

# Peripheral nerve injury in developing rats reorganizes representation pattern in motor cortex

(cerebral cortical development/motor system/motor control/neural plasticity)

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**ABSTRACT** We investigated the effect of neonatal nerve lesions on cerebral motor cortex organization by comparing the cortical motor representation of normal adult rats with adult rats that had one forelimb removed on the day of birth. Mapping of cerebral neocortex with electrical stimulation revealed an altered relationship between the motor cortex and the remaining muscles. Whereas distal forelimb movements are normally elicited at the lowest threshold in the motor cortex forelimb area, the same stimuli activated shoulder and trunk muscles in experimental animals. In addition, an expanded cortical representation of intact body parts was present and there was an absence of a distinct portion of motor cortex. These data demonstrate that representation patterns in motor cortex can be altered by peripheral nerve injury during development.

Genetic mechanisms expressed during development are believed to be important determinants of the functional organization of cortical areas. It is also clear that representation patterns can be modified, at least in sensory cortex. Anatomical and electrophysiological investigations of somatic sensory and visual cortices have shown that injury to peripheral receptors or even selective forms of experience in developing mammals result in expanded representations of intact parts (1–4) and concomitant changes in cortical connectivity patterns (5–9). Although developmentally induced modifications in representation patterns, intrinsic organization, or efferent connections of motor cortex could profoundly affect movement execution and control, the effect of peripheral nerve injury on these aspects of motor organization is largely unknown. In the present study we examined the effect of neonatal peripheral nerve injury on the somatic representation in the primary motor cortex (MI) in rats.

Among all cerebral neocortical areas, MI appears to be most closely related to the control of muscles. MI is necessary for the independent use of muscles in skilled voluntary movements, participates in movement initiation, and is involved in the elaboration of the complex repertoire of movements observed in mammals (10, 11). Generally, MI is defined in a variety of mammals as the cortical area in which movements can be evoked at the lowest levels of electrical stimulation (12). Intracortical microstimulation (ICMS) mapping reveals a topographically ordered pattern of representation in MI; focal sites in cortex are related to one or a few closely related muscles (13). Although electrical stimulation produces artificial patterns of neural activation, it appears to reflect the functional relationship of small regions of MI with muscles, since neurons at a cortical site discharge during movements of the same muscles that are activated by ICMS at that same site (14). In rats MI is largely comprised of a cytoarchitecturally distinct frontal agranular cortical field

that contains forelimb, head, and trunk representations. A portion of the immediately adjacent granular somatic sensory cortex (SI) is also included in MI because electrical stimulation in this region evokes movements at thresholds comparable to those in the agranular part of MI (15–17). This amalgamation of SI and MI, called the overlap zone, contains discrete motor and somatic sensory representations of the distal forelimb and hindlimb (15–19). The clearly defined organizational plan in MI suggests that rats would serve as a useful model to study the influences of environment or the consequences of injury on cortical development, organization, and function.

## METHODS

The organization of MI was studied in 12 normal adult rats and in 14 adult rats that had the right forelimb amputated on the day of birth. In rat pups less than 24 hr old, the right forelimb was removed surgically at the shoulder joint during hypothermic anesthesia. The wound was sutured closed and the pups were returned to the dam. Mapping was carried out at 2–4 months of age. Experimental animals were allowed to recover for 8–24 weeks, whereupon MI and the adjacent cortex were mapped with ICMS or extracellular recording techniques. The acute mapping procedures have been described (16). In brief, animals were anesthetized with ketamine hydrochloride (i.p., 100 mg/kg of body weight) and mounted in a stereotaxic frame. Microelectrodes (Pt/Ir, glass-insulated 0.5- to 2-M $\Omega$  impedance at 1 kHz) were lowered to a depth of  $1.8 \pm 0.1$  mm below the pial surface. Current trains (30-msec duration, consisting of 200- $\mu$ sec-long monophasic, cathodal pulses delivered at 300 Hz) of 5–60  $\mu$ A were routinely passed through the electrode tip while we examined the body visually and by touch to determine which body parts moved or muscles contracted. The resolution of the electrode penetrations was between 0.1 and 0.5 mm. We first defined the MI forelimb representation in normal animals and then mapped a similar cortical location in experimental animals. At each site of electrode penetration, the body part that was activated at the lowest current intensity was identified by a combination of visual inspection and muscle palpation; other body parts activated at higher currents up to 60  $\mu$ A were noted. At the termination of each experiment, lesions were made at selected sites by passing a dc current of 10  $\mu$ A for 10 sec. The animals were perfused, and the brains were removed and processed for histological localization of electrode penetration sites. These data were reconstructed as somatotopic maps; the area of a body part representation in this map was defined as the region where movement of that body part was evoked at the lowest current intensity. Map borders were defined as the midpoint between penetration

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Abbreviations: MI, primary motor cortex; SI, primary somatic sensory cortex; ICMS, intracortical microstimulation.  
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evoking movements of two distinct body parts. However, boundaries were drawn through points where movement of two body parts was evoked at similarly low thresholds (within  $\pm 5 \mu\text{A}$ ) at the same site.

