FUNCTIONAL RECOVERY OF FORELIMB RESPONSE CAPACITY AFTER FORELIMB PRIMARY MOTOR CORTEX DAMAGE IN THE RAT IS DUE TO THE REORGANIZATION OF ADJACENT AREAS OF CORTEX

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Abstract—Functional recovery after brain damage has been described frequently and different mechanisms have been proposed to account for the observed recovery. One possible mechanism involves the capacity of one part of the brain to take over the function of another. A possible area for this to take place is in the cerebral cortex, where a variety of reorganizational processes have been described after different manipulations. We show in the present study that the forelimb force and response capacity of the rat, which becomes highly impaired after the bilateral ablation of the forelimb primary motor cortex, is recovered when the animals receive an electrical stimulation in the ventral tegmental nucleus contingent to each forelimb response in the task. Microstimulation mapping of the cortical areas adjacent to the forelimb primary motor cortex revealed the appearance of an area located caudolaterally to the forelimb primary motor cortex, where forelimb movements could be evoked in recovered animals but to a lesser extent in non-recovered animals. A positive and significant correlation was observed between the size of the reorganized forelimb area and the behavioral performance of the animals. Ablation of the forelimb reorganized area in recovered animals reinstated the forelimb behavioral impairment, while the same lesion in normal animals had no effect on the behavioral performance.

The results indicate that recovery after bilateral forelimb primary motor cortex ablation may be due to the reorganization of specific adjacent areas in the cortex.

A variety of mechanisms have been proposed to account for functional recovery after brain damage.\(^2\)\(^3\)\(^7\) Whether recovery occurs through a mechanism which involves the ability of one part of the brain to take over the function of another has been a subject of controversy.\(^4\)\(^6\) Early work has led to the proposal of this type of mechanism,\(^7\) and recent evidence has accumulated favoring this hypothesis.\(^1\)\(^2\)\(^3\)\(^33\)

In the rat and other species a number of important advances have recently been made in the understanding of the capacity of the cortex to reorganize in adulthood after several manipulations (see Ref. 34 for review). In the motor cortex of the rat it has been shown that a dynamic reorganization occurs after peripheral manipulations.\(^3\)\(^4\) These reorganizational processes have been shown to occur rapidly\(^2\)\(^5\) and are apparently due to intracortical mechanisms in which the inhibitory circuitry plays a crucial role.\(^3\)\(^2\) Similar processes and mechanisms have been observed in the somatosensory cortex.\(^2\)\(^2\)\(^4\)\(^7\) However, less is known about the reorganizational processes which occur after central manipulations (i.e. cortical lesions). Several lines of evidence indicate that reorganizational processes are likely to occur after cortical lesions and probably mediate the subsequent functional recovery. Recently, Jenkins and Merzenich,\(^3\)\(^3\) based on the behavioral results described by Cole and Glees,\(^3\)\(^7\) showed that removing the somatosensory cortex responsive to a digit of the hand in owl monkeys induces a reorganizational process in areas surrounding the lesion, which involves the appearance of areas responsive to tactile stimulation of the digit whose representation had been ablated. Also, lesions of the primary motor cortex (MI) of monkeys induce the reorganization and subsequent appearance of activity in the supplementary motor area, which may account for the observed functional recovery.\(^1\)\(^4\) After lesions of the sensory or thalamic inputs to the motor cortex of the cat or monkey, the subsequent functional recovery observed has been shown to follow the reorganization of the alternative input to the cortex.\(^1\)\(^4\) In the macaque monkey, lesions of selective body part representations in the primary somatosensory cortex eliminate the representations of the same body part in the secondary somatosensory cortex.\(^4\)\(^0\) However,
several weeks after this lesion the area deprived of normal primary somatosensory cortex input is no longer silent and is occupied by an expanded representation of a different body part. In rats with neonatal unilateral cortical damage, the recovery of forelimb placing behavior has been demonstrated to involve the remaining hemisphere, where an aberrant ipsilateral corticospinal projection has been shown to provide the anatomical substrate through which the cortex affects recovery. However, recovery of neonatal unilateral cortical damage, the recovery of normal primary somatosensory cortex input is no several weeks after this lesion the area deprived of cortex maps, methods not based on electrophysiological deficits based on these procedures have determined that after bilateral lesions of the forelimb sensorimotor cortex of the rat, reorganizational processes measured at the level of gene expression take place in specific adjacent areas of the cortex in recovered animals. It seems that the reorganized area is essential for the recovery to persist, since ablation of the reorganized area abolishes the induced recovery.

