Unilateral lesion of primary motor cortex leads to interhemispheric disruption of neuronal density in supplementary motor area in macaque monkeys

C098

Schmidlin E., Contestabile A., Collanguilo R., Gindrat A. D., Hamadjida A, Kaeser M., Savidan J., Wyss A. F., and Rouiller E. M.
Domain of Physiology, Dept. of Medicine and Fribourg Center of Cognition, University of Fribourg, Switzerland

Introduction :

The motor cortex in primates is subdivided into primary motor cortex (M1), the premotor cortical area (PM), the supplementary motor area (SMA), itself subdivided into F3 (caudal part = SMA-proper) and F6 (rostral part = pre-SMA), and the cingulate motor area (CMA). The present study aimed at investigating morphological neuronal changes occurring in one of these premotor area, densely interconnected with the primary motor cortex, namely the caudal part of SMA or F3, after a unilateral and focal permanent chemolytic lesion of the hand representation in M1, in a large cohort of macaque monkeys (n=9). For comparison, the same analysis was conducted in 4 intact monkeys.

Hypotheses (based on connectional properties and diaschisis principle)

1. In intact monkeys, the density and dendritic branching properties of pyramidal neurons in F3 (SMA proper) and F6 (pre-SMA) is comparable on both hemispheres
2. Unilateral M1 lesion provokes a reduction of (excitatory) inputs from M1 onto F3 (diaschisis effect), more in the ipsilesional F3 than in the contralesional F3 (as the homolateral projection from M1 to SMA is stronger than the callosal one)
3. As a consequence, in the process of functional recovery from the lesion reflecting (at least in part) reactivation of the deafferented region, post-lesional plasticity leads to a neuronal imbalance between the 2 hemispheres in F3
4. In contrast, no interhemispheric imbalance in F6 after unilateral M1 lesion, as M1 and F6 are not interconnected (thus no distant effect of M1 lesion)
5. In F3, the distant effect of M1 lesion impacts on both layers III and V pyramidal neurons (stained here using SMI-32)
6. The amplitude of the interhemispheric neuronal imbalance in F3 depends on the M1 lesion volume

References

Long-term motor cortical map changes following unilateral lesion of the hand representation in the motor cortex in macaque monkeys showing functional recovery of hand functions
Wyss,A.F.; Hamadjida,A.; Savidan,J.; Liu,Y.; Bashir,S.; Mir,A.; Schwab,M.E.; Rouiller,E.M.; Belhaj-Saif,A.
Restor.Neurol.Neurosci. 2013 volume 31 issue 6

Selective long-term reorganization of the corticospinal projection from the supplementary motor cortex following recovery from lateral motor cortex injury
McNeal,D.W.; Darling,W.G.; Ge,J.; Stilwell-Morecraft,K.S.; Solon,K.M.; Hynes,S.M.; Pizzimenti,M.A.; Rotella,D.L.; Vanadurongvan,T.; Morecraft,R.J. J.Comp.Neurol. 2010 Volume 518

