Changes of cortico-cortical connections after unilateral lesion of the primary motor cortex leads to interhemispheric disruption of neuronal density in supplementary motor area in macaque monkeys.

Contestabile A., Collanguilo R., Gindrat A. D., Hamadjida A, Kaeser M., Savidan J., Wyss A. F., Rouiller E. M. and Schmidlin E.
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 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.

Design of the experiment

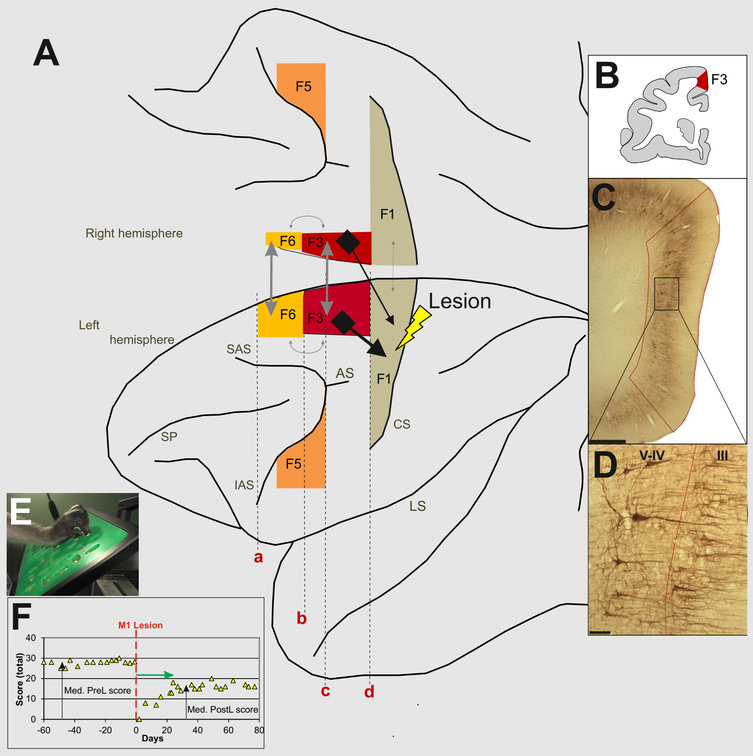


FIGURE 1

(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 straight double head arrows represent the interhemispheric connections, with the notion that M1 is less densely callosally connected that F3 and F6 with their counterparts. Furthermore (black arrows), F3 projects more strongly on the ipsilesional M1 than on the opposite M1. The curved double head gray arrows represent the connections between F3 and F6. 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

Hypotheses (connectionally based)

1. No interhemispheric difference of SMI-32 labelling in intact animals.
2. M1 lesion may provoke a loss of SMI-32 labelled neurons in F3 (retrograde degeneration).
3. No effect of the M1 lesion on F6 SMI-32 neurons, as there is no connection between M1 and F6.
4. Impact of the lesion is more prominent on SMI-32 neurons in F3 on the ipsilesional hemisphere than on the contralesional hemisphere.
5. The amplitude of the interhemispheric imbalance for SMI-32 staining in F3 is hypothesized to be directly proportional to the lesion volume in M1.
6. M1 lesion is expected to impact fairly equally on both layers III and V in F3.

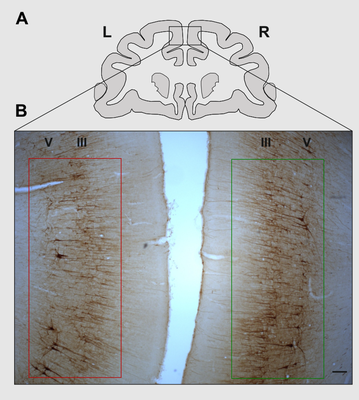


FIGURE 2

A: Drawing of a coronal section of monkey brain showing the analyzed cortical region.
B: Photomicrograph of SMA showing the ipslesional cortex (red) and the contralesional cortex (green)(scale bar 100µm), with SMI-32 positive pyramidal neurons in layers III and V.