To identify changes in the distribution pattern of corticospinal neurons resulting from the neonatal nerve injury, the corticospinal tract of four normal and four experimental adult rats was injected at the C4–5 level with 0.5  $\mu\text{l}$  of 30% horseradish peroxidase (HRP) using a 10- $\mu\text{l}$  Hamilton syringe. After 2 days, rats were killed and perfused with a mixed aldehyde solution. Tangential sections of the flattened cerebral cortex were reacted for HRP according to standard techniques (20). Cell distributions were charted on outline drawings of sections using a drawing tube attached to a microscope.

## RESULTS

The low-threshold cortical maps generated from one normal and one experimental rat are shown in Fig. 1 A and B. In intact rats the MI forelimb area is typically separated into distinct caudal and rostral regions (21). The larger caudal region comprises contiguous areas in the agranular part of MI and in the granular SI–MI overlap zone. The rostral part of the forelimb region is located exclusively in agranular cortex. In the normal rat, threshold intracortical stimulation within the MI forelimb area only activates muscles of the distal

forelimb, producing movements of the contralateral digits, wrist, or elbow. Muscles of proximal body parts are activated at some distal forelimb sites, but only at higher current intensities. We never observed a clear case of shoulder muscle activation at the lowest current intensity, although at one site the neck and shoulder muscles appeared to have the same threshold. We assume that the higher threshold for proximal movements represents either less effective synaptic connections or a greater number of relays between cortical sites and the proximal muscles. The possibility that the higher currents required to activate proximal limb muscles signify current spread is ruled out by the consistent finding that nowhere in MI of normal rats can a separate low threshold shoulder zone be found.

In experimental animals, we defined the MI forelimb region as that area in which intracortical stimulation activated muscles about the contralateral shoulder or ipsilateral forelimb. This region was smaller than that of normal rats [normal,  $4.0 \pm 0.52 \text{ mm}^2$  (mean  $\pm$  SD); experimental,  $2.26 \pm 0.77 \text{ mm}^2$ ;  $P < 0.05$ ]. Forelimb movements were never evoked by stimulation in the adjacent granular cortex. Thus, the smaller forelimb region of experimental animals resulted in part from the absence of the forelimb portion of MI located in granular cortex. We used electrophysiological recording methods to identify somatic sensory receptive fields in the area where the SI–MI overlap zone is normally found. Unlike the distal cutaneous receptive fields normally present in the

