulcus, centered at a medio-lateral coordinate (15 mm from midline) corresponding to the expected position of the hand area in M1. The dura-mater was incised and reclined in order to expose the central sulcus as well as the precentral and postcentral gyri. In monkey Mk-JH, ibotenic acid (Sigma 95%) was injected at six sites in the precentral gyrus, at a position roughly corresponding to the hand area based on its coordinate and the shape of the central sulcus at this location. The extent of the cortical territory, in which ibotenic acid was injected in Mk-JH, corresponded to the area of cortex captured by the charge-coupled device (CCD) camera for LSI measurements (Fig. 1). Acquisitions of cerebral blood flow with LSI (see below) were performed before and then after each individual injection of ibotenic acid. A volume of 3 μl of

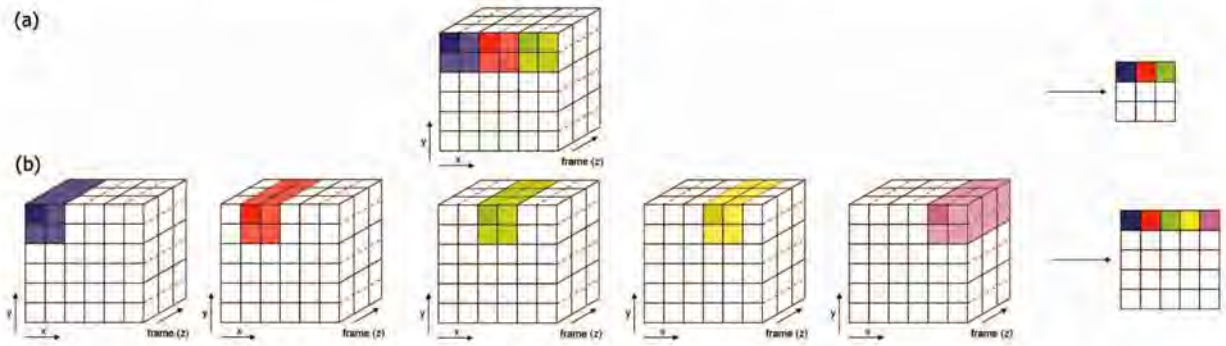

Fig. 2 (a) Standard (temporal) LSI scheme. (b) High resolution LSI introducing a sliding window in space.

ibotenic acid solution (10 μg/μl in phosphate-buffered saline) was injected at each site by using a Hamilton microsyringe, positioned at 2 mm below the pial surface (Fig. 1). The total volume of ibotenic acid injected in Mk-JH was 18 μl, an amount representative of volumes injected in monkeys used for behavioral studies.[4,11,12] In Mk-BI, from which only MRI data were derived, ibotenic acid was injected at 29 sites defined based on intracortical microstimulation,[11] at which a volume of 1 μl was infused (total amount 29 μl). The volume of ibotenic acid injected was larger in Mk-BI, because this animal was involved in the behavioral protocol,[12] requesting a lesion affecting the entire hand representation in M1. In contrast, Mk-JH was exclusively involved in the present LSI protocol and, therefore, the lesion was adapted to the cortical territory covered by the CCD camera rather than covering the entire hand representation in M1. In addition, in Mk-JH the infusion sites were somewhat more distant than in Mk-BI, and therefore, the volume injected at each infusion site in Mk-JH was larger. At the end of this experimental session aimed at lesioning the motor cortex, the dura mater was put back in place and sutured. The craniotomy was not closed. The muscle and skin were then sutured.

2.2 *Laser Speckle Imaging*

2.2.1 *Data acquisition and image processing*

For each subsequent LSI recording session conducted in Mk-JH only (at 1 and 3.5 weeks post-lesion, also under anesthesia as described above for the initial session), the skin was incised, the muscles reclined, and the dura was reopened as described above. For such a time interval between sessions, there were no adhesions between the dura and the blood vessels. A selected part of the surface of the cerebral cortex was homogeneously illuminated with a 785 nm single frequency laser (Toptica®, Munich, Germany), feeding a single mode fiber attached to a collimating lens (Schaefter + Kirchhoff®, Hamburg, Germany; Fig. 1). The whole illuminating device and the CCD camera have been positioned using a stereotaxic frame. The total laser power incident on the brain cortex was about 3 mW distributed over an area of several cm^2, thus preventing any physiological effects or superficial heating of cortical tissue. The diffuse reflected light was monitored in the image plane in the crossed polarization channel with a CCD camera (PCO Pixelfly, 640 × 480 pixels, 12 bit, exposure time 12 ms). A region of 10 mm by 7.5 mm of the cortex was imaged onto the 1/2″ CCD chip at 0.64× magnification.

Individual measurements of 15 s duration at 50 frames per second (total 750 frames) have been streamed at full resolution to the hard disk using the Streampix 3 software package (NorPix Inc.®, Montreal, Quebec, Canada). The local speckle contrast K was computed from a b × b square of pixels and typically 40 successive frames,[24,32,38] setting the actual time resolution of the experiment to 0.8 s. The image quality was further improved by implementation of a sliding window average (Figs. 2 and 3), where essentially all camera pixels are replaced by a metapixel (value K). Only a few pixels at the image border need to be excluded from the final image due to the lack of sufficient neighbors. For a raw image with 640 × 480 pixels, and typically b = 5, this procedure provides a speckle image with a nominal resolution of 636 × 476 pixels (Fig. 3). The Labview® and Matlab® source codes are available free of charge from the authors (with no support) at http://physics.unifr.ch/en/page/54/.

2.2.2 *Heartbeat filter*

During the experiment the recorded image sequences exhibited a small periodic movement of the cerebral cortex due to the heartbeat/blood pressure variation of the animal. This movement leads to a measurable change in contrast (roughly +/− 10%) and in turn slightly blurs the image, when averaging over a sequence of images. To minimize this effect, we have implemented a software filter that allowed us to select images at a certain reference point in the heart beat cycle (Fig. 4). As expected, we found that the contrast time course essentially follows the heart beat with a frequency of approximately 80 beats per minute. A high pass filter was applied to select only the frames of the sequence with an average region of interest (ROI) contrast higher than a specified threshold. All other frames were discarded for the processing of the laser speckle image, thus roughly reducing the number of analyzed raw images by a factor of four to five. As can be seen in Fig. 4, this procedure allows us to reduce the systematic error due to heartbeat from more than 10 to about 3%.

Residual movements of the skull were observed leading to minute shifts in *x*-/*y*-directions during a long time recording sequence. It was not straightforward to compensate these movements within our measurement scheme. The presence of these small drifts thus imposes a limit to the total recording time, and therefore, the statistical accuracy of the measurements, in order to avoid blurring effects. Here, we have chosen the image acquisition time such that the influence of these drifts is negligible.

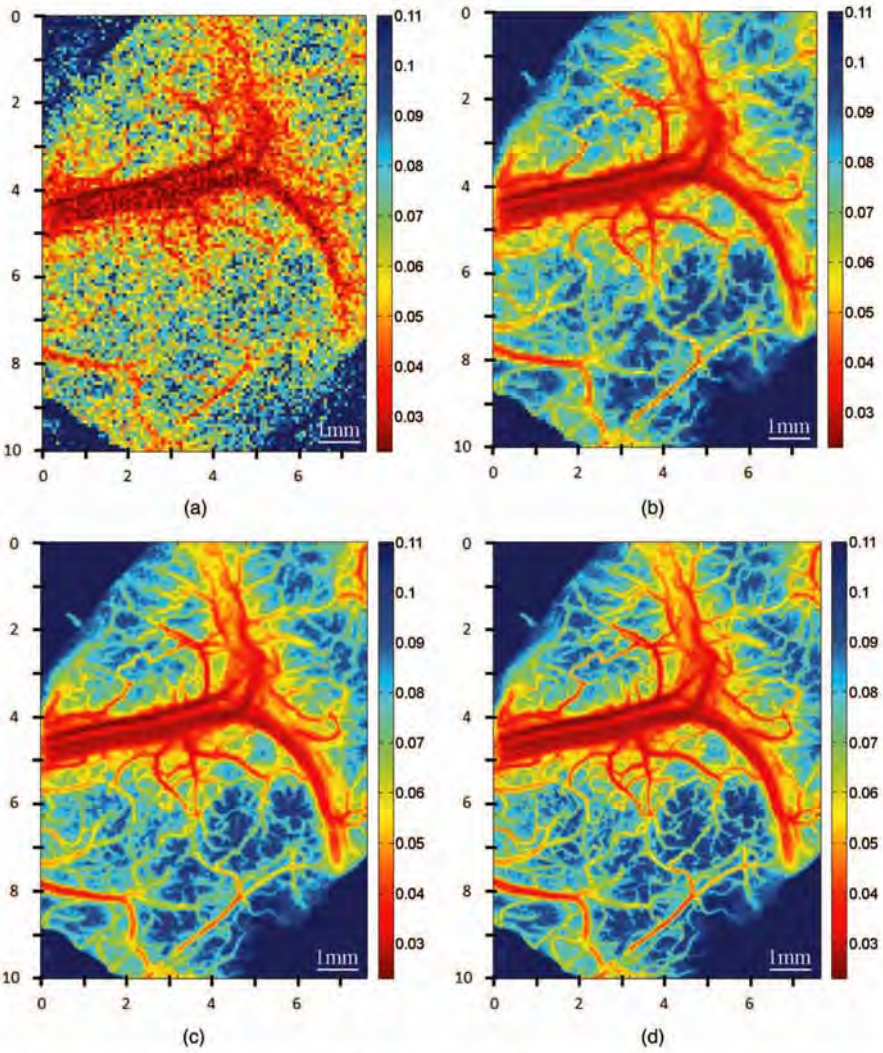

Fig. 3 Typical LSI in our experiment is constructed from 40 individual frames (resolution 640 × 480 pixels) and a spatial average of 5 × 5 camera pixels is performed at each point resolution in a final image with a nominal resolution of 636 × 476 pixels. Laser speckle images of the cerebral motor cortex calculated with different parameter settings: spatial averaging box size is set to 5^2 pixels for (a), (b), and (c); for (d) it is 2^2 pixels. (a) No time average, single frame, standard resolution scheme. (b) Time averaging over 40 frames, standard resolution scheme. (c) Time averaging over 40 frames, high resolution scheme (sliding window). (d) Time averaging over 250 frames, high resolution scheme (sliding window). The number of raw pixels used for the calculation of one LSI metapixels is 1000 for (b), (c), and (d) (example: box size * frames = 5^2 pixels * 40 frames = 1000 pixels). Axes labeling: *x-y* display the camera pixel at 0.64 magnification. The speckle contrast is color coded as shown by the color bar on the right of each LSI.

