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Dynamic organization of primary motor cortex output to target muscles in adult rats
I. Long-term patterns of reorganization following motor or mixed peripheral nerve lesions

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Summary. These experiments examined the ability of the adult motor cortex to reorganize its relationship with somatic musculature following nerve lesions. Cortical motor output organization was assessed by mapping the areal extent of movements evoked by intracortical electrical stimulation in anesthetized rats. Output patterns of the motor cortex of normal rats were compared with those of adult rats that had received either a forelimb amputation or a facial motor nerve transection 1 week to 4 months earlier. In both experimental conditions the extent of some representations increased. Stimulation thresholds required to elicit movements in expanded representations were at or below normal levels. After forelimb amputation, the area from which shoulder movements could be evoked at low thresholds enlarged. Sectioning of the branches of the facial nerve that innervate the vibrissa musculature enlarged the motor cortex forelimb and eye/eyelid output areas; these enlargements appeared to occupy the former vibrissa area. These results indicate that the amount of cortex controlling a group of muscles and the strength of the relationship between a cortical locus with its target muscles is modified by nerve lesions in adult mammals. They also show that motor nerve lesions are sufficient to produce this change and that the changes can appear as early as 7 days following a peripheral nerve injury.

Key words: Motor cortex – Motor representation – Peripheral nerve injury – Neural plasticity – Motor control

Introduction

Electrical stimulation mapping of movements and muscle activity has been used extensively to study the relationship between the primary motor cortex (MI) and various muscle groups and to describe the overall motor representation pattern in the cerebral cortex. From the earliest investigations of the relationship between MI and its target muscles there has been a suggestion that this relationship is flexible. Sherrington and his colleagues noted that strong electrical stimulation could alter the type of movement evoked from a cortical site (Brown and Sherrington 1912) and that different movements may be evoked from the same site when tested at different times (Leyton and Sherrington 1917). Further, Lashley (1923) suggested that a particular MI organizational pattern was a "temporary physiological condition". In contrast, Craggs and Rushton (1976) mapped MI of single animals repeatedly and concluded that the MI output organization is basically stable over time.

It has been demonstrated repeatedly that cortical circuits, at their input stages, are modified when normal sensory processes are disrupted. Reorganization of sensory representation patterns has been identified by receptive field mapping in various areas, including the visual (Hubel and Wiesel 1965), somatic sensory (Kalaska and Pomeranz 1979; Kaas et al. 1983) and olfactory (Wilson et al. 1987) cortex. For the visual system, there appears to be a critical period in the development of the animals during which manipulation of normal visual input modifies ocular dominance and orientation tuning properties of visual cortical neurons (Hubel and Wiesel 1970; Movshon and Van Sluyters 1981). In contrast, manipulations of somesthetic afferent input in either neonatal or adult mammals, particularly removal of a sensory surface by amputation or sensory nerve transection, produces a marked reorganization of the map of cutaneous inputs to the primary somatic sensory cortex (see Wall and Cusick 1986 for references).

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Recently, we found that cortical *output* relationships can be modified after peripheral manipulations similar to those that affect sensory input organization (Donoghue and Sanes 1987, 1988). These studies demonstrated that MI develops novel motor representation patterns subsequent to peripheral nerve lesions that occur early in development. We used intracortical electrical stimulation mapping techniques to evaluate the motor representation pattern in MI of normal adult rats and in adult rats that had one forelimb amputated on the first postnatal day. The neonatal forelimb amputation resulted in the development of larger than normal MI areas related to control of the shoulder and vibrissa musculature, lower stimulation thresholds needed to activate the shoulder muscles, and a more common pairing of shoulder and vibrissa movements at single sites than was normally observed. These experiments indicated that the amount of MI involved in controlling a muscle group can be enlarged and the strength of the connection between MI and somatic musculature can be increased after nerve injury early in development.