Cellular distribution section per section

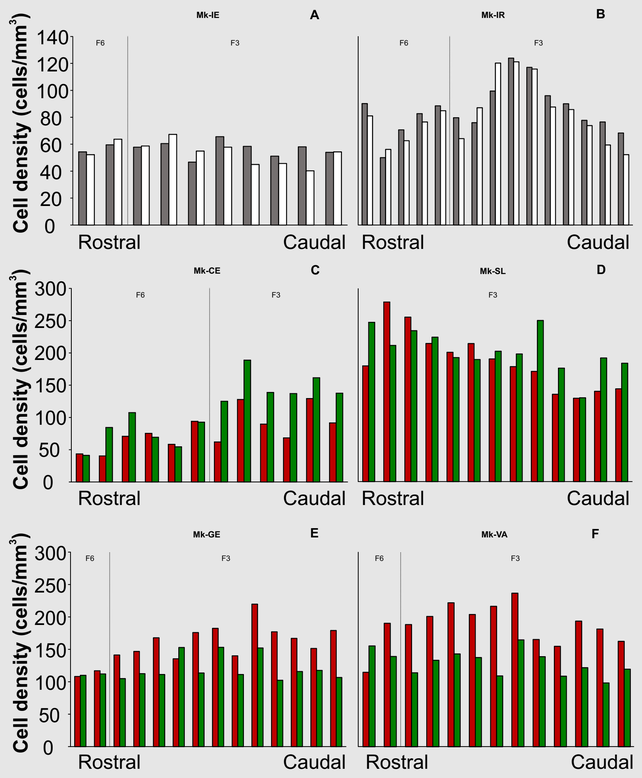


FIGURE 3

Histograms showing the rostro-caudal gradient of SMI positive cells' density in layer V derived from individual histological sections. The tentative limit between F6 and F3 are marked with a vertical dashed black line . Histograms **A and B** are derived from 2 intact monkeys in which the gray columns represent the SMI positive cells' density on the left hemisphere, whereas the white ones represent the cells' density on the right hemisphere. Histograms **C, D, E and F** illustrate 4 examples of monkeys subjected to a lesion of M1 in the hand region. In these 4 histograms, the columns in red represent the SMI positive cells' density on the ipsilesional (left) hemisphere, whereas the green columns represent the cell's density on the contralesional (right) hemisphere. The monkeys Mk-CE and Mk-SL illustrate a bias in F3 towards more SMI-32 positive cells in layer V on the contralesional hemisphere, whereas the other 2 monkeys (Mk-GE and Mk-VA) exhibit more SMI-32 positive cells in F3 in layer V on the ipsilesional hemisphere.