2.3 *Magnetic Resonance Imaging Acquisition*

The MRI data were acquired in Mk-JH and Mk-BI using a 1.5 Tesla Siemens© Symphony magnetic resonance scanner. To allow direct comparison of the extent of the cortical lesion at regular time points after the lesion, the anesthetized animal was placed in a decubitus ventral position with his head stabilized into a nonferromagnetic fixation frame with ear, mouth, and eye bars. MRI was performed under heavy sedation induced with subcutaneous injections of ketamine (Graeub® 10 mg/kg) associated with medetomidine (Graeub® 1 mg/kg). At the end of the acquisition, the anesthesia was reversed with Atipamezol (Pfizer®, 0.25 mg/kg). The acquisition parameters of the MRI data were the following: total of 19 images, slice thickness 2 mm, 2 TSE (TR 4500 ms and TE 129 ms), field of view 107 mm × 140 mm.

2.4 *Necropsy and Histology*

At the end of the experiment, the monkeys were sacrificed[5,7] for histological analysis of the lesion. Mk-JH was sacrificed a few days after the last LSI session, whereas Mk-BI was sacrificed several months post-lesion as it was involved in a study of functional recovery.[12] The monkeys were sacrificed under deep anesthesia [initiated first with an i.m. ketamine injection followed by an i.p. lethal dose of sodium pentobarbital (90 mg/kg)] by transcardiac perfusion with 0.9% saline (400 ml) continued with fixative (three liters of four paraformaldehyde in 0.1 M phosphate

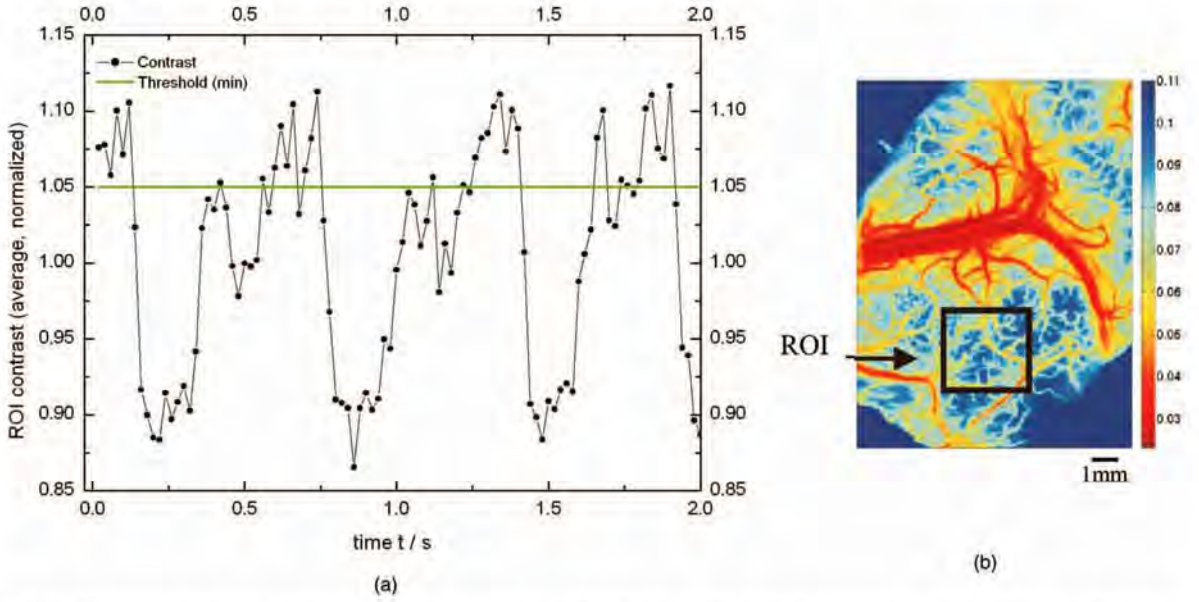

Fig. 4 (a) Heart beat signal computed from the average contrast in a ROI of 100 × 100 pixels from successive individual frames. The data were normalized and baseline corrected. The green line shows the minimum threshold, all other frames below that line will not be used to calculate a high resolution contrast image. (b) LSIs of the cerebral motor cortex calculated from 5^2 pixels and 40 successive frames. The ROI analyzed for the heartbeat correction is indicated by the black square.

buffer, pH = 7.6) and solutions (two liters each) of the same fixative containing increasing concentrations of sucrose (10, 20, and 30%). The brain was removed, dissected, and stored in a sucrose solution (30%) for 1.5 to 2 weeks. Frozen sections of the brain were then cut in the frontal plane at a thickness of 50 μm. Eight series of sections were collected with a cryotome (HM560, MICROM®, Switzerland). Among these series, one was Nissl-stained and one was immunocytochemically treated (SMI-32 antibody against a non-phosphorylated neurofilament epitope), as previously reported.[7,39] Furthermore, additional series were processed for other markers, such as Neuronal Nuclei [(NeuN) neuronal marker] and glial fibrillary acidic protein [(GFAP) glial marker], following previously described protocols.[40]

3 Results

3.1 *Injections of Ibotenic Acid*

In the first LSI recording session conducted in Mk-JH, LSI acquisition was immediately made after incision of the dura in order to establish the cerebral blood flow level corresponding to the reference state under stable propofol anesthesia. Then, this first session comprised the procedure of ibotenic acid infusion in the motor cortex in order to produce a permanent cortical lesion, comparable to lesions made in the course of previous studies in our laboratory.[4,11,12] Six penetrations with the needle of a Hamilton syringe were performed (Fig. 5), in the part of cerebral cortex rostral to the central sulcus, corresponding to M1.

3.2 *Speckle Contrast Imaging (Laser Speckle Imaging)*

The LSI in the reference state exhibited a high contrast [Figs. 6(a) and 7], corresponding to a moderate blood flow. As expected, the infusion of ibotenic acid at the first site of injection (Fig. 5) produced an immediate decrease of contrast, in line with an increase of cerebral blood flow (Fig. 7). Note, however, that the contrast slightly increased in the few minutes following the infusion of ibotenic acid at one site, as shown by the three LSI data points taken at 1.5 min intervals following the first post-infusion LSI acquisition. The same time course of contrast change was observed at all six injection sites during the few minutes following the actual infusion [Fig. 7(a)]. The next infusion at sites #2 and #3 produced a further decrease of contrast (increase of blood flow) [Fig. 7(a)]. As of the fourth injection site, the contrast reached a stable lowest level (maximal blood flow), although the small rebound of contrast was still

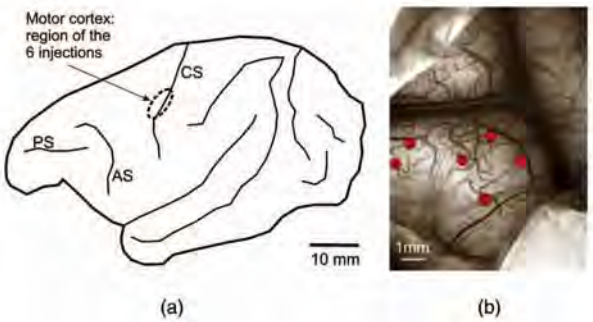

Fig. 5 (a) Lateral view of the left hemisphere of Mk-JH, showing the cortical territory (circle) in which a lesion of the motor cortex was performed with infusion of ibotenic acid along six penetrations in the pre-central gyrus. The ROI is presumably located in the zone corresponding to the hand area. (b) CCD image (slightly tilted to the left) of the cortical surface in the pre-central gyrus, with location on the cortical surface of the six syringe penetrations (one to six). Same orientation as in the right panel of Fig. 1.

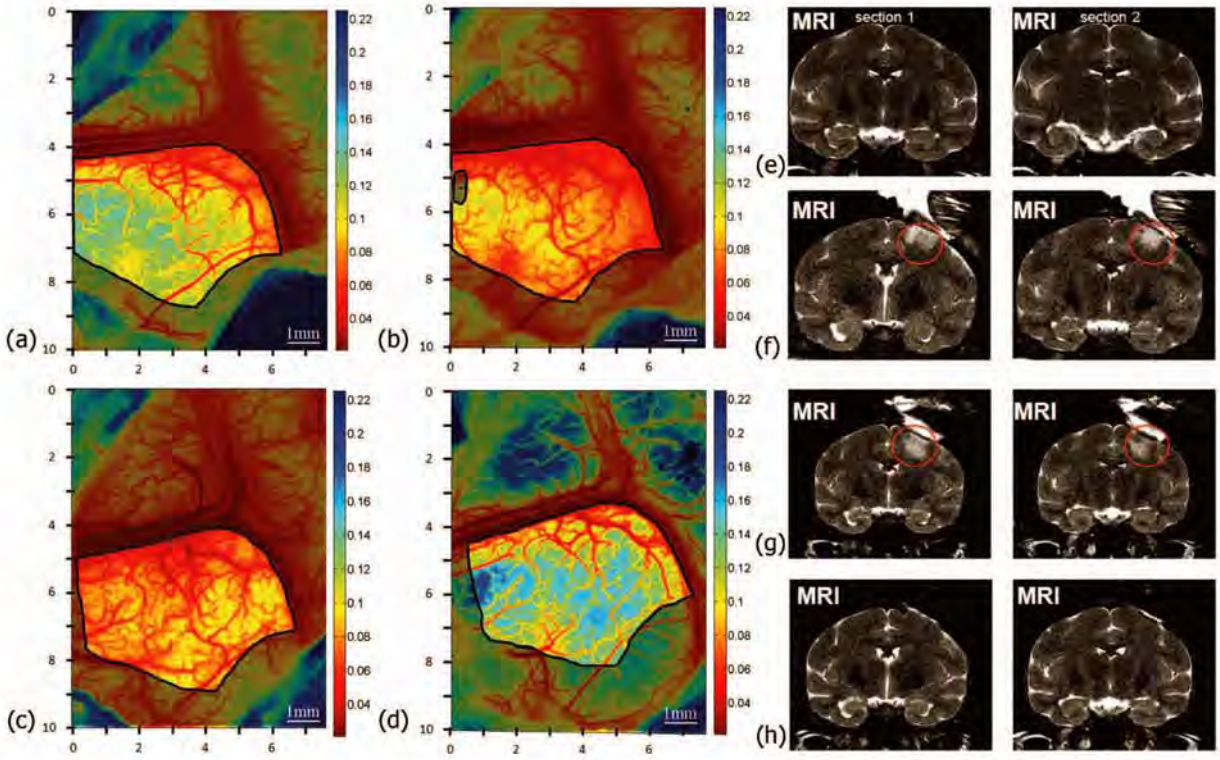

Fig. 6 LSI [(a),(b),(c),(d)] and MRI [(e),(f),(g),(h)] are compared in Mk-JH within a time frame of 3.5 weeks; (a), (e) reference before the injections; (b), (f) LSI taken 60 min post-lesion, MRI at one day post-lesion; (c), (g) LSI and MRI at one week post-lesion (one day apart from each other); (d), (h) LSI and MRI 3.5 weeks post-lesion (one day apart from each other). The highlighted area in the LSI figures shows the ROI wherein the average contrast was calculated for the quantitative data shown in Fig. 7. Calculation parameters for the LSI: 250 frames out of 750, HBC filter, sliding box, box $= 5^2$ pixels. The left and right MRI images on the same horizontal panel are taken at two rostro-caudal levels, distant by 2 mm, intercepting the ROI in the motor cortex.