The removal of the sensorimotor cortex of the rat induces deficits in a variety of tasks. Animals with sensorimotor cortex lesions show deficits in tactile discrimination and placing tasks, in handedness tasks, and in some aspects of lever pressing. In these studies the lesions were not specific to the primary motor cortex or to any of its representations. In a recent study we have performed specific lesions of the forelimb MI (FLMI) representations and were able to establish the importance of the forelimb representations and their variable features (i.e. size) in paw preference tasks. Studies of cortical-induced deficits based on these procedures have not been performed and most studies have relied on cytoarchitectonic criteria to define the lesion site. However, due to the extensive variability in the motor cortex maps, methods not based on electrophysiological mapping procedures to define the MI should not be considered adequate to study the effects of lesions of specific motor cortex representations. An important aspect would be to determine if lesions circumscribed to specific MI representations induce deficits in specific aspects of behavioral performance.

We have recently developed a method based on the contingent electrical stimulation of the ventral tegmental nucleus (VTN) as an effective model to induce recovery in different behavioral tasks after damage to different cortical sites. The motivational and cue properties of this stimulation seem to mediate the recovery induced by the stimulation.

In the present study, we were interested in the possibility that a behavioral deficit, measured in a force task, due to the bilateral ablation of the FLMI could be reversed by the contingent electrical stimulation of the VTN. Furthermore, we investigated if the recovery induced by this method would produce reorganizational processes in adjacent areas of the cortex, which would consist of the appearance of a reorganized FLMI, and that ablation of this area should reinstate the behavioral deficit in recovered animals, while it should have no effect in normal animals. Some of the results have been presented in abstract form.

**EXPERIMENTAL PROCEDURES**

**Preoperative behavioral training**

Thirty male Wistar rats (250–300 g body weight, in-house colony) were trained in a force task. Over a period of seven days the animals were trained to press two independent isometric levers placed outside one of the walls of the behavioral compartment. In order to access each of the levers the animals had to reach through a rectangular (11 × 15 mm) hole to access a lever placed outside the wall. The spatial arrangement of the levers permitted the animals to respond on lever I exclusively with the left forepaw and on lever II exclusively with the right forepaw. Thus, each lever permitted the independent evaluation of each forelimb. The levers were connected to force transducers and these were connected to a recording system. Each time the animal pressed the lever over a force of 2 g the system recorded the peak force of every response exerted upon the lever. Responses were recorded for a constant period of time (150 s/lever per day), during which the animals were actively dedicated to responding upon the lever. Therefore, the duration of a session (150 s/lever) was a constant period during which the animals were actively performing the tasks (i.e. pressing the lever), and the time dedicated to other behaviors (i.e. eating, moving around the cage) was not included in this constant period of time. This feature of the task allowed us to consider the number of responses performed during the constant time period as a reflection of the timing of the responses (i.e. response velocity). If rats made few (e.g., they were distracted in the cage for periods of time) but fast responses, the task would not end until the total time spent pressing the lever was 150 s per lever, and thus this animal would have a large number of responses at the end. Thus a large number of responses implies that the responses were fast, while a low number of responses would imply that the responses were slower. The recording system also calculated the number of responses over and under an established force threshold of 10 g. A peak force of 10 g was the force threshold to produce a positive response, and a fixed rate of five responses over the threshold was necessary to obtain a food pellet through a feeder placed in the cage. A behavioral session involved placing the animal in the behavioral chamber and opening the access to one of the levers. The animal would start responding upon the lever and the system would record during the time the lever was pressed (over 2 g) during a total of 150 s. Then the access to this lever would be closed, the access to the other lever would be opened and the recording session would start for the other forelimb. Thus, the behavioral parameters that the recording system calculated for each session were: the mean peak force of all the responses, the number of responses over the force threshold (10 g) and the number of responses under the force threshold. The average duration of a behavioral session per animal and day was 15 min. Animals were handled according to National Institutes of Health guidelines on animal care.

**Microstimulation mapping and forelimb primary motor cortex lesions**

Twenty-four hours after the last day of training the animals were divided into three groups. A group of animals was submitted to bilateral microstimulation mapping of the primary motor cortices and received bilateral lesions of the forelimb representation (FLMI group, n = 10). A second group of animals was also submitted to bilateral microstimulation mapping of the primary motor cortices, received bilateral lesions of the forelimb representations and were implanted unilaterally with an electrode in the VTN (FLMI + VTN group, n = 10). A third group of animals...
was submitted to sham surgery or left intact (Sham group; \( n = 10 \)).