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

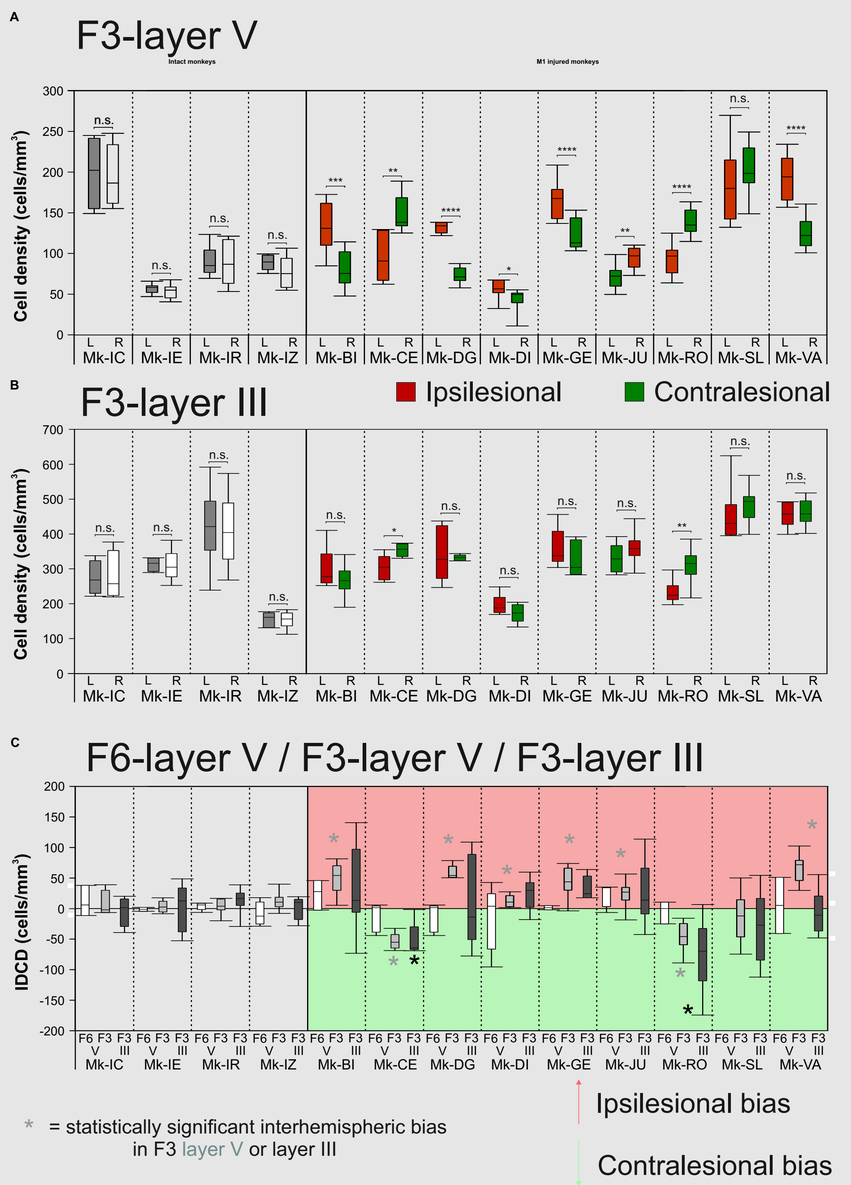
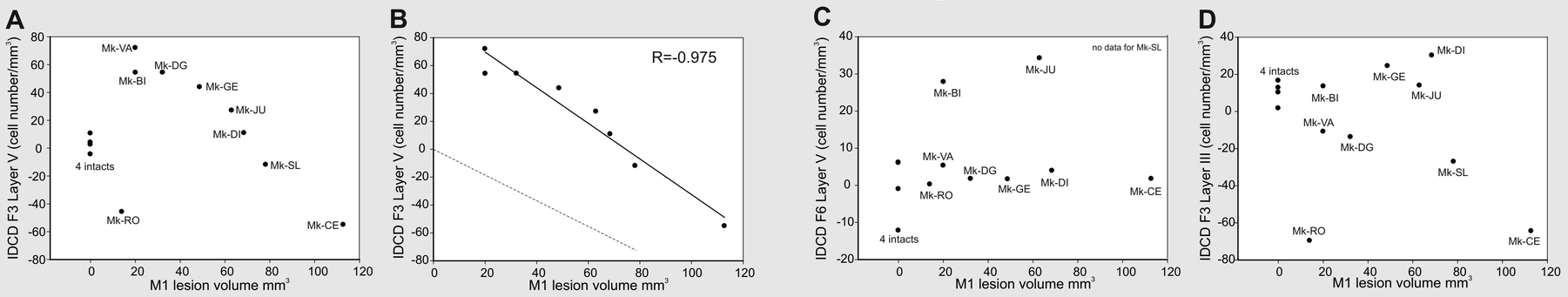


FIGURE 4

(A) and **(B)** Box plots showing the distribution of SMI-32 positive cell's density in F3 on both hemispheres for each monkey included in the present study, in layer V (panel A) and in layer III (panel B). As in Figure 3, 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 (white boxes), in F3 for layer V (gray boxes) and in F3 for layer III (black boxes), for each monkey included in the present study.