present in the few minutes following each individual injection [Fig. 7(a)]. LSI was acquired during 25 more minutes after injection at the last site (site #6), taken at roughly 5 min intervals: there was a slow increase of contrast, finally reaching a stable level after about 75 min, situated in between those observed after the second and third injections of ibotenic acid. This contrast level at plateau is illustrated for the ROI in Fig. 6(b), showing that it was clearly diminished as compared to the reference state [Fig. 6(a)].

At that step, the dura was sutured (together with muscles and skin), the anesthesia was discontinued and the monkey was returned to the animal room. LSI was reacquired one week later and the contrast level was comparable to that observed about one hour after lesion [Figs. 6(c) and 7(b)]. The next LSI data point was acquired 3.5 weeks post-lesion, showing a dramatic increase of contrast, reaching a value higher than the initial reference value [Figs. 6(d) and 7(b)]. These data are indicative of a substantial decrease of blood flow at 3.5 weeks post-lesion, in line with the notion that ibotenic acid infusion produces a permanent lesion of the cortical tissue.

3.3 *Comparison of Laser Speckle Imaging, Magnetic Resonance Imaging and Histology*

LSI and MRI were not acquired the very same day as the two facilities are located at distant sites. The first MRI image [Fig. 6(e)] was taken two days before the first LSI recording acquisition [Fig. 6(a)], both representing the reference state pre-lesion. The second MRI image was acquired one day after the first LSI session during which the ibotenic acid was infused at six sites. The zone of infusion corresponds to a marked hypersignal [red circle in Fig. 6(f)] present in the pre-central gyrus. The hypersignal in the MRI was found to be maintained in the same area [red circle in Fig. 6(g)] one week later, in parallel to the decrease of contrast in the LSI [Figs. 6(c) and 7(b)], associated to an increase of cerebral blood flow. The last MRI image was taken 3.5 weeks post-lesion [Fig. 6(h)], showing a complete disappearance of the hypersignal, in parallel to the dramatic decrease of cerebral blood flow at the same time point, reflected by the increase of LSI contrast [Figs. 6(d) and 7(b)]. The LSI and MRI signals in the ROI follow a parallel time course, at least for the four time points considered in the present study (reference, briefly after lesion, one week post-lesion, and 3.5 days post-lesion). The lesion generated by ibotenic acid infusion in the motor cortex is visible with both LSI and MRI only during a few days (at least one week). Later on, after 3.5 weeks, the signals in both LSI and MRI returned in the direction of the reference appearance pre-lesion [compare Figs. 6(a) and 6(d) for LSI; Figs. 6(e) and 6(h) for MRI], although the LSI signal had a higher contrast, corresponding to a decrease of cerebral blood flow. Evidence for the presence of a permanent cortical lesion was provided by the histology post-mortem for Mk-JH (Fig. 8). As shown in SMI-32

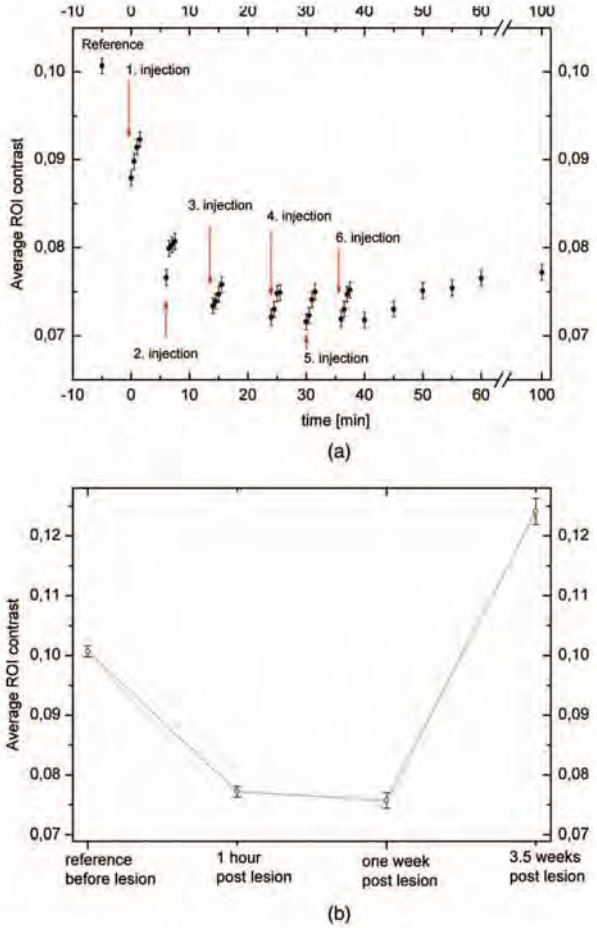

Fig. 7 Time course of maximum, minimum, and average ROI contrast on (a) the day of ibotenic acid injection in Mk-JH as well as (b) at two further distant time points. In (a), the data points related to the six infusions of ibotenic acid were acquired after offset of the injection (the needle was removed from the tissue). In (b), the data point at reference and at one hour post-lesion corresponds to the data point at reference and at 100 min in the upper panel (errors bars were estimated from the time course of the measurements). The data at one and 3.5 weeks were derived from six and five independent measurements, respectively.

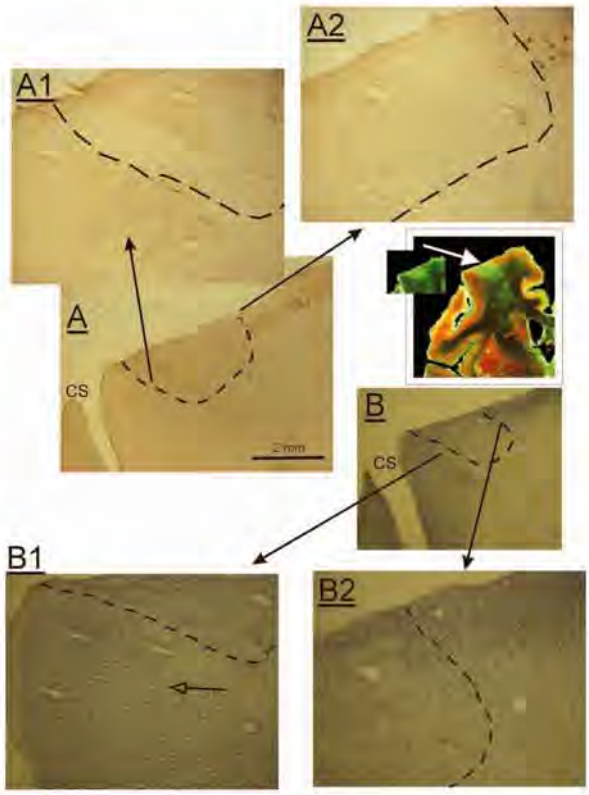

Fig. 8 Histological assessment of the permanent lesion in the motor cortex in Mk-JH, on a frontal section of the motor cortex. Panel A is a low magnification of the lesion area, as seen with the marker SMI-32. In the top row, the panels A1 and A2 are higher magnification of the lateral and medial edges of the lesion territory, respectively, also in SMI-32 material. The damaged territory corresponds to an interruption of the layers III and V pyramidal neurons stained with SMI-32. In the middle, panel B is a low magnification in Nissl material of the lesion area. The panels B1 and B2 in the bottom row show at higher magnification the edges of the lesion with Nissl staining. In all panels, the dashed line delimits the lesion territory. The open arrow in panel B1 points to large Nissl stained neurons in layer V in an intact territory located slightly more lateral than the lesion. The inset in the middle at the right is a low magnification view of the lesion (white arrow), as seen in a combined NeuN (red) and GFAP (green) stained material. The corresponding lesion area is shown for GFAP labeling alone (in green), next to the white arrow. CS = central sulcus.

stained material (Fig. 8), the infusion of ibotenic acid produced a clear disruption of the cortical layers in the lesion site (delineated with the dashed line) without SMI-32 labeled pyramidal cells, whereas in the intact tissue adjacent to the lesion territory the cortical layers III and V are clearly visible by the presence of pyramidal neurons stained with SMI-32 (panels A1 and A2 in Fig. 8 at higher magnification). The appearance of the lesion is similar to that previously shown in monkeys subjected to such lesion and involved in our functional recovery protocols.[11] The focal cortical lesion does not generate a cavity in the gray matter but, as seen on Nissl staining, it corresponds to a homogeneous territory with loss of neuronal cell bodies in all cortical layers (Fig. 8, panels B1 and B2; comparable to a previous description in the rat: see Fig. 1 in Leroux et al.[41]). Furthermore, the lesioned territory seen with the above two markers (SMI-32 and Nissl) matches the zone corresponding to an interruption of the neuronal marker NeuN and, in contrast, an increase of the glial marker GFAP (Fig. 8, middle inset). The MRI data obtained in Mk-BI (not shown) exhibit a similar time course of the hypersignal associated to the ibotenic acid lesion in M1, as illustrated for Mk-JH in Fig. 6.