Microstimulation mapping and FLMI lesion procedures were similar to those described previously. Briefly, animals were anesthetized with ketamine (100 mg/kg, i.p.) and placed in a stereotaxic apparatus. Both frontal neocortices were exposed by making a cut that extended from 0.2 mm posterior and 5 mm anterior to bregma and from 0.5 to 5 mm lateral from the midline. The dura remained intact and its surface was covered with 1.5% agar dissolved in 0.9% saline. Platinum-iridium glass insulated electrodes with 15 x 40 \( \mu \)m tips (0.8 - 1.2 M\( \Omega \) impedance at 1 kHz) were used for stimulation. The electrode penetrations were regularly spaced with a grid of penetrations separated by 500 \( \mu \)m. For intracortical electrical stimulation, current trains (100 ms train duration, 300 Hz, 200 \( \mu \)S pulse duration, monophasic cathodal pulses) of a maximum of 60 \( \mu \)A were passed through the electrode at a minimum interval of 1.5 s. The body parts activated by the penetration were identified by visual inspection and/or muscle palpation. In every case where two movements were observed after stimulation in a penetration site the current intensity was lowered until only one of the movements was evoked and this movement was the one considered to be represented at that cortical site. Thus, the threshold values described in the present study are the minimum currents required to evoke the movement represented at each cortical site.

The lesions of the FLMI representation were performed bilaterally. The reason for this was in order to avoid the consequences upon limb preference which are known to occur after unilateral lesions (see Ref. 15). At the conclusion of the mapping procedure a map of the forelimb representation was constructed. The boundaries of the forelimb representation were determined as the midpoint between two electrode penetrations, one that elicited a forelimb movement and one that elicited a movement from another body part. The electrode served to delimit the area of the forelimb representation by moving it along the boundary of the representation. This served as a reference to the investigator of the tissue that was to be damaged and therefore aided in the correct placement of the lesions. After removal of the dura the lesions were induced by delicate suction down to the underlying white matter. Care was taken not to damage the white matter. A surgical microscope aided the investigator in this procedure. The lesions were then filled with hemostatic sponge and the wound was sutured. The dura was also removed in a group of animals (\( n = 5 \)) in the Sham group which were submitted to surgery. Immediately after each lesion remapping was conducted in order to ensure that no cortical tissue corresponding to the FLMI (i.e. from where forelimb movements could be elicited) was left intact.

The electrode implantation in the VTN was performed after the lesions. A twisted bipolar stainless steel electrode entirely insulated except at the cross-sectional area of the tips was implanted unilaterally in the VTN (5.5 mm posterior to bregma, 0.7 lateral to the midline and 8.0 mm below the skull surface). The animals were allowed seven days to recover from the surgical procedure.

Postoperative behavioral training

Seven days after surgery the animals were submitted to a postoperative behavioral period which lasted eight days (postoperative period with intracranial stimulation). The behavioral procedure followed was identical to the one in the preoperative period, with the only difference being that the animals that had been implanted with an electrode received electrical stimulation in the VTN (100 Hz rectangular pulses of 0.2 ms) each time they pressed the lever. The train duration of stimulation was the time during which the animal pressed the lever (maximum of 1 s). The intensity of the current increased in a linear fashion with the force that the animal exerted upon the lever and decreased in the same way. The current increased from zero up to a maximum current established for each animal. The maximum current level was reached for a force of 60 g or over (maximum intensity range = 0.8 - 1.5 mA). The intensity of the current was selected prior to postoperative training for each animal to induce a rewarding effect by placing them in a behavioral chamber and delivering VTN stimulation while observing the effect on the animal. The effect would be considered rewarding if it was possible to make the animal perform a behavior (i.e. bar press in a common operant chamber) by stimulating contingently to this behavior. The current level selected (which was the current toward which the stimulation increased linearly as the animal pressed the lever with greater force) was selected as three times the current level required to produce a reliable rewarding effect. This session lasted approximately 10 min and was performed one day prior to the beginning of the postoperative behavioral training period. Electrical stimulation of the VTN was performed unilaterally due to the fact that unilateral stimulation is sufficient to induce a robust rewarding effect.

All the animals were submitted to a second postoperative period which lasted four days (postoperative period without intracranial stimulation). During this period the animals in the FLMI + VTN group did not receive electrical stimulation for pressing the lever.

Microstimulation mapping and hindlimb primary motor cortex lesions

Based on the maps of the MI constructed previously, a group of animals which had been trained in the behavioral task was submitted to bilateral lesions of the hindlimb MI (HLMI group; \( n = 5 \)). An additional group of animals belonging to the FLMI + VTN group described previously were also submitted to bilateral lesions of the HLMI [(FLMI + VTN) + HLMI group; \( n = 5 \)]. Six days after surgery these animals were submitted to performance in the behavioral task. The behavioral procedure followed was the same as that used during the preoperative training procedure.

An additional group of animals from the FLMI group (\( n = 5 \)) and from the FLMI + VTN group (\( n = 5 \)) were submitted to microstimulation mapping of the motor cortex posterior to the FLMI lesions. The procedures followed were the same as those described previously.