The ability for the relationships between cortical outputs and their targets to reorganize in adult mammals would suggest a flexible role for cortical participation in motor control throughout life. Therefore, we tested the hypothesis that the potential for reorganization of the cortical motor representations persists into adulthood. Intracortical electrical stimulation mapping techniques were used to compare the output organization of MI in normal adult rats with animals that had a forelimb amputated when they were mature adults. Since this manipulation involved damage to both sensory and motor nerves, we further tested the effect of a lesion restricted to a motor nerve on MI output organization. For these experiments, the facial nerve branches that supply the mystacial vibrissa were sectioned because these branches are free of sensory fibers (Semba and Egger, 1986) and because the mystacial vibrissa have a relatively large representation in MI cortex. Portions of these results have been presented previously (Suner et al. 1986; Sanes et al. 1988).

Material and methods

Animals

Experiments were performed on adult albino rats (Sprague Dawley) of both sexes weighing 150 to 225 g. Electrical stimulation mapping of the region of MI cortex (Donoghue and Wise 1982; Wise and Donoghue 1986) was performed in rats that had previously received either a forelimb amputation (n = 6) or a transection of the facial nerve branches that innervate the mystacial vibrissa (n = 10), and in 22 age-matched unoperated normal rats.

General surgical procedures

Aseptic techniques were used for survival surgery. Rats were anesthetized initially with ketamine HCl (100 mg/kg) with supplemental doses (i.p. or i.m.) as needed to suppress the hindlimb withdrawal reflex.

Forelimb amputation

After surgical exposure, the nerves of the brachial plexus and the adjacent vessels were ligated using 6-0 suture (ethylon), and the vessels, nearby nerves, and muscles were cut at the level of the shoulder joint. The right forelimb was removed, and the skin and underlying tissue were closed with 6-0 suture. The rats were kept on a heating pad until they were ambulatory.

Facial nerve section

The motor innervation of the mystacial vibrissa was interrupted unilaterally by cutting the buccal and marginal mandibular branches of the right facial nerve near their exit from the parotid gland. The proximal ends of the nerve branches were ligated with silk suture to reduce the likelihood of peripheral sprouting, and the distal ends of the nerve were evulsed. The wound was closed with suture, and the animal was returned to the home cage after recovery from anesthesia. All of these animals lacked vibrissa movements on the side of the lesion at the time of mapping. Inspection of nerve stumps at perfusion showed no evidence of sprouting, but we could not rule out that collateral branches had grown into intact, more proximal branches of the facial nerve.

Electrical stimulation mapping

Movements evoked by intracortical electrical stimulation at sites in the frontal agranular cortex were mapped after a 1-week to 5-month recovery period. Mapping procedures were similar to those that have been described previously (Donoghue and Sanes 1988). Animals were anesthetized with ketamine, as described above, and mounted in a Kopf stereotaxic frame. Rectal temperature was monitored and stabilized at 37–38°C with a hot water heating pad. The left frontal cerebral neocortex was exposed by making a craniotomy that extended from about 2 mm posterior and 5 mm anterior to bregma and from 0.5 to 5 mm lateral from bregma. The dura remained intact and its surface was covered with 1.5 to 2% agar dissolved in 0.9% saline. Mapping sessions lasted for up to 7 h.

Glass-insulated PtIr electrodes (1–2 MΩ impedance, measured at 1 KHz) were used for stimulation. An electrode was lowered vertically to 1.8 mm below the cortical surface, and then, while stimulating, it was adjusted up or down 200 μm to find the depth for the lowest threshold of electrical stimulation to evoke movement at that penetration site. The electrode depth was verified by direct measurements in histological sections with marking lesions that had been placed at selected sites at the completion of mapping experiments. Electrode penetrations were spaced regularly to cover the entire MI with a grid of penetrations separated by 500 μm, then intermediate points were spaced as close as 100 μm apart, but more typically 250 μm, to yield higher resolution maps. Alterations in the coordinate grid were sometimes necessary to avoid electrode penetrations through surface blood vessels.