3.4 *Comparison of the Lesion Size Assessed with Magnetic Resonance Imaging, or Post-mortem on Histological Sections or with Laser Speckle Imaging*

Based on consecutive histological sections as the one illustrated in Fig. 8 and using an ad-hoc function of the Neurolucida software (based on the Cavalieri method) as previously described,[11] the volume of the histological permanent lesion affecting the

gray matter was estimated to be 18.8 mm^3 in Mk-JH (after sacrifice, one month post-lesion). For comparison, the volume of the lesion as reflected by the hypersignal in the MRI scan (computed with the software OsiriX®) was 436 mm^3 at one day post-lesion, 405 mm^3 after one week, and 341 mm^3 after two weeks. After 3.5 weeks, the lesion was no longer visible on the MRI scan [Fig. 6(h)]. In the second monkey (Mk-BI), the volume of the lesion assessed based on the hypersignal on the MRI scan was 262 mm^3 one day post-lesion, 146 mm^3 one week after lesion, 135 mm^3 two weeks after lesion, and 97 mm^3 three weeks after the lesion, whereas, 10 months post-lesion, the volume of the permanent lesion was estimated at 20.1 mm^3 on SMI-32 stained histological material.

As expected, mostly due to edema in the days following the cortical lesion, the volume of the lesion derived from the MRI scans is clearly larger than the final volume histologically determined, by a factor of about 10 to 20. The difference may also comprise a deviation between the two methods to measure the volume of the lesion: although the infusion was aimed to the gray matter, some spread to the white matter is likely, which may be detected on the MRI scans, whereas the histological assessment was limited to the gray matter.

Finally, for comparison, the volume of the lesion was tentatively calculated from the LSI data at the time point at which a territory with reduced blood flow was observed, corresponding to the lesion. Based on an estimation of the surface of the cortical territory with reduced cerebral blood flow as seen after 3.5 weeks in Mk-JH [Fig. 6(d)] and considering an average cortical thickness of 2 mm, the volume of the cortical lesion as derived from LSI data is estimated to be around 58 mm^3. Again, this figure is larger than the actual volume of the lesion histologically determined, but only by a factor of two to three in the comparison between LSI after 3.5 weeks and histology after four weeks. As the LSI approach does not permit an assessment of the cortical thickness in which cerebral blood flow is modified, one may compare in Mk-JH the extent of cortical surface exhibiting an increase of cerebral blood flow detected with LSI during the excitotoxic phase (immediately after infusion of ibotenic acid and one week later) with the cortical surface of the hypersignal observed from MRI one day and one week post-lesion. The cortical surface of the MRI hypersignal was 78.3 mm^2 at one day and 71.7 mm^2 at one week post-lesion, whereas the zone of increased blood flow corresponded to a cortical surface estimated at 22 mm^2, both on the day of ibotenic acid infusion and one week later.

4 Discussion

The present study, based on a parallel assessment of a cortical lesion using LSI and MRI in nonhuman primates, provides evidence that LSI and MRI are tools suitable to monitor the time course of a cortical lesion, for subsequent correlation with the behavioral recovery curve, at least in the present experimental conditions (monkey, ibotenic acid cortical lesion). Although the time course of LSI and MRI signals change in parallel in a time window of a few weeks post-lesion, there is no evidence for a direct relation between LSI and MRI signals, as the former measures cerebral blood flow whereas the latter reflects mostly edema (see below) and tissue infarct – degeneration. The real time LSI data during the surgery show that, when ibotenic acid is infused in the cerebral cortex at multiple adjacent sites in a bit less than an hour, there is an immediate increase of cerebral blood flow, which is still present after one week (Fig. 7). As far as the extent of the cortical lesion is concerned, the present study provides estimates of the ratio between the sizes of the lesion derived from LSI data and from MRI data during the few weeks following the damage, as well as with the size of the corresponding permanent lesion histologically assessed.

Moreover, the LSI and MRI data may contribute to a better understanding of mechanisms underlying functional recovery after cerebral cortex lesion, such as collateral blood or reperfusion after ischemic stroke.[28,30] The LSI method may also be suitable to assess whether cortical areas adjacent to the lesion change their activity, as they may contribute to the recovery, as shown in a rat model of M1 lesion.[26] The improvements of the LSI analysis method [heart beat compensation (HBC) filter, high resolution and low noise LSI] have successfully been applied to the present data set, revealing detailed and additional information on cerebral blood flow that are not visible in the MRI. The LSI adapted technique permitted here to monitor online the cerebral blood flow while ibotenic acid was infused in the cerebral cortex of macaque monkey to produce a permanent lesion. At the onset of ibotenic acid infusion, the cerebral blood flow dramatically increased almost immediately, followed by a slight reversal during the next five minutes (Fig. 7).

Follow-up studies are available in human subjects after stroke, during time windows ranging from 12 h,[42] one month,[43] and up to four months.[44] MRI is a standard tool used in the clinical evaluation of acute stroke, mainly based on the weighted-diffusion imaging setting, offering the best sensitivity to assess acute ischemic lesions for instance.[45,46] In the present study, the T2-weighted imaging setting was chosen as it provides better sensitivity and resolution to detect a chronic cortical lesion and, therefore, is better suited to conduct a longitudinal study over several weeks. Nevertheless, the T2 setting allowed detection of the ibotenic acid lesion in Mk-JH and in Mk-BI already relatively early, about 24 h post-lesion. This observation contrasts with previous T2 data in humans after stroke, exhibiting no hypersignal either immediately post-infarct or after 24 h.[43] This discrepancy may be explained by the different type of lesion, stroke in humans, and ibotenic acid in the macaque monkeys Mk-JH and Mk-BI. More consistent is the situation observed after about a week, with a hypersignal detected with T2 in both humans[43] and monkeys Mk-JH and Mk-BI (present study). After three to four weeks, there was again a discrepancy, as the T2 hypersignal was present in human subjects[43] but no longer in Mk-JH and Mk-BI. In human subjects after stroke, a T2 hypersignal was still present after four months.[44] However, the latter authors reported that the volume of the infarct lesion in humans significantly diminished in the chronic phase, possibly due to lesion consolidation-related changes. In Mk-JH and Mk-BI, similar changes may have happened, but even more rapidly in the present case of a much smaller ibotenic acid lesion, corresponding to a total disappearance of the lesion after 3.5 weeks in MRI. In macaque monkeys, in contrast to the present study, an ibotenic acid lesion in the hippocampus remained visible about 4 years later,[47] but the lesion was performed at the neonatal stage (12 to 16 days after birth), whereas in Mk-JH and Mk-BI the lesion took place at the adult stage, thus possibly accounting for this discrepancy (at least in part). After lesion of the hippocam-

pal formation in adult macaques with ibotenic acid infusion,[48] a prominent hypersignal was found in T2-weighted images after one week post-lesion (as is the case in the present study), but the hypersignal then weakened at two weeks post-lesion, before it gradually disappeared. The optimal time for post-lesion scan was thus identified as less than two weeks.[48]

The time course of the neurotoxic lesion observed here in the nonhuman primate is generally consistent with that reported from MRI data for the same type of lesion in the rat, either in the cerebral cortex[49] or in the striatum,[50,51] namely a hypersignal during a few days, followed then by a progressive decrease after one to two weeks. From the time course of the cortical lesion in the rat derived from MRI,[49] it was concluded that functional and behavioral investigations should be initiated about two weeks after the neurotoxic lesion to avoid transient confounding factors.

As far as cortical lesion produced by ibotenic acid injection in monkeys is concerned,[4,11,12,16,52–54] the present parallel LSI and MRI study extends previous information in the time dimension. As a result of ibotenic acid infusion, the excitotoxic increase of activity, as reflected by an increase of cerebral blood flow visualized with LSI, does not last only a few hours but it is still present after at least a week. The clinical consequence of the ibotenic acid infusion in M1 in monkeys has a very rapid time course as the flaccid paralysis of the contralateral hand occurs already 10 to 15 min after the infusion of ibotenic acid.[4] Based on the parallel time course of both LSI and MRI, the present data suggest that the MRI hypersignal is possibly associated, at least in part, to the excitotoxic activity of the ibotenic acid injections. After 3.5 weeks, the excitotoxic activity has disappeared in both LSI and MRI. The disappearance of the hypersignal in T2-weighted images could be correlated with the decrease of the cytotoxic edema, as previously reported in the rat.[49] This observation would then be in line with the time course reported after ibotenic acid injection in the hippocampal formation,[48] as well as with excitotoxic lesion in rats.[49–51] It remains to be determined at which precise time point (between one and 3.5 weeks) the excitotoxic activity disappears, to turn into a cortical region with diminished cerebral blood flow, as compared to the pre-lesion reference level. However, as previously claimed based on ibotenic acid lesion in the hippocampus of monkeys, the edema associated to the lesion may, to a large extent, contribute to the hypersignal in the MRI imaging during a few weeks post-lesion.[48] Interestingly, the same authors found that T2-weighted imaging obtained after one to two weeks post-lesion was an accurate predictor of the extent of the lesion determined a year later from post-mortem histology. In a study on macaque monkeys subjected to ibotenic acid lesion of the cortical areas MT (middle temporal) and MST (medial superior temporal),[54] the hypersignal in T2-weighted MRI imaging associated to the lesion remained visible after several months. However, the lesions were much larger than the present lesions in M1, as the total amount of ibotenic acid injected in MT and MST were about five to eight times bigger.

LSI was previously used in rats (ministroke models) to monitor the changes of cerebral flow at the surface of the cerebral cortex during and after blood vessels occlusion,[25–29] as well as the reperfusion after reversal of vascular ligation.[28] In these studies, the blood flow was measured with LSI during minutes and hours after the lesion, and up to 24 h in the reperfusion model.[28] Besides the very different type of lesion, ibotenic acid initially provoking (for at least a week) an increase of cerebral blood flow reflecting over-excitation, whereas vessels' occlusion generates a dramatic decrease of perfusion, the present study provides LSI measurements at much more distant time points from the lesion (one and 3.5 weeks). The permanent lesional property, characterized by a reduced cerebral blood flow, is established only between one and 3.5 weeks after infusion of ibotenic acid.

Due to limitations, mainly for ethical reasons, on the use of nonhuman primates in biomedical research, the present pilot experiment aimed at applying LSI on macaque monkeys was restricted to a single animal (acute experiment lasting about one month). As a consequence, no statistical data on LSI measurements could be provided. Based on the present pilot LSI data, the next step may be to develop a LSI chronic recording site from the cortical surface (below a transparent artificial dura, as previously reported[55–58]), including the lesion site (for instance M1) as well as adjacent cortical areas possibly contributing to the functional recovery (e.g., the premotor cortex). This approach may replace the monitoring of the cortical lesion based on MRI, in case the latter facility is not accessible for nonhuman primates, with the restriction however that LSI is invasive (chronic recording chamber). As LSI and MRI provides parallel data with respect to the time course of changes of the cortical lesion, if available, MRI may be preferred as it is noninvasive. Moreover, the present study gives an approximation on how much the size of the cortical lesion assessed by MRI during the few weeks post-lesion overestimates the lesion extent observable post-mortem on histological sections.