Map construction

Maps of the penetration grid were constructed by making the anterior-posterior and midline position of each electrode penetration according to the micromanipulator that measured the distance from bregma. All the maps shown in the study were constructed by mapping the location of the penetrations from which movements of the forelimb or hindlimb were obtained at a current intensity of 60 \( \mu \)A or less. Movements of other body parts were elicited adjacent to the forelimb and hindlimb areas. These representations were not mapped in sufficient detail to determine its size. The movements elicited about some body parts (i.e. moth and vibrissae) in the border penetrations which served to delimit the boundaries of the forelimb representations are also shown in some maps. Surface reconstructions of the lesions were derived during the process of remapping the motor cortex in previously damaged animals. This was done by plotting the extent of the ablation by measuring the damaged area in the horizontal plane with the aid of the stereotaxic coordinates. These plots were further compared with the histological analysis.

Histological analysis

At the conclusion of the experiments, all the lesioned animals received an overdose of sodium pentobarbital and some were perfused through the heart with saline followed by paraformaldehyde (4%), while others were decapitated. The brains were removed and placed in fixative. Sections of the brain were cut in the sagittal plane at 50 \( \mu \)m in a freezing microtome and stained with Cresyl Violet.
RESULTS

Primary motor cortex representations in rats

In the rat, the organization of the motor cortex has been described extensively. The area from which forelimb movements are evoked at threshold currents is located laterally in the motor cortex and bounds medially with a large area from which vibrissae movements are evoked. Hindlimb movements are evoked caudal to the forelimb representation. As described previously, considerable variation is observed in the size of the forelimb representations, while the general location and the boundary relations are consistent features of the map. Figure 1 shows examples of FLMI and HLMI representations and of the movements obtained in the boundary penetrations of the FLMI.

Forelimb primary motor cortex lesions and contingent electrical stimulation of the ventral tegmental nucleus

Figure 2 shows the number of responses over the force threshold per group (Fig. 2a), the number of responses under force threshold per group (Fig. 2b) and the mean peak force of the total number of responses per group (Fig. 2c) during three periods: the preoperative period (three days are shown), the postoperative period with intracranial stimulation (eight days) and the postoperative period without intracranial stimulation (four days). The number of responses over force threshold was the parameter used for statistical analysis, since this parameter clearly reflected the effects of the lesion and of the contingent VTN stimulation. Analysis of variance (ANOVA) indicated that the number of responses over force threshold of the FLMI group differed between days ($F_{[4,145]} = 12.12; P < 0.0001$). The multiple comparison test of Tukey showed that in the FLMI group the number of responses over the force threshold for each preoperative day differed significantly from each postoperative day ($P < 0.01$). Thus, the FLMI group showed a marked behavioral impairment in the performance of the task during the postoperative period. ANOVA indicated that the number of responses over the force threshold of the FLMI + VTN group differed between days ($F_{[4,135]} = 20.91; P < 0.0001$). The Tukey test showed that in the FLMI + VTN group the number of responses over the force threshold for each preoperative day differed significantly from the first ($P < 0.01$) and second ($P < 0.01$) postoperative days. Thus, recovery for the animals in the FLMI + VTN group was evident on the third day of postoperative period with intracranial stimulation. ANOVA indicated that the number of responses over the force threshold of the animals in the Sham group did not differ significantly from the levels of performance reached during the postoperative period with intracranial stimulation.

The number of responses under force threshold (Fig. 2b) showed an opposite tendency to the number of responses over force threshold. Thus, the animals

![Figure 1](image-url)
in the FLMI group showed a larger number of responses under force threshold compared to the control and FLMI + VTN groups. Therefore, the animals in the FLMI group had no problem in performing low force responses upon the lever. Finally, the peak force of the total number of responses (Fig. 2c) clearly reflected the effect of the lesion since the animals in the FLMI group showed a lower peak force than the animals in the control group. The animals in the FLMI + VTN group showed a clear tendency to perform responses with a larger peak force than the animals in the FLMI group, and during postoperative days 5–8 these animals increased the peak force above the control animals. This effect of increase in the peak force above the control animals returned to the control level once the VTN stimulation was withdrawn (days 9–12 of the postoperative period).

The histological analysis revealed that the lesions of the FLMI reflected the size and shape of the representations determined with the mapping technique. Thus, the lesions were adequately placed in the forelimb representation. In a few cases white matter damage was observed. This did not reflect any variability in the behavioral performance of these animals compared with those that had not received white matter damage. Moreover, in preliminary studies we determined that damage of the underlying white matter does not affect the behavioral consequences (i.e. measured in the present task) of lesions of the FLMI. Figure 3 shows an example of a lesion of the FLMI and Figure 7 shows surface reconstructions of the lesions. Two additional animals received lesions that included not only the FLMI but also the HLMI (i.e. most of the sensorimotor cortex was ablated). These animals were subjected to the same procedures as the animals in the FLMI + VTN group. In contrast with the results shown by the animals in the FLMI + VTN group, these two animals did not show any evidence of recovery during the postoperative period. Their behavioral performance resembled that shown by the animals in the FLMI group (not shown, but similar to FLMI group postoperative performance; Fig. 2).