Design of the experiment

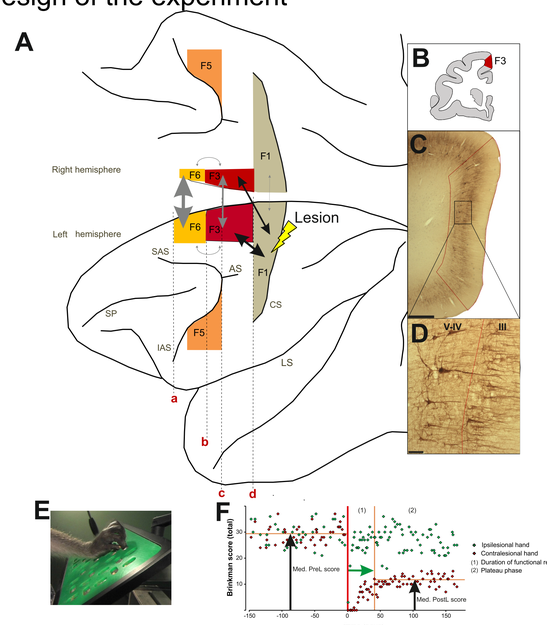


FIGURE 1

(A) Schematic representation of pre-SMA (area F6 in yellow), SMA-proper (area F3 in red), and the primary motor cortex (M1 = area F1). The black double head arrows represent the M1 - SMA interconnections, with the notion that M1 is less densely callosally connected with SMA than homolaterally. Furthermore (black arrows), F3 projects more strongly on the ipsilesional M1 than on the opposite M1. Importantly, F6 does not project to M1 and reciprocally. As explained in the methods, the limit between F3 and F6 was pushed somewhat rostral to the genu of the arcuate sulcus, in order to ensure full inclusion of F3 in the histological analysis.
(B) Schematic coronal section of a macaque monkey's hemisphere. F3 is indicated in red. **(C)** Photomicrograph of a coronal section of macaque monkey's hemisphere in the region of SMA (scale bar: 500 µm), stained with SMI-32. The red dashed line delineates the SMA cortical area (F3).
(D) Magnification, as indicated, of a portion of the section in panel C (scale bar: 100 µm). The layers III and V are visible with the corresponding pyramidal cells stained with SMI-32. The red dashed line follows the bottom of layer III.
(E) Lateral view of the modified Brinkman Board task.
(F) Schematic representation of typical behavioral data in adult macaque monkeys, as assessed using the modified Brinkman board task. The manual dexterity is given by the score (number of pellets retrieved in 30 seconds from wells); as a function of time (days) before and after a unilateral lesion of the contralateral hand area in M1. Relevant for the present report are the scores pre-lesion and post-lesion, as well as the time (duration) of functional recovery of manual dexterity (green arrow) after the lesion. The day of the lesion is shown by the vertical dashed red line.

Neurons counting section per section

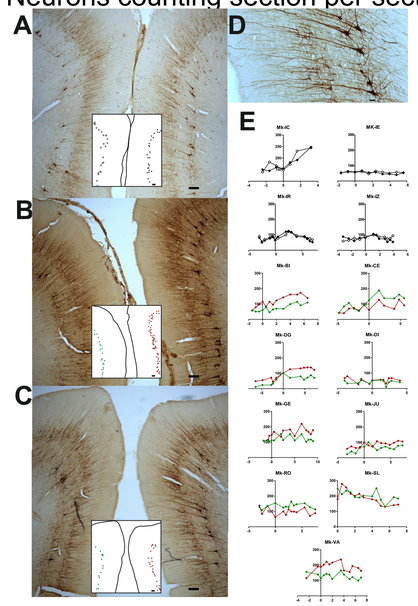


FIGURE 2

(A), (B) and (C) Photomicrographs of a coronal section of an intact macaque monkey (A: Mk-IR), an injured monkey (B: Mk-GE) and an anti-Nogo-A antibody treated macaque monkey (C: Mk-VA) stained with SMI-32 (scale bar: 100 µm). The white insets are a representation of layer V SMI-32 positive neurons taken into account. In the figures (B) and (C), the SMI-32 positive neurons in the injured hemisphere (right in the picture) are indicated with red dots, whereas the ones in green belong to the intact hemisphere (left in the picture). (D) Higher magnification photomicrograph of a coronal section of F3 in macaque monkey (scale bar: 20 µm). The layers III and V are visible with the corresponding pyramidal cells stained with SMI-32. (E) Dispersion graphs of the rostro-caudal gradient of SMI-32 positive cell density in layer V of all monkeys. The cell density for each hemisphere is represented with reference to the rostral limit of F3 (3mm rostrally to arcuate genu). The symbol # was used to indicate that the analyzed cortex region is not complete, but no other sections on the rostro-caudal axis were available for the analysis. In the figure 2A, the white diamonds show the cell densities of the left hemisphere and the black ones indicate the cell densities of the right hemisphere. In the figure 2B and 2C, the green diamonds show the cell densities of the contralesional hemisphere and the red ones indicate the cell densities of the ipsilesional hemisphere.

Statistical analysis of Interhemispheric Difference of Cellular Density (IDCD) in SMA