5 Conclusion

Using in-parallel morphological (MRI) and functional imaging (LSI) methods in a macaque monkey, the present study shows that a restricted excitotoxic lesion of the motor cortex in nonhuman primate leads to a marked hypersignal in parallel to a dramatic increase in blood flow, at least up to a week post-lesion. Traces of the lesion were still detectable using LSI after several weeks (about three to four weeks) in the form of a diminished cerebral blood flow as compared to pre-lesion, a time point at which the MRI signal had already returned to baseline. The presence of the lesion on a stable and long-term basis was corroborated by the histological post-mortem analysis.

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Annexe 5.4

Video Article

Behavioral Assessment of Manual Dexterity in Non-Human Primates

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Abstract

The corticospinal (CS) tract is the anatomical support of the exquisite motor ability to skillfully manipulate small objects, a prerogative mainly of primates[1]. In case of lesion affecting the CS projection system at its origin (lesion of motor cortical areas) or along its trajectory (cervical cord lesion), there is a dramatic loss of manual dexterity (hand paralysis), as seen in some tetraplegic or hemiplegic patients. Although there is some spontaneous functional recovery after such lesion, it remains very limited in the adult. Various therapeutic strategies are presently proposed (e.g. cell therapy, neutralization of inhibitory axonal growth molecules, application of growth factors, etc), which are mostly developed in rodents. However, before clinical application, it is often recommended to test the feasibility, efficacy, and security of the treatment in non-human primates. This is especially true when the goal is to restore manual dexterity after a lesion of the central nervous system, as the organization of the motor system of rodents is different from that of primates[1,2]. Macaque monkeys are illustrated here as a suitable behavioral model to quantify manual dexterity in primates, to reflect the deficits resulting from lesion of the motor cortex or cervical cord for instance, measure the extent of spontaneous functional recovery and, when a treatment is applied, evaluate how much it can enhance the functional recovery.

The behavioral assessment of manual dexterity is based on four distinct, complementary, reach and grasp manual tasks (use of precision grip to grasp pellets), requiring an initial training of adult macaque monkeys. The preparation of the animals is demonstrated, as well as the positioning with respect to the behavioral set-up. The performance of a typical monkey is illustrated for each task. The collection and analysis of relevant parameters reflecting precise hand manipulation, as well as the control of force, are explained and demonstrated with representative results. These data are placed then in a broader context, showing how the behavioral data can be exploited to investigate the impact of a spinal cord lesion or of a lesion of the motor cortex and to what extent a treatment may enhance the spontaneous functional recovery, by comparing different groups of monkeys (treated versus sham treated for instance). Advantages and limitations of the behavioral tests are discussed. The present behavioral approach is in line with previous reports emphasizing the pertinence of the non-human primate model in the context of nervous system diseases[2,3].

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=3258

Protocol

The overall scheme of the experiment is depicted in Figure 1.

1. Animal preparation and transfer to the behavioral laboratory

1. In the laboratory, prepare the behavioral set-up: fill the wells of the different test boards (tests 1 to 3 below) with the pellets, which serve as reward during the behavioral tests.
2. Transfer the monkey from the group housing room into a transfer cage. The monkey is trained to enter a tunnel giving access to the primate chair, with subsequent positioning of the head. The monkey's weight is measured, before transfer in the primate chair to the laboratory.

2. Test 1: Modified Brinkman board

1. This test, modified and adapted from previous reports[4,5], is the basic behavioral task of reference, to be conducted on every behavioral session. Initiate the video recording with the digital camera above the set-up (possibility also to place 2 additional cameras, one on each side of the board) and place the monkey in front of the Brinkman board.
2. Open for instance the right window on the primate chair to give access to the right hand. Using the right hand, the monkey retrieves the food pellets from the 50 slots (25 vertical and 25 horizontal).
3. After completion of the test, close the right window and refill the board with pellets.
4. Open the left window and repeat the test for the left hand.

5. Reward the animal at the end of the test with a few dried raisins or an almond, a procedure to be repeated at the end of each test to maintain the motivation during the whole daily session.

Additional information: Three digital video cameras are used to record the sequence for off-line processing, placed one above the board and one on each side of the board (to precisely assess the position of the fingers while performing the grasping). Within the same daily session, the monkey can perform another task (either task 2, and/or task 3 and/or task 4; to be distributed among the different days of the week). For the test 1, if the monkey started with the right hand on day 1, start with the left hand on day 2 and so on.

6. Optional: While the test performed above with one or the other hand separately allows comparing the performance of the left hand to the right hand (to identify the "dominant hand"), it is also possible at an early stage of the training to let the monkey perform the task with both hands simultaneously. If one hand is used more often than the other to grasp pellets, then it may be considered as the "preferred hand".

3. Test 2: Brinkman box (with and without visual control)

1. The Brinkman box comprises 20 wells (10 vertical and 10 horizontal). As compared to test 1, the monkey has to control the hand in a limited space, with reduced degrees of freedom to perform the precision grip movement. Fill the board with pellets and close the upper facet of the box, in order to conduct first the test in absence of visual control (relying on tactile exploration).
2. Place the monkey in front of the Brinkman box. Open the left window of the primate chair to test the left hand in absence of visual control. The monkey tries to retrieve the 20 pellets, while the sequence is recorded from a digital camera placed below the box.
3. Close the left window of the primate chair. Refill the board with pellets. Open the right window of the primate chair. The monkey repeats the test with the right hand in absence of visual control. Close the right window of the primate chair.
4. To test the ability to grasp pellets in the Brinkman box under visual control,open the top facet of the box.
5. Refill the box with pellets and the monkey performs the test using the right hand.
6. Close the right window of the primate chair. Refill the box with pellets.
7. Open the left window of the primate chair. The monkey performs the test using the left hand.Repeat step 2.5.

4. Test 3: Rotating Brinkman board

1. This test is comparable to the Brinkman board task (test 1), except that the board is rotating, forcing the monkey to anticipate the displacement of the board in one (clockwise) or the other (counter-clockwise) direction. Fill the board with pellets. The sequence is recorded with a digital camera placed above the set-up (possibility also to place 2 additional cameras, one on each side).
2. Open the right window of the primate chair. The monkey retrieves the pellets from the 32 wells, distributed on four concentric rows, while the board is turning clockwise.
3. Close the right window. Refill the board with pellets.
4. Open the left window of the primate chair. The monkey performs the test as in 4.2 with the left hand.
5. Repeat points 4.2 to 4.4, while the board is turning counterclockwise (one hand after the other). Repeat step 2.5.

5. Test 4: Reach and grasp drawer task

1. To combine prehension ability with the capacity to generate force, this test (derived from previous versions[6-11]) was designed so that the monkey has to open a drawer by exerting first a grip force on the knob of the drawer, followed by a load force to open the drawer, giving access to a pellet placed inside the drawer. The pellet is retrieved with the same hand while the drawer remains open, again using the precision grip. A digital camera is placed on top of the drawer, to record the trials for off-line control of the data (e.g. detection of erroneous trials).
2. Open the right window of the primate chair. The monkey performs 10 trials at each of the 5 different levels of resistance, using the right hand (to have at least 5 correct trials for each resistance level).
3. Repeat the test (50 trials) with the left hand. Repeat step 2.5.

Additional information: On the next behavioral daily session, alternate the hand with which the animal did the test first on the previous session.

6. End of behavioral session

1. After completion of the tests foreseen on that day, feed and reward the monkey with food in addition to the pellets received during the tests. Typically, the monkey receives cereals and fruits.
2. The monkey is returned to the group housing room with the mates.

The precise temporal sequence of the various tests performed is written on the protocol form.

7. Representative Results

The four behavioral tests illustrated above (**Figure 1**) have been used extensively in our laboratory in the context of studies aimed at investigating the functional recovery from lesion of the cervical spinal cord (**Figure 2A**) or of the motor cortex (**Figure 2B**), in absence or in presence of a treatment applied to enhance the spontaneous recovery[12-19].

For test 1 (*modified Brinkman board*), the analysis is focused on two parameters (**Figures 3 and 4A**): i) the score, given by the number of pellets retrieved by the monkey in the first 30 seconds, counted separately for the vertical slots and the horizontal slots (by replaying off-line the recorded video sequence); ii) the contact time (CT), defined as the time (duration) of contact between the fingers and the pellet (see also Figure 6, bottom series of pictures). It is the time interval between the insertion of the first finger (usually the index finger) into the slot to touch the pellet and the onset of retrieval of the pellet out of the well. The time interval is measured by replaying frame by frame the video sequence. The CT is measured for the first five vertical slots and the first five horizontal slots aimed by the monkey[16-18]. The graphs of the score illustrate the initial training phase, the pre-lesion plateau, the dramatic drop of score (usually to zero) immediately after the lesion, the progressive (spontaneous) functional recovery towards the post-lesion plateau. The functional recovery is expressed in % by the ratio of the median post-lesion score at plateau divided by the median pre-lesion score (plateau) *100 (**Figures 3 and 4A**). For the CT, as an increase reflects a deficit, the functional recovery expressed in % is

the ratio of the median pre-lesion CT (plateau) divided by the median post-lesion CT at plateau *100. The effect of treatments post-lesion, demonstrated based on the test 1, are illustrated in detail in previous reports from this laboratory[14,15,17]. A further analysis may address the issue of the strategy, namely the temporal sequence of slots visited by the monkey (**Figure 4B**).

For test 2 (*Brinkman box*), although the score can also be established as in test 1, a more meaningful parameter is the "total time", defined as the time interval between the picking of the pellet in the first slot and the picking of the pellet in the last (20th) slot (**Figure 5**). A functional recovery can be computed and expressed in %. It is calculated using the median total time pre-lesion (plateau after training phase) divided by the median total time post-lesion at plateau *100.

For **test 3** (*rotating Brinkman board*), although the score can also be established as above (test 1), a sensitive parameter is the contact time (CT, defined as above in test 1), measured for the first ten slots (**Figure 6**).