For intracortical electrical stimulation, current trains (30 ms duration, 300 Hz, 200 μs long, monophasic cathodal pulses) of 5 to 60 μA were passed through the electrode at 1.2 s intervals. Identical procedures were used for mapping MI of normal and

experimental animals. Two investigators were needed to make the threshold determination. At each penetration site, all body parts that were activated at $\leqslant 60\ \mu A$ were identified by a combination of visual inspection and muscle palpation by one investigator. Then, the current intensity was decreased by the second investigator, and the threshold for movement of each body part was noted. The first investigator observed the movements without detailed knowledge of the actual current intensity. The second investigator changed the current level, using the descending method of limits, with $\sim 2\ \mu A$ decrements and with $\sim 20\%$ catch trials, until the movement examiner reported a near 50% occurrence of movement with repetitive stimulation. The second examiner recorded this current as the "threshold" for that particular movement. Sites were classified as negative if no movement was elicited at a stimulation current of 60 μA. If three negative sites were recorded in succession within the presumed region of MI, the electrode was returned to a region between two previously positive sites and within 100 to 250 μm of one of them. If this site was also negative, the experiment was terminated and all sites after the last recorded positive site were not considered for analysis. Electrolytic marking lesions were made by passing 10 μA DC for 10 s at the end of the mapping session.

Two sets of control rats were mapped. The first set ($n = 13$) was used for comparison in the forelimb amputation study. In these animals, eye/eyelid movements which typically occur at sites located medial to the vibrissa zone were not examined and all sites in this region were considered to be negative. The second set of control rats ($n = 9$) was paired with the rats with a facial nerve transection. In these animals, we also mapped the medial frontal cortex and carefully searched for eye/eyelid movements using a surgical microscope. We commonly noted eye or retrobulbar swelling 2 to 4 h after the mapping began. This swelling may have prevented observation of the typically small eye/eyelid movements that we studied. Consequently, the most medial parts of frontal cortex were mapped early in the session, although some medical sites were examined throughout the mapping session.

Histology and electrode localization

At the conclusion of the experiment, the animal received an overdose of sodium pentobarbital and was perfused intracardially with 0.9% saline followed by 10% formol saline. Sections of the brain and spinal cord were cut in the frontal plane at 50 μm on a freezing microtome and stained with thionin using routine procedures. The depth and areal location of lesion sites was verified from these histological sections.

Map construction

Maps of the penetration grid were constructed on graph paper during the experiment by marking the AP and ML position of each electrode penetration according to the micromanipulator coordinates that measured the distance (in millimeters) from bregma. The accuracy of the absolute and relative location of each site was also verified through direct comparison of the penetration marks created by the electrode shaft as it passed through the agar that covered the cortical surface, blood vessel location and from the reference point at Bregma. Histological reconstructions were used in some cases to identify the areal location of penetrations. From this comparison we estimate that the two dimensional maps contain 100–300 μm distortions in the actual separation of the most rostral-lateral penetrations as a result of the curvature of the hemisphere in this region. That is, penetrations in the rostral-lateral part of the hemisphere (where the jaw, lip and tongue are represented) were deeper than at other sites, and hence farther apart than shown, because the electrode did not traverse the layers radially. Since all penetration maps were made in the same manner and the area of interest did not include this part of the map, we feel that this distortion is negligible for the present experiments. For subsequent illustration and analysis a series of maps was created from this penetration grid map. *Low threshold maps* were created by enclosing the cortical area from which movement of each body part was evoked at the lowest current intensity at individual sites. Map borders were defined as the midpoint between penetrations evoking movements of two separate body parts (e.g. vibrissa and forelimb) at the lowest threshold. However, boundaries were drawn through points where movements of two body parts were evoked at similarly low thresholds (within $\pm 1\ \mu A$ of the lowest threshold movement at that site). If two data points were separated by more than 1 mm, or if a point had no boundary, a boundary was drawn 250 μm from the data point. This boundary was required for areal measurements. The arbitrary value of 250 μm was selected as one that minimized the inclusion of cortical area where data was missing and when data points were separated by more than 1 mm, without placing the point at a border. Typically this rule was applied at the medial edge of the hemisphere where the marked curvature prevented accurate stimulation of layer V. Since mapping strategies were similar in all experiments, the criteria for map construction were applied uniformly across all cases, and missing data points were infrequent in central regions of the map, there would appear to be no strong bias toward under- or over-representation of areas either in control or experimental animals. Maps of the *total* representation of a particular body part were constructed by plotting all the points from which movements of this part were evoked at $\leqslant 60\ \mu A$. The size, shape, and location of maps, movement thresholds (in μA), and types of movements evoked at stimulation sites in experimental rats were compared to normal rats. Sizes of MI body output zones were measured using a digitizing tablet and area measurement software.