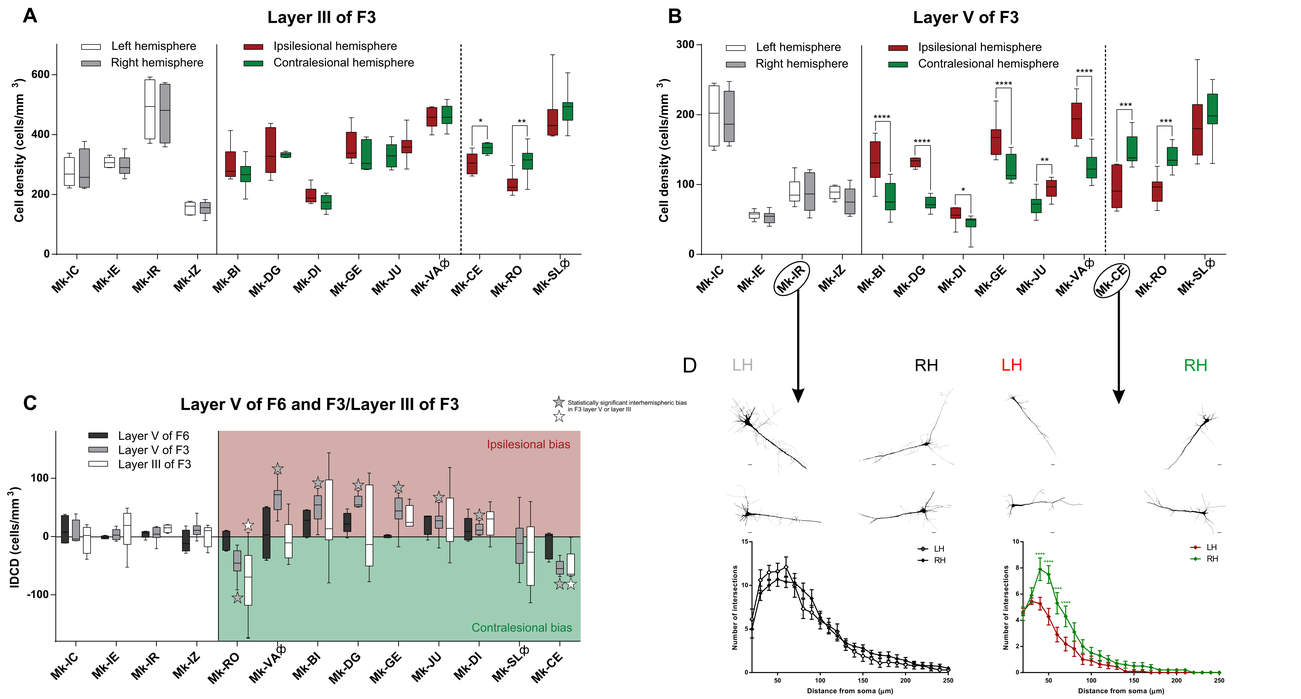
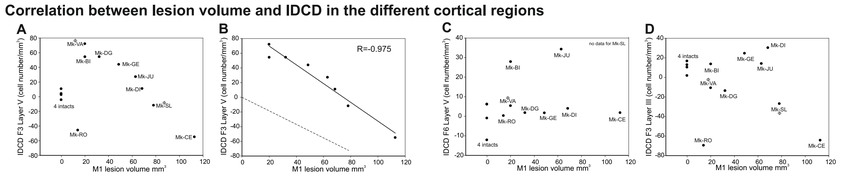


FIGURE 3

(A) and **(B)** Box plots showing the distribution of SMI-32 positive cells density in F3 on both hemispheres for each monkey included in the present study, in layer III (panel A) and in layer V (panel B). As in Figure 2, for the intact monkeys, the gray boxes are for the left hemisphere whereas the white boxes are for the right hemisphere. In the 9 monkeys subjected to unilateral M1 lesion, the red boxes are for the ipsilesional hemisphere, whereas the contralesional hemisphere is depicted by green boxes. The p-values, calculated with a paired t-test or a Wilcoxon-test by comparing in each individual section the cells' density obtained in one hemisphere with that obtained in the other hemisphere, are annotated for each monkey.
(C) Box plots showing the distribution of interhemispheric difference of cell density (IDCD) for SMI-32 positive neurons in F6 for layer V (black boxes), in F3 for layer V (gray boxes) and in F3 for layer III (black boxes), for each monkey included in the present study.
(D) Preliminary data of dendritic branching pattern. Upper panels: histological reconstruction of two SMI-32 stained pyramidal neurons in each hemisphere: left: intact monkey (MK-IR) and right: injured monkey (MK-CE). Scale bar: 20 µm. Lower panel: Quantifed branching pattern of dendrites in selected neurons (12 neurons per hemisphere) as a function of the distance from the soma.

Correlation between lesion volume and IDCD in the different cortical regions



Functional recovery time and extent

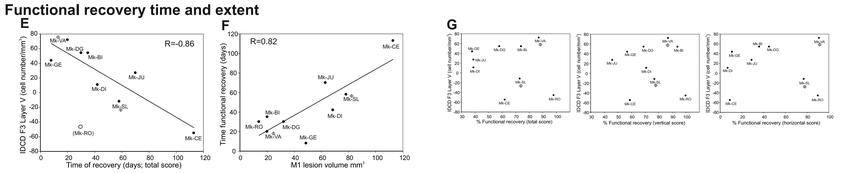


FIGURE 4

Panels A to G are various correlations between different parameters assessed in the present study, as explained in the results. Panel A and B are comparable, except that panel A shows the IDCD data in F3 layer V for all animals, whereas in panel B the intact monkeys have been omitted, as well as Mk-RO considered as an outlier (different lesion procedure). In panel B, the dashed line represents an hypothetic loss of neurons in the ipsilesional F3, increasing with lesion volume. The actual data were fitted with a regression line, with the corresponding coefficient of correlation (R=). In panels A, C and D, the intact animals appear with a lesion volume of zero (absence of lesion); note in panel C that 2 intact monkeys have identical IDCD values (superimposed). In panel E, Mk-RO was also considered as an outlier (not taken into consideration for the regression line and coefficient of correlation). In panel G, the extent of functional recovery in % is shown separately for the total, vertical and horizontal scores derived from the modified Brinkman board task (see methods).