For **test 4** (*reach and grasp drawer task*), the set-up comprises several detectors, recording discrete events such as trial initiation (the hand interrupts a light beam placed in front of the panel), hand touching the knob, onset of drawer pulling, end of drawer opening, hand entering the slot (pick-in time), hand withdrawal from the slot (pick-out time). Force transducers on the knob allow measuring the grip force (exerted by the thumb and index finger pressing on the knob) and the load force (exerted to pull the drawer, to counteract different levels of resistance imposed on the drawer opening). The different levels of resistance opposing the opening of the drawer were obtained by changing the intensity of current applied to a rotative electromagnetic motor attached to the back of the drawer. The set-up is designed to apply a resistive force in parallel to the orientation of the opening of the drawer. Detailed scheme of the drawer set-up is available on request to the corresponding author. All these data are collected with the interface and software *Spike 2* and displayed as illustrated in **Figure 7**.

As previously reported[14,15], these tasks represent the behavioral basis to investigate whether the spontaneous recovery from a lesion of the cervical cord may be enhanced with a specific treatment aimed at promoting axonal regeneration (**Figure 8**).

The retrieval score and the contact time parameters reflect different components of the manual dexterity: the first one includes the entire motor sequence (reaching, grasping, withdrawal of the hand, transport of the pellet to the mouth), whereas the second one is focused on the grasping phase only. These two parameters are particularly accurate and complementary for the modified and the rotating Brinkman board tasks. Whereas the score represents largely redundant information in these two tasks, the contact time in contrast provides more task-specific information due to the variability of the slots' positions in the rotating Brinkman board task, as compared to their static position in the modified Brinkman board task. The Brinkman box task differs from the two above mentioned tasks, as the degrees of freedom of movements with the hand are limited by the closed space. As a consequence, the positions of the different slots on the board within the Brinkman box play an important role in terms of difficulty to perform the manual prehension, due to the exiguity of the box. For instance, the grasping with the hand from the slots located on the right side of the box interferes with right lateral wall.

Consequently, the assessment of retrieval score limited to 30 seconds as in the modified Brinkman board would be biased depending on the position of the slots actually visited by the monkey during this restricted time period, generating a substantial variability from one session to another. For this reason, it is more appropriate to involve all slots (n=20) and therefore the parameter total time was chosen. In the modified Brinkman board, the total time was not considered, as the monkey may in some case loose motivation (for instance post-lesion) due to the large number of slots to be performed (n=50). Along the same line, the analysis of the contact time would implicate to take all the slots of the Brinkman box into consideration (whereas only the first five slots in the modified Brinkman board were considered as the position of the selected slots has little, if no impact on this parameter). Therefore, for the Brinkman box, we recommend in a first approach to determine the total time, as it was observed to be a highly pertinent and informational parameter, at least in our studies with monkeys subjected to a motor cortex lesion (no data available for spinal cord lesioned monkeys). Nevertheless, the contact time may be considered for the Brinkman box, but in a second step including all twenty slots, separately however for the horizontal and vertical slots (not shown).

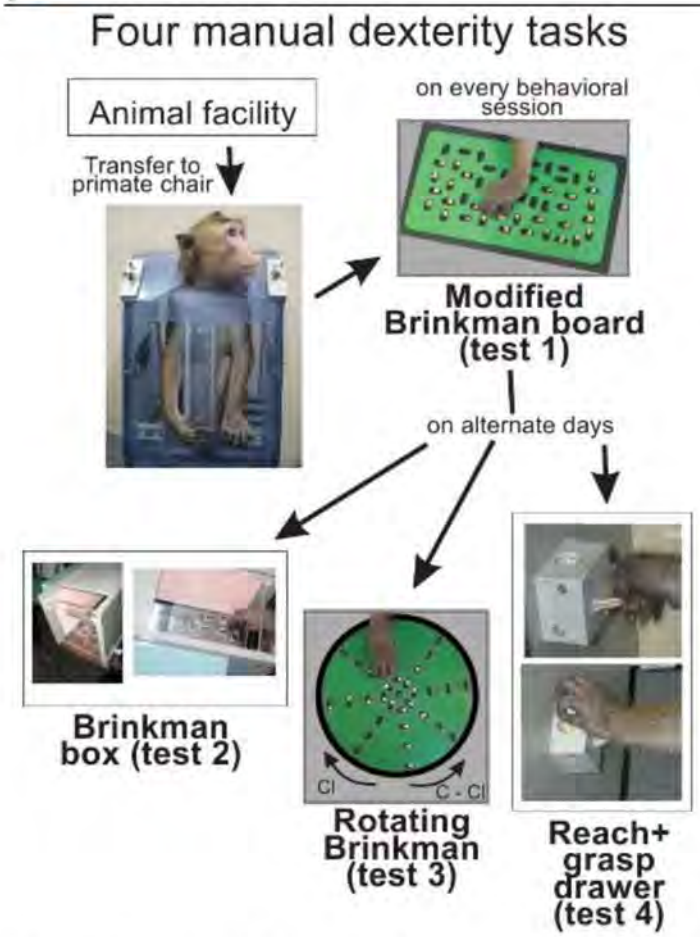

Figure 1. Overall scheme of the experiment. The animal is transferred from the animal facility into the primate chair, transported then to the behavioral laboratory. On each daily session, the monkey performs the test 1. On alternate days, the monkey then performs test 2, and/or test 3, and/or test 4. Some monkeys (especially motivated) may perform all tests on the same daily session. Food pellets (used as reward) were made of dried banana or glucose powder that is compressed in a round shape of about 4 mm in diameter. In all our behavioral tests, we use dustless precision pellets (45 mg) provided by BioServ, One 8th street, Suite One, Frenchtown, NJ 08825, USA.

The dimension of the modified Brinkman board is 240mm long and 140 mm wide, whereas the dimension of the slots is 15 mm long, 8 mm wide and 6 mm deep. The diameter of the board in the rotative Brinkman board is 114 mm.

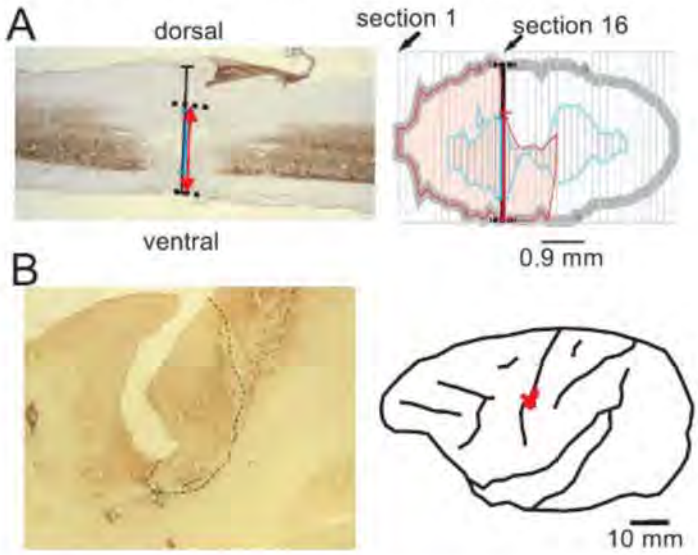

Figure 2. Representative (surgical) lesion of the cervical cord (panel A; modified from[15]) and (chemical) lesion of the motor cortex (panel B; modified from[16]), derived from corresponding histological sections, processed for SMI-32 staining. The maximal extent of the cervical cord lesion has been reconstructed from consecutive sagittal sections of the spinal cord (panel A), whereas the extent and position of the motor cortex lesion has been reconstructed from consecutive frontal sections of the brain and re-positioned on a lateral view of the corresponding brain hemisphere (red spot in panel B). The cervical cord lesion resulted from a transection with a surgery blade at the level C7-C8, resulting in a sub-hemisection interrupting unilaterally the main CS tract component in the dorsolateral funiculus, above the motoneurons controlling hand muscles[14,15,20]. The permanent cortical lesion was produced by infusion of ibotenic acid[13,16,17], at sites covering the hand representation previously established using intracortical microstimulation (ICMS, see[16,21,22]). The cortical lesion territory appears as an abrupt interruption of the SMI-32 staining of neurons in layers III and V in the rostral bank of the central sulcus (area delineated with the dashed line in panel B).

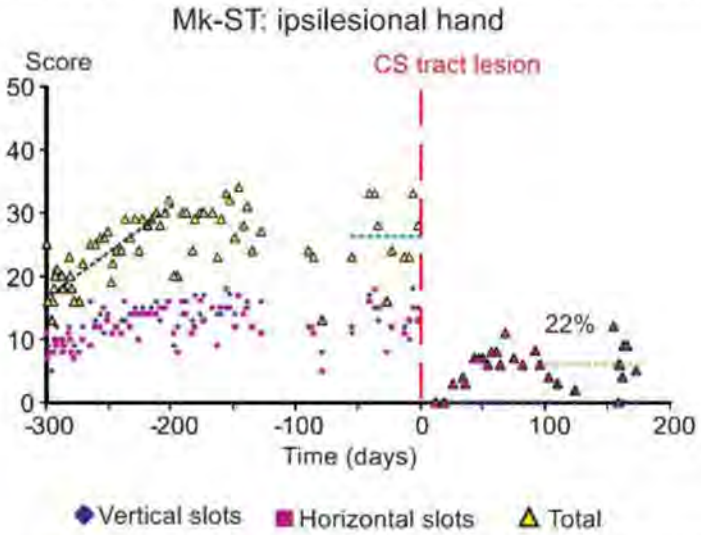

Figure 3. Representative data derived from the modified Brinkman board task (test 1) performed by a monkey subjected to a lesion of the spinal cord (as illustrated in Figure 2A). The graph shows the score (ordinate), separately for the vertical slots (blue symbols) and the horizontal slots (red symbols). The yellow symbols are for the sum of vertical and horizontal scores on a given daily session. In the abscissa, the time is for the consecutive days of the behavioral sessions. The vertical dashed redline (day 0) is the day at which the lesion was performed. Three different periods are highlighted: the first one (black dashed line) corresponds to the training period, the second one (blue dashed line) to the plateau of performance before the lesion, and the third one (green dashed line) to the plateau of recovered performance. Data modified from[15].