Correlation between lesion volume and IDCD in the different cortical regions



Functional recovery time and extent

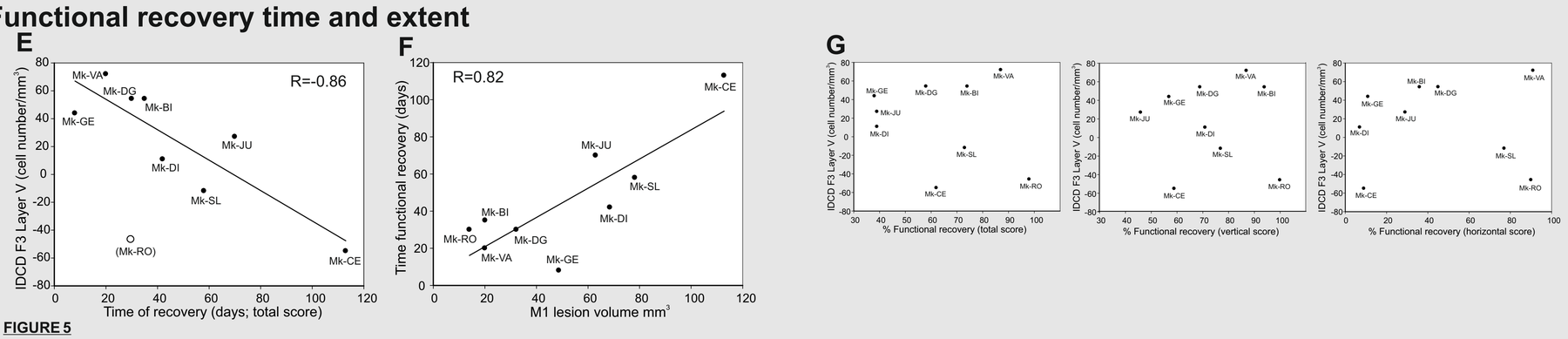


FIGURE 5

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 the expected result based on the hypothesis elaborated in the introduction; 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 (see methods).

Results

1. No interhemispheric difference of SMI-32 labelling in intact animals. **Verified** (Fig. 4C).
2. M1 lesion may provoke a loss of SMI-32 labelled neurons in F3 (retrograde degeneration). **Not verified**, no apparent loss of F3 neurons, as compared to intact animals (Fig. 4 A-B).
3. No effect of the M1 lesion on F6 SMI-32 neurons, as there is no connection between M1 and F6. **Verified** (Fig. 4C).
4. Impact of the lesion is more prominent on SMI-32 neurons in F3 on the ipsilesional hemisphere than on the contralesional hemisphere. **Not verified**, interhemispheric imbalance goes in both directions (Fig. 4C; Fig. 5B).
5. The amplitude of the interhemispheric imbalance for SMI-32 staining in F3 is hypothesized to be directly proportional to the lesion volume in M1. **Partly verified**, strong correlation between IDCD and volume of the lesion (Fig. 5B).
6. M1 lesion is expected to impact fairly equally on both layers III and V in F3. Not verified, impact essentially on layer V (Fig. 4; Fig. 5D).

Discussion

The impact of the M1 lesion on SMI-32 neurons in F3 cannot be explained based on connectional interpretations (see hypotheses).

As a result of the unilateral M1 lesion, the phenotype of Layer V pyramidal neurons in F3 is modified, possibly corresponding to a change of immunoreactivity to SMI-32 antibody. This effect is strongly influenced by the volume of the lesion, a parameter known to also dictate various scenarii of functional recovery from motor cortex lesion.

Material and 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).

I**nterhemispheric 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).