Results

MI representation pattern in normal rats

Figure 1 summarizes the general topographic organization of the rat motor cortex. The representation pattern shown is based on recent studies where movements evoked by intracortical electrical stimulation were mapped in rats (Hall and Lindholm 1974; Donoghue and Wise 1982; Neafsey and Sievert 1982; Sanderson et al. 1984; Neafsey et al. 1986; Donoghue and Sanes 1988). In normal rats the territory from which forelimb movements are evoked at threshold currents is located laterally in the motor cortex and is bounded medially by a relatively large area from which vibrissa movements can be evoked. The eye/eyelid movement area is located along the medial aspect of the vibrissa area. Although the size and exact location of these areas show considerable individual variation, their general location and boundary relationships are relatively consistent features of the map. Figure 2 shows maps obtained from two normal rats in which the topographic relationships of the forelimb, vibrissa, and eye/eyelid areas are indicated. The forelimb area of MI comprises the region from which movements

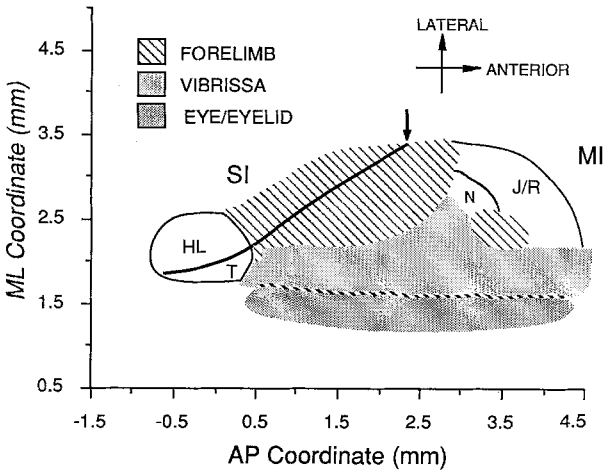

Fig. 1. The typical output representation pattern in MI. This schematic map illustrates the size, the location and the topographic relationships of areas from which movements are evoked by low levels of electrical stimulation in the rat motor cortex. This and all subsequent maps illustrate a dorsal view of the left hemisphere; coordinate axes are in mm, with (0, 0) being the location of bregma on the skull. Note that the eye/eyelid, vibrissa and forelimb areas roughly form strips that are elongated in the rostral-caudal dimension. Forelimb and vibrissa areas occupy the largest part of MI. The solid line (arrow) indicates the border between MI and the primary somatic sensory cortex (SI); the dashed line marks the border of the vibrissa and eye/eyelid. HL, hindlimb area; T, trunk area; N, neck area; J/R, jaw/tongue/rhinarium area