Effect of hindlimb primary motor cortex lesions

Figure 4 shows examples of typical HLMI representations. Figure 5 shows the number of responses over the force threshold before and after bilateral lesions of the HLMI representations in animals from the FLMI + VTN group [(FLMI + VTN) + HLMI] and in normal animals that had been trained in the behavioral task (HLMI group). ANOVA indicated that the number of responses over the force threshold did not differ significantly between days. ANOVA also indicated that the number of responses over the force threshold for the (FLMI + VTN) + HLMI group differed significantly between days ($F_{(5,245)} = 40.5; P < 0.0001$). The Tukey test indicated that each of the preoperative days differed significantly from
each of the postoperative days ($P < 0.01$). Therefore, lesions of the HLMI do not impair the ability of the animals to perform the task, while this same lesion in animals recovered from FLMI lesions impairs their ability to perform the task.

The histological analysis revealed that the lesions of the HLMI reflected the size and shape of the representations determined previously with the mapping technique. Thus, the lesions were adequately placed in the HLMI representation, as shown in Fig. 6.

**Posterior primary motor cortex microstimulation mapping in animals with forelimb primary motor cortex lesions**

The microstimulation mapping of a group of animals from the FLMI + VTN group revealed a forelimb representation in an area caudolateral to the FLMI lesion (Fig. 7). Microstimulation mapping of a group of animals from the FLMI group showed very rare forelimb movements. The FLMI + VTN group ($\text{mean } \pm \text{S.E.M.} = 0.85 \pm 0.09 \text{ mm}^2$) and the FLMI group ($0.125 \pm 0.1 \text{ mm}^2$) differed significantly in the size of the area posterior to the FLMI lesion from which forelimb movements were evoked ($F_{0.15} = 16; P < 0.0001$). Figure 8 shows an example of an electrode penetration which evoked a forelimb movement in an area adjacent to the FLMI lesion, and an example in the same animal (FLMI + VTN group) of a penetration which evoked a hindlimb movement.

To further establish if a direct relation existed between the area from which forelimb movements were obtained in animals lesioned in the FLMI and behavioral performance in the task, we calculated a correlation between the size of this area and the behavioral performance for the animals in the FLMI and FLMI + VTN groups. As the behavioral performance parameter we used the number of responses over force threshold corresponding to the last day of behavioral performance. The data showed a significant positive correlation between both parameters ($r_{17} = 0.94; P < 0.0001$). Therefore, the size of the FLMI lesion (Fig. 7).
reorganized forelimb area and the behavioral abilities of the animals are directly related. It is important to note that this correlation was calculated considering each limb independently with its contralateral reorganized area. This indicates that the behavioral asymmetry in performance correlates with the asymmetry observed in the reorganization of the motor cortex and further indicates a relation between both parameters. In addition, this asymmetry did not correlate with the placement of the electrode in the VTN.

Current thresholds and number of penetrations for forelimb and hindlimb movements induced by microstimulation mapping

Table 1 shows the mean current thresholds to induce forelimb movements and hindlimb movements in the FLMI and FLMI + VTN groups before and after damage to the FLMI. Also shown are the total number of microelectrode penetrations which elicited these types of movements. The animals in the FLMI group showed a large decrease in the number of penetrations that elicited forelimb movements after a lesion of the FLMI, but not in the number of penetrations or mean current thresholds to elicit hindlimb movements. Also, the current thresholds of a few penetrations that elicited forelimb movements in the animals of the FLMI group after a lesion of the FLMI were very large (57.3 μA). On the contrary, the animals in the FLMI + VTN group showed a lower decrease than the FLMI group in the total number of penetrations that elicited forelimb movements after a lesion of the FLMI. The mean current threshold to elicit forelimb movements in the FLMI + VTN group (47.3 μA) was higher than the thresholds observed in the normal FLMI (34.9 μA), but significantly lower than those observed in the FLMI group after the lesion. Finally, the number of penetrations that elicited hindlimb movements in the animals of the FLMI + VTN group showed a slight decrease after the lesion, indicating that a possible reorganization occurred in the motor output of the FLMI. Moreover, this effect was not followed by a change in the mean threshold to elicit hindlimb movements after damage to the FLMI.

The area that showed a reorganization of movements evoked by microstimulation mapping in the FLMI + VTN group, prior to the FLMI lesion, was comprised of penetrations which elicited no movements or hindlimb movements (see Figs 1 and 7). Some of the penetrations which in this region showed low threshold hindlimb movements prior to the lesion also showed forelimb movements at higher thresholds (22% of the penetrations). After the FLMI lesion the animals in the FLMI + VTN group showed forelimb movements which were frequently (40% of the penetrations) followed by hindlimb movements at higher current levels. Thus, in the reorganized area of the FLMI + VTN group, penetrations which elicit both forelimb and hindlimb movements are more frequent than in the normal MI. It is important to indicate that a direct comparison between exact electrode penetrations before and after the lesions (i.e. comparing Figs 1 and 7) should not be considered accurate, since tissue distortion following the lesion should clearly vary the location of each penetration site. Thus, even though the exact absolute stereotaxic coordinate may not be useful, the general location may be comparable since the locations of other representations were not affected after the lesion. In the present study the filling of the lesions with hemostatic sponge may have helped in avoiding further distortion of the tissue.