Discussion

A unilateral M1 lesion provoked a neuronal interhemispheric imbalance in F3 in its layer V, both in terms of pyramidal cells' density and dendritic branching pattern (Fig. 3C and D). The distant effect of the M1 lesion on F3, with interhemispheric imbalance, possibly corresponds to a change of phenotype of layer V pyramidal neurons, but not to cell loss (Fig. 3B). The interhemispheric imbalance in F3 is strongly linked to the M1 lesion volume, suggesting that it may reflect distinct mechanisms of the functional recovery process (also dependent on the lesion size). The present post-lesional neuronal plasticity in F3 (layer V) is consistent with the reported contribution of the corticospinal (CS) tract originating from F3 in the functional recovery from unilateral M1 lesion in macaques (McNeal et al., 2010): sprouting of CS axons originating from the ipsilesional F3 (at spinal levels C5-T1), but not of those originating from the contralesional F3.

Methods

Thirteen adult macaque monkeys: 4 intact monkeys and 9 animals subjected to a unilateral lesion of M1 (hand representation) were involved in this study. Cortical lesion was performed by intra-cortical infusion of Ibotenic acid (10µg/µl), resulting in loss of neurons in the hand representation in M1 and a dramatic deficit of manual dexterity of the contralateral hand, followed by incomplete functional recovery (Fig. 1F). SMI-32 staining is a marker of long projecting neurons, corresponding to pyramidal neurons in cortical layers III and V. Microscopic analysis of lesion volume and SMI-32 cellular density were performed using Neurolucida software, at 100X magnification, on histological sections (50 µm thick).
Interhemispheric difference of cellular density (IDCD): The density of SMI-32 positive neurons in contralesional SMA was subtracted from the SMI-32 cellular density in the ipsilesional SMA. Positive IDCD means more cells in the ipsilesional SMA. Negative IDCD means more cells in the contralesional SMA.
Functional recovery was assessed using the modified Brinkman board task (Fig. 1E), consisting for the animal to retrieve 50 pellets located in 50 wells randomly distributed in a Perspex board, 25 being vertically oriented and 25 being horizontally oriented. The behavioral score corresponds to the number of successfully retrieved pellets within 30 seconds. The percentage of functional recovery is the post-lesion score at plateau divided by the pre-lesion score * 100 (Fig. 1F). The time of recovery corresponds to the time interval in days between the day of the lesion and the beginning of the post-lesion plateau (Fig. 1F).
To quantify the dendritic branching pattern, we selected three neurons in each of four sections in each hemisphere in Layer V, with the following criteria: SMI-32 stained, identified nucleus, no interrupted dendrite, and penetration of apical dendrite in layer III. The branching pattern was then counted every 10 microns from the soma limit and averaged per section per hemisphere.
Two monkeys (ϕ) were treated with the Anti-Nogo-A antibody (refs. Wyss et al., 2013)

Results

1. No interhemispheric imbalance of SMI-32 stained neurons in F3 in intact animals, both in layers III and V. Verified (Fig. 3C).
2+3. As a result of unilateral M1 lesion, there was a neuronal density interhemispheric imbalance in F3. Verified (Fig. 3C).
4. In contrast, there was no neuronal density interhemispheric imbalance in F6. Verified (Fig. 3C).
5. The distant effect of M1 lesion on F3 was clearly more prominent on layer V pyramidal neurons than on layer III ones. Partly verified (Fig. 3A and B; Fig. 3C).
6. The neuronal density interhemispheric imbalance in layer V of F3 was strongly related to the M1 lesion volume (in eight out of nine M1 lesioned monkeys. Verified (Fig. 4A and B).

Acknowledgments: Mattia Luchini, Amanda Capobianco
This work was supported by: Swiss National Science Foundation, Grants No 31-61857.00, 310000-110005, 31003A-132465, 3100308-149643 (EMR), No 320030-160229 (ES), the National Centre of Competence in Research (NCCR) on "Neural plasticity and repair"; Novartis Foundation; The Christopher Reeves Foundation (Springfield, NJ, USA); The Swiss Primate Competence Centre for Research (SPCCR: http://www.unifr.ch/neuro/rouiller/SPCCR/welcome.html).