Figure 4A

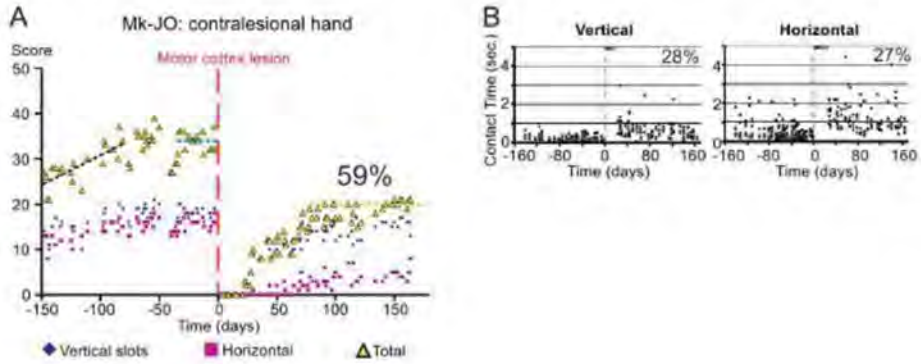

Figure 4B

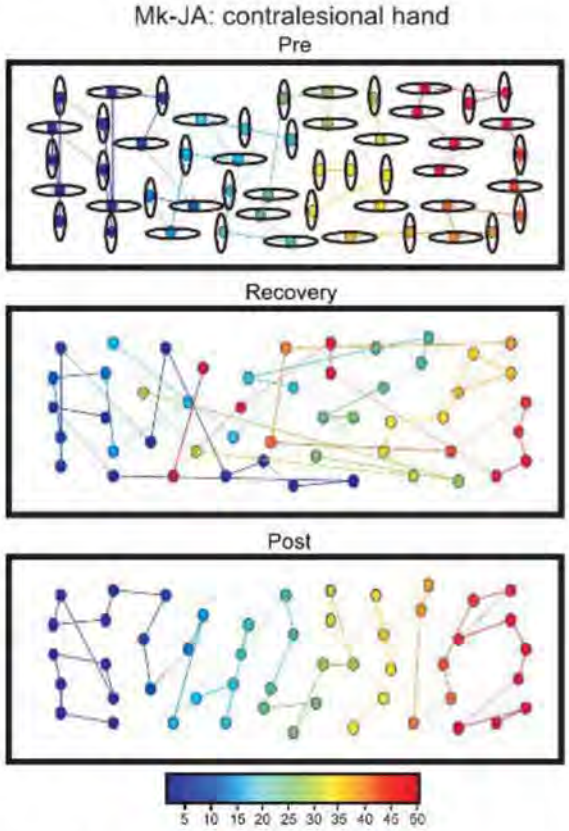

Figure 4A. Same as in Figure 3 (**test 1**), but in a monkey subjected to a lesion of the motor cortex (as illustrated in Figure 2B). The graphs show the score (panel A; same conventions as in Figure 3) and the contact time (CT, panel B) data. In the abscissa, the time is for the consecutive days of the behavioral sessions. The vertical dashed redline (day 0) is the day at which the lesion was performed. In panel B, each dot corresponds to the time of contact between the finger and the pellet in one slot (5 trials per orientation for each session; the grey bar represents the median value). Note that for the trials in which the animal could not perform the task (immediately after the lesion), the CT appears as a saturated value at 5 seconds. Data modified from[16,17].

Figure 4B. Analysis of strategy adopted in the modified Brinkman board task performed with the contralesional hand, before lesion of the motor cortex (Pre), during the recovery phase (Recovery) and post-lesion at plateau (Post). The color of each slot indicates the sequential order of the slots visited by the monkey in one session (the first slot visited is depicted by the darkest blue and the last slot visited by the darkest red). Note that pre-lesion, the monkey started on the left side of the board and scanned systematically towards right. During the recovery, the sequential order was changed. At plateau post-lesion, the strategy adopted pre-lesion re-appeared (systematic scan from left to right).

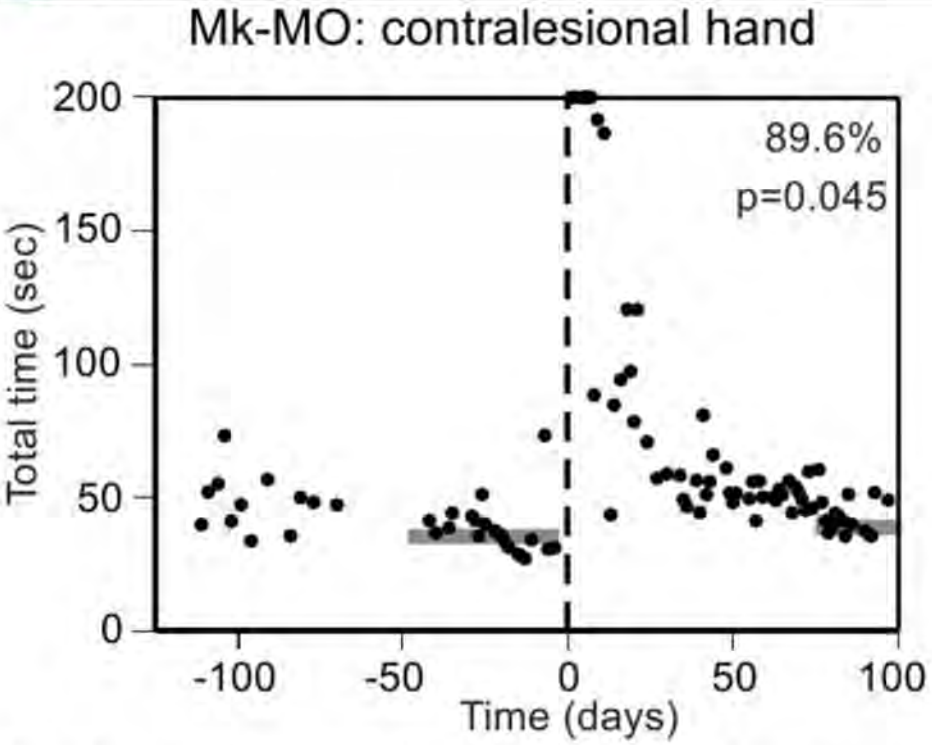

Figure 5. Representative data derived from the Brinkman box (test 2), for a monkey performing the task under visual control. The ordinate is the total time needed to empty the 20 wells along the daily sessions (abscissa), conducted before and after a lesion of the motor cortex (vertical dashed line). The dimensions of the accessible volume within the box are 1360 cm^3 (120mm*110mm*103mm). Note an initial phase of training, characterized by a larger variability of the total time from one session to the next. Immediately after the cortical lesion, the monkey was not able to perform the task (data points saturated at 200 seconds). The p value is statistically significant for the difference between the median total time pre-lesion (middle of the horizontal gray rectangle at the left) and the median total time post-lesion in the last sessions (middle of the horizontal gray rectangle at the right). The percentage of functional recovery is 89.6% whereas the volume of the motor cortex lesion was 41.8 mm^3. Data modified from[19].

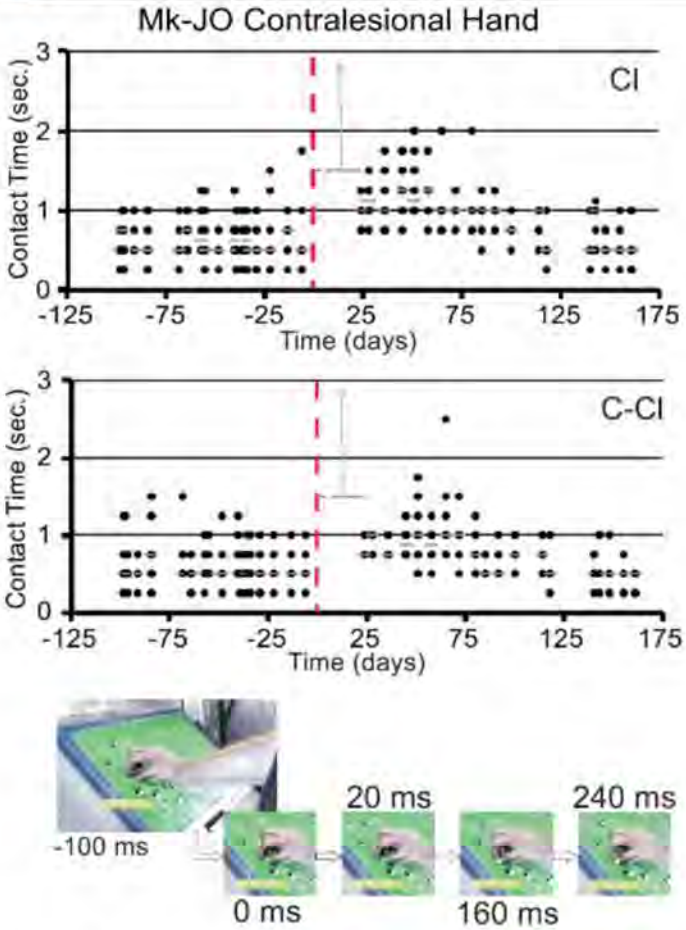

Figure 6. Representative results (top two graphs) derived from the rotative Brinkman board task (**test 3**), with illustration of the contact time measured pre-lesion and post-lesion, with the same conventions as in Figure 4A (panel B), for a monkey subjected to a lesion of the motor cortex (data modified from[17]). The top graph is for a clockwise ("CI") rotation of the board, whereas the bottom graph is for a counterclockwise ("C-CI") rotation of the board. The two vertical gray arrows indicate that the contact time was infinitely long in few sessions immediately after the lesion, as the monkey was unable to perform the task with the contralesional hand. The series of pictures at the bottom of the figure illustrate the method to measure the contact time (valid for both the modified Brinkman board and the rotating Brinkman board). The leftmost picture shows the hand approaching the slot containing the pellet (100 ms before contact between the index finger and the pellet). The next frame on the right corresponds to the time point of contact (0 ms). Then the contact time is defined as the time interval (in ms) running until reaching the frame (rightmost one) corresponding to the time point at which the pellet is taken out of the slot. The contact time here is 240 ms.