DISCUSSION

The present study shows that bilateral lesions of the FLMI of the rat induce a deficit in the performance of a force task. Functional recovery from this behavioral deficit is induced by a contingent electrical

![Fig. 5. Mean number of responses (± S.E.M.) over a 10 g force threshold for three days before and four days after lesions of the HLMI. Surgery consisted of a bilateral ablation of the HLMI in a group of animals in which recovery had been induced after a lesion in the FLMI [(FLMI + VTN) + HLMI group] and in a group of normal trained animals (HLMI group).](image-url)
problem is the degree of current spread from the stimulating microelectrode. In the present study the current spread has been minimized by several factors. First, the microelectrodes used had small tips that minimized current spread. Second, the current level was never increased above 60 μA. Third, the electrode tips used in the present study did not increase the current density above the threshold to induce stimulation in the VTN. After recovery has been induced, microstimulation mapping of areas adjacent to the ablated FLMI reveals an area from where forelimb movements can be elicited. This reorganized zone is located in an area caudolateral to the FLMI lesions. The area coincides with the location from where hindlimb movements are evoked in normal animals and with primary somatosensory cortex. Furthermore, lesions of this area in recovered animals reinstate the behavioral deficit, while they have no effect in normal animals. The results lead to the conclusion that functional recovery after FLMI lesions is due to the reorganization of adjacent areas of the cortex.

**Primary motor cortex representations: technical considerations**

Microstimulation mapping techniques used to define the representations of the MI suffer from a number of problems that need to be considered in order to produce accurate results. One potential
Table 1. Mean current thresholds ± S.E.M. to induce forelimb and hindlimb movements in the primary motor cortex of animals from the forelimb primary motor cortex group and from the forelimb primary motor cortex + ventral tegmental nucleus group, before and after a bilateral lesion of the forelimb primary motor cortex

<table>
<thead>
<tr>
<th>Type of movement</th>
<th>Before lesion</th>
<th>After lesion</th>
<th>Before lesion</th>
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<tr>
<td>Forelimb</td>
<td>35.7 ± 1.5</td>
<td>57.3 ± 1.7*</td>
<td>34.9 ± 1.4</td>
<td>47.3 ± 2.5*+</td>
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<td>(19.7 ± 1.1)</td>
<td>(0.6 ± 0.2)</td>
<td>(18.0 ± 1.2)</td>
<td>(3.4 ± 0.6)</td>
</tr>
<tr>
<td>Hindlimb</td>
<td>37.4 ± 1.2</td>
<td>38.8 ± 1.9</td>
<td>36.8 ± 1.7</td>
<td>37.7 ± 1.8</td>
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<td></td>
<td>(9.9 ± 0.8)</td>
<td>(8.4 ± 0.6)</td>
<td>(8.7 ± 0.5)</td>
<td>(6.1 ± 1)</td>
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</table>

Figures in parentheses are the average number of penetrations which elicited these movements for cerebral hemisphere ± S.E.M. of five animals per group from which the current thresholds were calculated. *P < 0.01, significantly different from current thresholds before the lesion; †P < 0.01, significantly different from current thresholds of the FLMI group after the lesion.

damage in the stimulated tissue, as determined by the fact that no bubbles were observed when the electrode tip was immersed in saline and stimulated with the highest train and current used in the present study. A second potential problem when using microstimulation techniques is the possible indirect stimulation via polysynaptic pathways due to the use of stimulus trains. In the present study, a 100 ms stimulus train was used in every penetration site. Other groups have used either shorter stimulus trains (30–50 ms) or much larger ones (300 ms). In preliminary mapping experiments, we determined that changes in the type of movement were not observed using different train sizes (i.e. comparing 30 to 100 ms trains). The only difference observed was the duration of the evoked movement. This may also be the case for longer train durations, as others have found previously. A third problem may arise from the anesthetic state of the animal. In the present study we have done everything possible to standardize the anesthesia procedure by using supplement doses at regular intervals and monitoring tail pinch and eyelink reflexes regularly. Despite these and other possible potential problems inherent in the microstimulation procedure, it seems that they were adequately controlled in the present study, since the relative location, size and boundaries of the maps described here do not differ significantly from previously published studies using the same procedures.