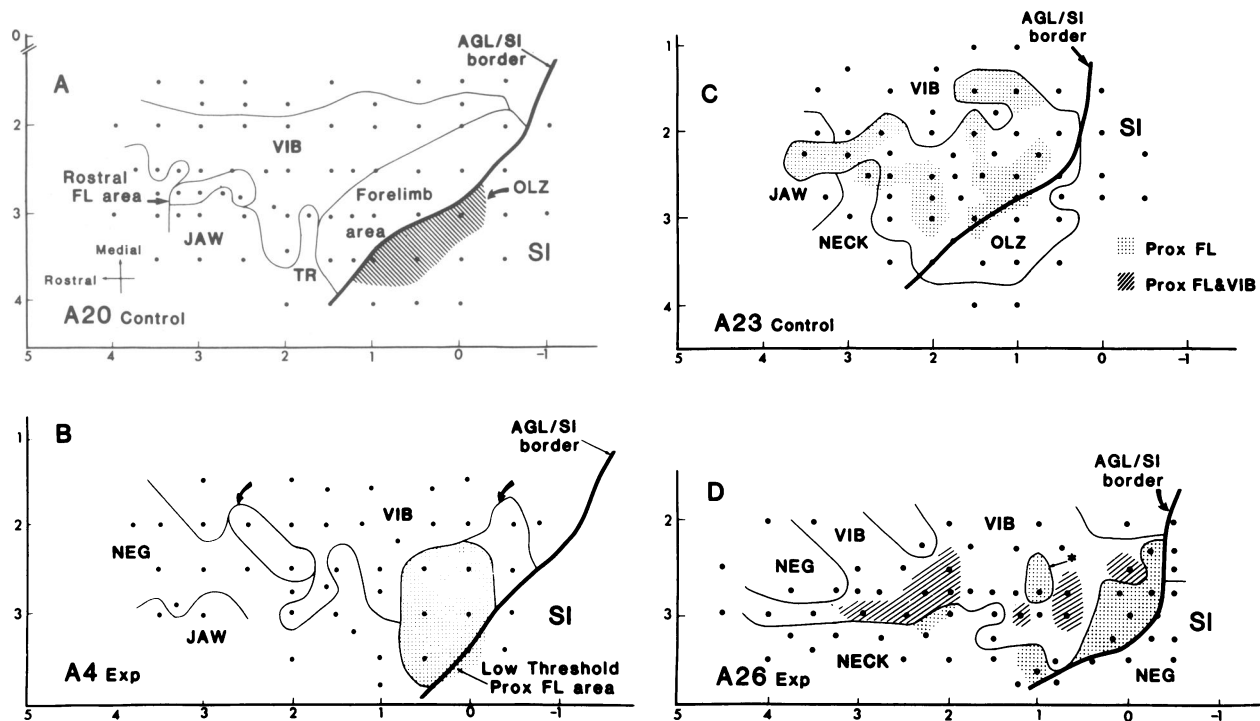


FIG. 1. Microstimulation maps of forelimb region of MI in two control (A and C) and two experimental (B and D) rats. In the four panels, each dot marks a stimulation site and thin solid lines mark boundaries between somatotopic subdivisions of MI. The border between the frontal agranular and granular SI cortex is marked by a heavy line. Numbers on axes are in millimeters, with bregma as the zero point. (A) Pattern of representation at the lowest current intensities showing the location of the forelimb representation in MI. The forelimb is divided into two regions; the caudal section ("forelimb area") is contained largely in frontal agranular cortex (AGL) but also extends into the adjacent granular SI cortex (OLZ, diagonal hatching). A second forelimb region ("rostral FL area") is also contained within frontal agranular cortex. Stimulation at any site within the total extent of the forelimb region produces distal forelimb movements. (B) Lowest threshold map from an experimental rat. Movements of contralateral shoulder musculature (stippled) and of the ipsilateral forelimb (enclosed areas marked by curved arrows) are now evoked at the lowest currents. No forelimb movements are evoked within SI. In normal rats, ipsilateral sites coincide with some contralateral distal forelimb sites (J.P.D. and B. L. Schlaggar, unpublished observations). (C) Coincidence of distal forelimb and shoulder sites in a normal rat. Shoulder movements (stippled area) are found at higher thresholds at some sites within the distal forelimb area. One exception is seen at the border with the neck area. (D) The abnormal coincidence of vibrissa and shoulder points in an experimental rat. Shoulder movements were evoked at lowest threshold within the stippled area. The diagonally hatched areas mark the regions where vibrissa movements were elicited at the lowest threshold, but at higher current intensities shoulder movements were evoked at these same sites. Note that one shoulder site is surrounded totally by vibrissa sites, which is never seen in normal MI. Prox, proximal; FL, forelimb; NEG, negative; VIB, MI vibrissae area; TR, trunk.

overlap zone (15, 17), cells in this region in experimental rats ( $n = 3$ ) were activated by cutaneous inputs from the trunk and shoulder regions. This cutaneous representation formed part of an enlarged SI territory for these body parts.

By contrast, stimulation at most sites in agranular MI cortex of experimental rats activated muscles at low thresholds. Uncharacteristic of the normally high threshold for proximal forelimb movements in normal rats, we found many sites in amputated animals in which shoulder movements were evoked at the lowest threshold. These thresholds were often comparable to those necessary for activation of more distal forelimb muscles in intact animals and were lower than shoulder movement thresholds in normal rats [control ( $n = 28$ ),  $50.07 \pm 12.5 \mu\text{A}$ ; experimental ( $n = 78$ ),  $37.99 \pm 14.46 \mu\text{A}$ ;  $P \leq 0.0005$ ]. This finding suggests that neonatal forelimb nerve injury increased the strength of the connection between MI and the proximal muscles.