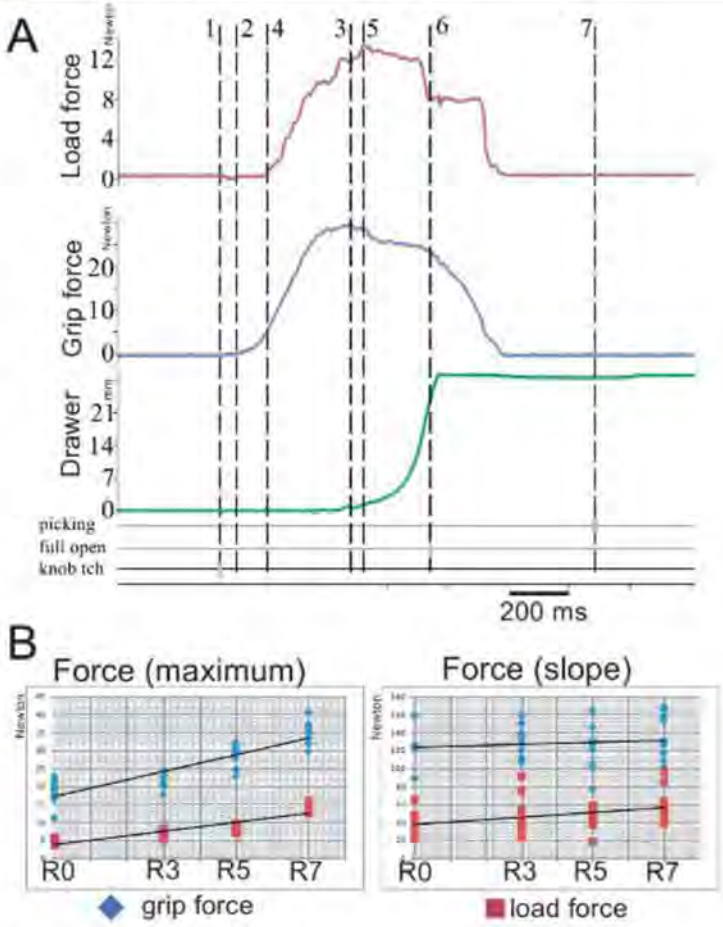

Figure 7. Representative data derived from a session performed by one monkey on the reach and grasp drawer task (**test 4**).

Panel A: Raw data corresponding to three parameters acquired online during a single trial: load force in red, grip force in blue and displacement of the drawer in green. Several markers were also acquired during the task: picking time corresponds to the time of the reward grasping, full open to the full opening of the drawer and knob touch (tch) to the time point when the animal first touches the knob. For the analysis, seven cursors were placed at critical time points in the unfolding task (e.g. 3 gray cursors on the bottom three horizontal lines): 1) time locked to the touch of the knob by the animal; 2) onset of grip force; 3) maximal grip force; 4) onset of load force; 5) maximal load force; 6) time locked when the drawer is fully open; 7) time locked to the picking time.

Panel B: Representation of the quantitative results for two parameters recorded during the task: the grip force (force used to grasp the knob between the index finger and the thumb) and the load force (force used to open the drawer) in two diagrams: the maximal value (left graph) and the slope value (from the onset to the max.; right graph).

Four out of the five different relative levels of resistance have been illustrated here: R0 (0 Newton), R3 (1.4 N), R5 (2.75 N) to R7 (5 N). The knob of the drawer has a triangular and flat shape. The base of the triangle attached to the drawer measures 20mm and the top (15 mm from the base) consists in a circular contour of 7 mm of diameter. The drawer itself has the following dimensions: length=50 mm; width=27 mm and height= 45mm.

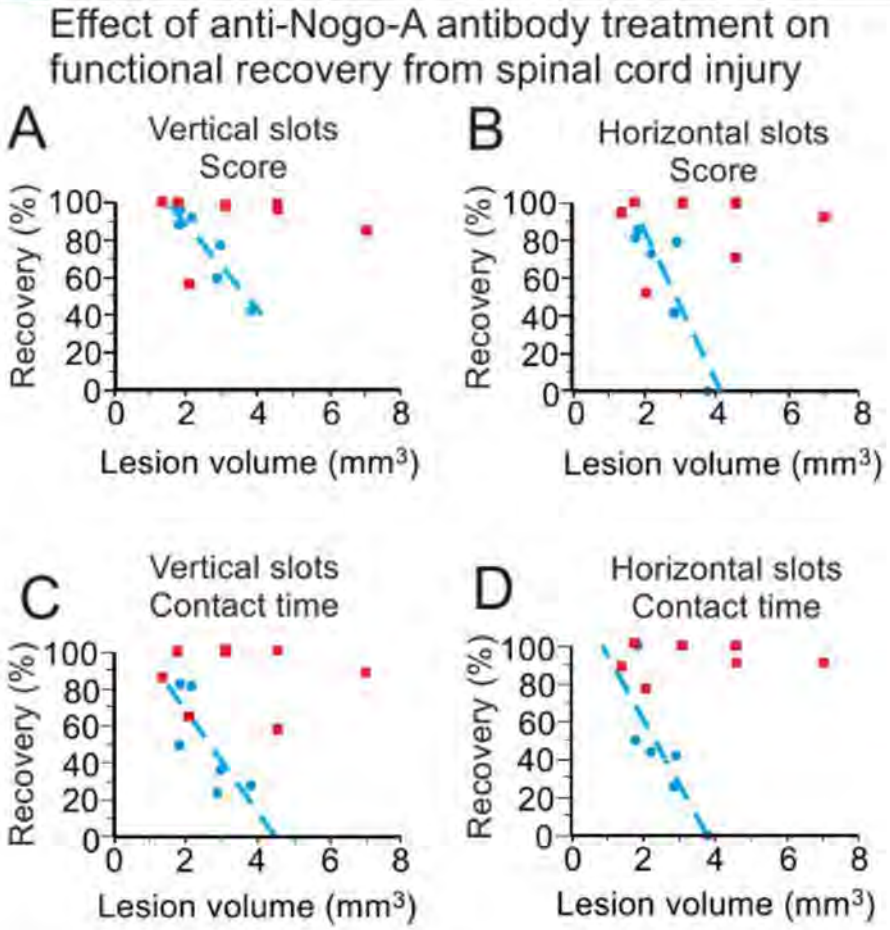

Figure 8. Reminder (modified from[15]) of the use of the behavioral **test 1** to investigate the possible effect of a treatment (anti-Nogo-A antibody) on the functional recovery from cervical cord lesion. For both scores and contact time, as well as for both slot orientations, the group of control antibody treated monkeys (blue symbols; n=6) recovers manual dexterity less well than the group of anti-Nogo-A antibody treated monkeys (red symbols; n=7), especially for large volumes of lesion. The 2 groups differ significantly with p=0.035 (panel A), p=0.022 (panel B), p=0.035 (panel C) and p=0.008 (panel D).

Discussion

Although the present behavioral tasks have been considered so far in our laboratory in the context of studies related to lesion of the cervical cord or to lesion of the motor cortex with the aim to test various treatments (see[14,15,17] and http://www.unifr.ch/neuro/rouiller >select "research" in the top bar menu, then > "motor system" > "recovery after lesion"), they may also have a broader application, as manual dexterity is also an aspect to consider in other pathologies, such as Parkinson disease (MPTP monkeys) or in case of sensory de-afferentation affecting proprioception and/or the sense of touch (especially the **test 2** in absence of visual control).

The behavioral tests proposed here are suitable to investigate the motor control of distal movements of the forelimb, as involved in manual dexterity. The specificity of the tests is demonstrated by the absence of deficit (except for a couple of days) in case of a lesion which does not impair relevant components of the control system: indeed, in case of a lesion placed more caudal than the motoneurons controlling hand muscles, there was no deficit. The pertinence of the test 1 can be appreciated by comparing on video sequences taken at two time points of the post-lesion recovery curve, which differ by an enhancement of functional recovery of 25%, possibly in relation to a cell therapy treatment[17].

In spite of some initial, relatively short training phase at beginning (lasting generally 2-3 months), the behavioral tests proposed here are relatively "natural" and straightforward, as compared to complex (e.g. conditional) tasks for which the training of the monkey may take nearly a year or more. The positive reinforcement is based on solid food, which is less sensitive on the ethical point of view than water deprivation, usually used in more complex tasks[23]. There is no need to deprive the monkeys from food to obtain stable and consistent results. The pellets received during the tasks represent the first access to the food on the day of the behavioral session (assuming that the monkey does not eat during the preceding night; however additional food may be given until the end of the afternoon of the preceding day). It is crucial that each monkey performs the behavioral session at the same time of the day, as well as respecting the same sequential order between the different monkeys forming a group in the housing room. As the monkeys are sensitive to external disturbing events, the behavioral tasks should be conducted in presence of background music, masking potential disturbing noise coming from neighboring rooms or laboratories. It is crucial that during the whole duration of the experiment (from initial training up to the last daily experimental session, a period of several months if not years), a given monkey is placed daily under the supervision of the same experimenter.

The present behavioral tests, used since several years in our laboratory to quantify manual dexterity, are to some extent comparable to other tests of manual dexterity recently reported in the literature[24-28]. There is however a crucial need to standardize tests across different laboratories (for better comparison), which is a tentative goal of the present report. On demand, the detailed properties of the set-ups illustrated here for tests 1-4 can be provided by the corresponding author, in order to replicate them. Beyond the issue of regenerative medicine (recovery from lesion of spinal cord or cerebral cortex), the present palette of tests may be suitable to address in normal non-human primates developmental issues (e.g. time course of motor development of dexterous movements), to investigate lateralization aspects (hand preference/dominance) and to decipher evolutionary questions by comparing the motor abilities of different species of primates, including human subjects. Note however that the dimensions of the apparatuses should be adapted according to the digits' size (thickness and length) of the primate species, as it may influence the task performance. In the present study, the tests were conducted on macaca fascicularis monkeys, ranging from 2.5 to 8 years old and weighing between 2.5 and 8 kg. The length of the index finger (used first to manipulate the pellets) ranges from 32 to 35 mm, whereas the circumference of the distal phalanx (tip) of the index finger was between 22 and 25 mm in the monkeys included in our studies. As tested in previous experiments, the same grasping tests are suitable for macaca mulatta as well.

All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996) and approved by local (Swiss) veterinary authorities. All experimental procedures on the monkeys, as well as the detention conditions in the animal facility, were described in detail in recent reports from our laboratory: see references 12-18.

Disclosures

No conflicts of interest declared.

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Déclaration des contributions personnelles

Le present travail de thèse s'inscrit dans un vaste projet de recherche du laboratoire sur le long terme. Il en résulte que le travail expeérimental est avant tout un travail d'équipe, plus particulièrement s'agissant du modèle du primate non-humain.

De manière générale, les contributions des différents membres du laboratoire apparaissent sous la forme de la liste et de la position des auteurs pour les articles correspondants aux différents chapitres des résultats.

Pour plus de précision, l'auteur de la présente thèse a contribué de manière prédominante aux chapitres de la thèse No 3, 4 et 5, tant pour l'analyse des données que pour la rédaction. De même, l'auteur de la thèse a joué un rôle prédominant dans l'analyse des données du chapitre de la thèse No 2. Enfin, au même titre que les autres co-auteurs, l'auteur de la présente thèse a contribué de manière significative au chapitre No 1, ainsi qu'aux articles en annexe.