Effect of forelimb primary motor cortex lesions upon forelimb response capacity

It has been well established that sensorimotor cortex lesions in rats lead to a variety of deficits measured in a number of behavioral tasks. Also, different metabolic mapping and morphological techniques have been used to show the involvement of the motor cortex in different motor behavioral tasks, and some have shown the involvement of specific subdivisions of the motor cortex in motor performance. Accordingly, studies of the behavioral consequences of damaging different subdivisions of the motor cortex have not been extensive. The importance of considering the specific subdivisions of a cortical area with respect to the consequent differential behavioral impairments observed after damaging
These subdivisions have been shown in the somatosensory cortex of monkeys. Recently, it has been shown that lesions of different subdivisions of the sensorimotor cortex of the rat defined cytoarchitectonically or of the FLMI defined electrophysiologically lead to specific behavioral impairments. One of the most enduring effects described after sensorimotor cortex ablation has been a permanent impairment in the forelimb force capacity of the rat. The results of the present study clearly indicate that the deficit observed in the former study was the consequence of damaging the FLMI.

The MI has been extensively involved in the coding of force. The rate of motor cortical cell discharge varies with the magnitude and direction of the force exerted. If the MI codes for force it should be expected that lesions of this area lead to a deficit in force capacity. This seems to be supported by the present study. It has also been suggested that lesions of the FLMI may lead to a deficit in response velocity. Therefore, in the present study the deficit observed is probably not only the consequence of an impairment in force capacity, but also in response velocity, and most likely in the capacity to produce rapid force responses. In agreement with this idea is the recent evidence which indicates that the motor cortex does not code for the total force performed by the subject but for the change in force. In our study the capacity to perform responses under the force threshold was not affected by the FLMI lesion. The animals with lesions had no problems pressing the lever, but showed a great impairment in their capacity to perform rapid force responses. Since the task was limited by a constant time period, the response frequency can be considered an index of the velocity of the responses. It seems that the FLMI lesion induced a deficit characterized by an impairment in the capacity to produce rapid force responses. Another possibility is that the force capacity of the animals was not affected by the lesions and only the capacity to respond upon the lever was affected, and that recovery of force capacity is only a consequence of the recovery of the ability to press upon the lever. It is difficult to differentiate between both possibilities with the procedures used in the present study. Thus, we hypothesize that the impairment and recovery described in the present study involved the capacity to produce rapid force responses, and we conclude that recovery was due to improved forelimb response capacity of the animals.

**Functional recovery after forelimb primary motor cortex lesions**

In the present study animals with FLMI lesions are recovered from their behavioral impairment in the force task by a contingent electrical stimulation in the VTN. It is also shown that once the stimulation is withdrawn the beneficial consequences of it persist and the animals perform the task adequately. This result is in accordance with recent data showing that the behavioral deficits induced after lesions of different cortical areas may be recovered by a contingent electrical stimulation of the VTN. The beneficial effects of the method have been attributed to the motivational and cue properties of the stimulation, and it has been determined that the effect of the stimulation upon recovery is specific to the motivational effects (i.e. rewarding) of the VTN. Thus, brain areas which produced aversive effects upon stimulation did not induce functional recovery in an avoidance task in a shuttle box after damage of the frontal cortex, and clearly impaired the animals' performance. Moreover, electrodes which are not adequately placed in the VTN and do not produce a rewarding effect do not induce functional recovery in the present task or in any other task tested. The motivational properties of the contingent electrical stimulation seem to be a crucial factor in producing the beneficial effects reported.

**Primary motor cortex reorganization after forelimb primary motor cortex lesions**

In the present study it is shown that, in animals in which functional recovery has been induced after FLMI lesions, forelimb movements can be evoked in an area located caudolaterally to the FLMI ablation. This effect was mainly observed in the animals in the FLMI + VTN group. The fact that the effect seems specific to recovered animals (i.e. animals in the FLMI + VTN group) indicates that it is a consequence of the functional recovery. The relation between the behavioral recovery and the reorganization of the MI is also suggested by the fact that a significant positive correlation was observed between these measures even when considering each limb independently, which suggests that the variability in performance is related to the variability in reorganization of the MI. Alternatively, it could be that the area that shows reorganization was not ablated by the prior FLMI lesion. However, this is unlikely since only the recovered animals showed a reorganized forelimb area. Also, after each lesion remapping of the adjacent cortical tissue assured that no FLMI was remaining. Even if a small piece of FLMI remained it should have been very small and of similar size in both groups. In this case, it is possible that the remaining area expanded extensively in recovered animals, while it remained at its initial size or decreased in size in non-recovered animals. The area where reorganization was observed in recovered animals is comprised, in normal animals, of a region from where hindlimb movements are elicited and a more lateral region from where forelimb movements can be elicited at high threshold currents (above 60 μA). This high threshold area corresponds to the somatosensory cortex of the rat. Thus, it is possible that in the recovered animals the observed reorganization is due to a decrease in the thresholds to induce forelimb movements in the more lateral region and
also to a change in the motor output of part of the hindlimb motor cortex. We have recently shown that recovery from lesions of the forelimb sensorimotor cortex of the rat lead to an increase in the number of cells with c-fos immunoreactivity in specific layers of the same area as the one observed to be reorganized in the present study. The correlation between both studies is clear and it seems that the induction of functional recovery is mediated through the occurrence of plastic changes in an area posterior to the FLMI. Thus, this plastic reorganization can be demonstrated at the level of gene expression and at the electrophysiological level. Similar reorganization processes have been described previously after cortical lesions and recent results also agree with ours in the sense that these processes do not seem to occur to a great extent in the absence of some type of post lesion training. We have discussed in the previous section that functional recovery as measured in the behavioral task was dependent upon the VTN stimulation producing a rewarding effect. However, it could be suggested that this stimulation, independently of its rewarding effect and behavioral contingency, even though not inducing behavioral recovery, would induce reorganization of the motor maps described in the present study. This issue was partly dealt with in a previous study, where analysis of c-fos expression of the area shown to reorganize in the present study in recovered animals was not affected by non-contingent electrical stimulation of the VTN.