A significant feature of the organization of MI in the normal rat is the distinct segregation of body parts. Thus, although stimulation at a single site will often activate muscles of, for example, the distal and proximal forelimb (Fig. 1C), it is rare to find the forelimb and vibrissa, as another example, represented at the same site. The only exception to this appears to be at borders between representations for two body parts. However, in experimental animals (Fig. 1D) the shoulder and vibrissa frequently shared common sites in the forelimb region. Vibrissa movements most commonly had the lower threshold at these sites. This intermingling of representations indicates that the vibrissa representation expanded into the presumptive forelimb zone, while the relationship of MI with proximal forelimb muscles was preserved within this same area. A possible mechanism of the expanded MI vibrissa representation could be a shift of corticospinal projections from the cervical cord to brainstem targets that control vibrissa movements. However, injections of HRP into the cervical spinal cord showed a similar distribution of corticospinal neurons in normal and experimental rats. Additionally, MI cytoarchitecture examined in thionin-stained sections lacked any obvious change that might suggest anatomical reorganization.

In contrast to the integrity of the general aspects of MI cytoarchitecture and corticospinal projections, forelimb removal at birth resulted in marked anatomical changes within the spinal cord and the primary afferent pathway. Sections through the spinal cord showed a near total absence of large, darkly staining cells where distal forelimb motor neuron pools are normally found; these pools appeared to be normal on the side of the intact limb. There was an obvious shrinkage of the ipsilateral dorsal columns and a loss of cells in the ipsilateral cuneate nucleus (data not shown).

## DISCUSSION

Our results indicate that peripheral nerve injury during development influences the pattern of movement representation in motor cortex. We conclude that forelimb removal in neonatal rats results in three forms of change in MI representation patterns identified by mapping with intracortical stimulation. First, MI in these rats has more effective or stronger connections with proximal muscles than in normal rats. Second, cortically adjacent representations expand into the presumptive forelimb territory. Third, in the absence of normal target structures, the region of the cutaneous part of the MI forelimb area, the MI-SI overlap zone, fails to develop. However, cutaneous input to this cortical zone is preserved through the expansion of the SI trunk and shoulder representations.

The basis for MI reorganization is uncertain, though several different mechanisms might be involved. The threshold change for proximal body parts could represent a

strengthening of already existing synaptic connections between MI and alpha motor neurons for the shoulder musculature. Alternatively, new connections between MI neurons and their target cells or between MI target cells (such as interneurons) and their targets may have been formed. The "expansion" of other body parts into the presumptive forelimb region may also result from changes in functional connectivity between MI and its targets. However, another likely explanation for the expanded vibrissa representation is that forelimb removal at birth preserves widespread connections that are transiently formed during normal development and then lost (8, 22-25). Thus, individual cells in MI may project subcortically to vibrissa and to proximal and distal forelimb motor control structures early in development and then be restricted largely to one target as the organism matures. This hypothesis requires testing. Our anatomical finding that the corticospinal cell distribution was not altered by neonatal forelimb amputation may suggest that some cells in the mixed vibrissa-shoulder area retain weak or ineffective projections to the spinal cord.

The absence of the overlap zone in MI may require a different explanation than retention of developing pathways. Functionally, this area responds to cutaneous inputs much like other regions of SI but also contributes directly to motor output, as demonstrated by microstimulation-induced movement. It is possible that depriving this area of its normal peripheral somesthetic input disrupts the formation of usual efferent connections. The large agranular part of MI in the rat is not responsive to cutaneous stimulation (15-17, 26), so this mechanism could not cause the observed alterations in MI output organization, but aberrations in other inputs could induce change. Alternatively, damage to motor nerves could form a basis for motor system reorganization.

It is important to recognize that the changes in MI representation we observed may reflect subcortical reorganization. One could devise various patterns of connective reorganization in the brainstem that would produce the changes in MI maps we have described, without requiring any change in cortical connections. Determination of reorganization sites will require mapping of subcortical structures and, perhaps, further anatomical studies.

Both lesions of peripheral receptors and sensory deprivation are known to modify patterns of cortical sensory representation (1-4). These changes result from reorganization of input pathways at the cortical level and, in many cases, at the subcortical levels as well (27, 28). Our results indicate that motor cortical representation pattern is affected by peripheral nerve damage. It appears that a general principle of the modifiability of cerebral cortex, whether it is related to sensation or motor output, is emerging. An implication of these results is that MI and perhaps the entire cortex may be undergoing continual reorganization. In general, cortical modifiability may depend on the availability of target cells, inputs from other brain regions, and inputs from peripheral end organs. An example of this suggestion can be found in a clinical study in which the somatic sensory evoked potentials of human amputees changed over time (29). Another example would be in cases of cerebral hemorrhagic infarcts when humans, who often are initially paralyzed, recover varying degrees of motor function. The mechanisms for such recovery are uncertain, but they could involve changes in the somatotopy of MI.

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