**Hindlimb primary motor cortex lesions after recovery from forelimb primary motor cortex lesions**

The present results show that HLMI lesions in normal animals do not induce any impairment in the performance of a force task. However, this same lesion in animals functionally recovered from FLMI lesions reinstates the behavioral impairment of these animals in the force task. This result is further supported by the fact that lesions of the prefrontal cortex spared by the FLMI lesions do not reinstate the behavioral impairment and that unilateral HLMI lesions in recovered animals reinstate the behavioral impairment only in the forelimb contralateral to the HLMI lesion (unpublished observations). The HLMI lesions of the present report were performed based on the map constructed during the initial microstimulation mapping session, and as we have shown above, it includes the area reorganized (i.e. area in which forelimb movements are evoked in animals recovered after FLMI lesions) in recovered animals. These results and the fact that two cases with extensive lesions of the sensorimotor cortex showed no recovery, even though they were submitted to contingent electrical stimulation of the VTN, indicate that the reorganized area is essential for recovery to occur, since its ablation after recovery or its ablation before postoperative training blocks the recovery process.

Possible mechanisms involved in cortical reorganization

Recent reports indicate that the motor cortex has the capacity to functionally reorganize after different experimental manipulations. The reorganization consisting of the expansion of motor cortical representations has been attributed to a mechanism which involves cortical disinhibition. The organization of intracortical circuits forms a substrate for reorganization where the balance between the inhibitory and excitatory circuits plays a crucial role. In the motor cortex, the expression of horizontal axon collaterals of pyramidal cells are normally suppressed by inhibition. Thus, these horizontal connections form a substrate for reorganization to take place. The mechanism through which these processes occur may involve the long-term potentiation of the synaptic efficacy of these connections and/or processes of disinhibition where relief of inhibition can facilitate the strengthening of these connections. The mechanism through which the relief of inhibition occurs in the motor cortex is currently unclear. In the hippocampus, it has recently been shown that the release of inhibition can occur by leaving inhibitory interneurons dormant through a process which has been suggested to involve the retraction of excitatory axon collaterals upon inhibitory interneurons. Alternatively, a decrease in the number of GABA-stained neurons may account for the relief of inhibition, as has been reported recently. Finally, we have also recently shown that a long-term depression in the release of GABA in another system (cerebellum) depends on the action of specific growth factors. These possible mechanisms of disinhibition should also be tested further in the cerebral cortex. The relief of inhibition by these or other mechanisms would then account for the increased expression of the horizontal excitatory connections and allow the activation of motor cortical output cells from a different motor cortex representation. We have recently shown that these excitatory connections expressed after disinhibition are mediated mainly through non-N-methyl-D-aspartate glutamate receptors. Thus, the reorganization of the balance between GABAergic and glutamatergic connections within the cortex is a likely mechanism to explain these processes. In our study, these types of reorganizational processes may be facilitated by the beneficial effects of VTN stimulation upon the performance of the force task. In the recovered animals the remaining forelimb motor cortex output would be activated from other cortical areas through intracortical horizontal connections. However, alternative mechanisms possibly involving additional processes cannot be discarded at present.

**CONCLUSIONS**

The bilateral ablation of the FLMI of the rat induces a deficit in the performance of a force task.
Functional recovery from this behavioral deficit is induced by a contingent electrical stimulation of the VTN. After recovery has been induced, microstimulation mapping of areas adjacent to the ablated FLMI reveals an area from where forelimb movements can be elicited. This reorganized area is located caudolateral to the FLMI lesions. Ablation of this area in recovered animals reinstates the behavioral deficit, while it has no effect in normal animals. The results lead to the conclusion that functional recovery after FLMI lesions is due to the reorganization of adjacent areas of the cortex.

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REFERENCES


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