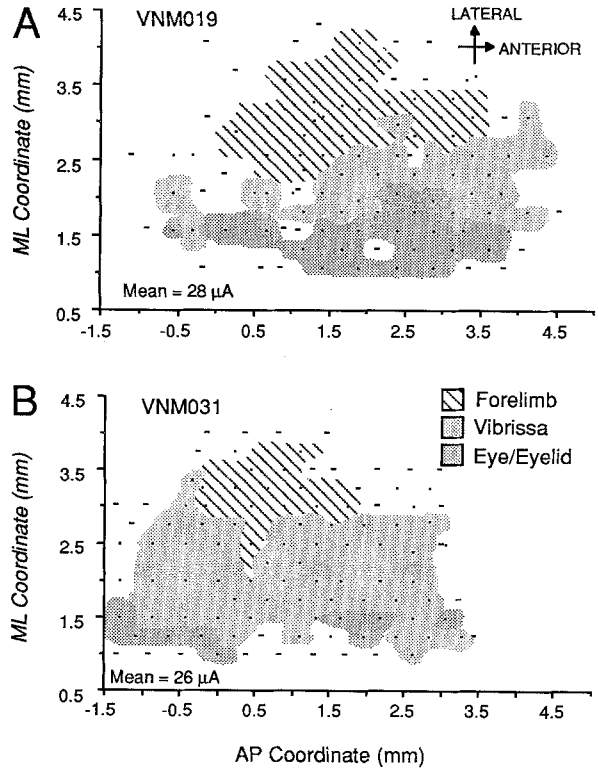

Fig. 2A,B. Low threshold electrical stimulation maps of forelimb, vibrissa and eye/eyelid representations. Each dot marks an electrode penetration site from which a movement was evoked at $\leqslant 60$ μA. Each dash marks a site from which no movement was evoked at 60 μA. The different shadings enclose areas from which forelimb, vibrissa, or eye/eyelid movements were evoked (see key). Note that most of the forelimb representation is separated from the eye/eyelid area by the vibrissa area. The mean current for activating low threshold movements throughout MI for a rat is given in the lower left hand corner of each map. The map illustrated in **A** is representative of most animals. The map shown in **B** is an atypical variation in which the forelimb representation is substantially smaller than usually found in normal animals and the vibrissa representation is large and extends far lateral from its typical boundary. This format is used for all subsequent figures that show MI maps from experimental or normal rats

about the shoulder, elbow, wrist, or metacarpophalangeal joints are evoked at the lowest current intensity. Most commonly elbow, wrist, or digit movements are evoked at threshold stimulation. In this study, these movements are collectively defined as *distal* forelimb movements, whereas shoulder movements, which may be elicited by activity in arm, shoulder girdle, or neck musculature, are defined as *proximal* movements (Donoghue and Sanes 1988). In normal rats, proximal forelimb movements were almost always evoked in conjunction with other movements, and within the forelimb area, the distal forelimb movement nearly always had a lower threshold than the proximal forelimb movement. No attempt was made to segregate classes of vibrissa movements; threshold stimulation could evoke movements of a group or, occasionally, a single contralateral vibrissa. Bilateral and exclusively contralateral sites were also observed. The eye/eyelid zone was defined as the region from which movements of the eyeball or contraction of the periorbital musculature were evoked (Hall and Lindholm 1974). The movements observed included low amplitude contralateral or bilateral horizontal saccades, twitching of a portion of one eyelid, or bulbar retraction. These responses could occur in isolation or, more commonly, together in various combinations, even at threshold currents, and they were time-locked to the stimulus.

MI organization following forelimb amputation

General organization. Movements were evoked by electrical stimulation throughout MI of rats that had one forelimb amputated after any of the survival times tested (1 to 5 months). A total of 404 penetrations was made in the region of MI from six amputated rats. Movements were evoked at currents of $\leqslant 60$ μA at 297 (73.5%) of these sites. A total of 966 penetrations was made in the 13 control rats that were used for this experimental group, and stimulation at a

similar percentage of these sites (73.7% or 712) elicited movements. In the amputated rats, stimulation evoked movement within the region of the frontal agranular cortex that would normally correspond to the forelimb representation (Fig. 3). Some negative sites were observed within this region, but negative sites were also encountered within the normal MI at about the same frequency (Fig. 2A). Low threshold shoulder or vibrissa movements were evoked at currents $\leqslant 60$ μA at many sites that would have been expected to fall within the distal forelimb area in normal animals. The form of the shoulder output zone mapped in different cases showed considerable variability. This representation sometimes consisted of small, widely separated foci (Fig. 3A) or of a single larger contiguous area (Fig. 3B). These shoulder sites always fell within the general region from which the forelimb movements would be evoked in normal rats. Vibrissa sites bounded most of the shoulder representation.

Size of representation areas. The shoulder area increased in size by an average of 221% after forelimb amputation (Fig. 4), although there was considerable individual variability in its size (normal area, 1.06 ± 0.19 mm^2 [mean $\pm$ SEM], range, 0.25–2.32 mm^2; experimental, 2.25 ± 0.33 mm^2, range, 1.47–3.28 mm^2, $p \leqslant 0.005$, t test). The shoulder area of all six experimental animals was larger than the mean size of the shoulder area of the normal rats. Another indication of the enlargement in the shoulder area was the increased occurrence of shoulder movements in experimental animals. Low threshold shoulder sites accounted for only 0.7% (7 of 712) of all positive sites in normal rats: this increased to 15.2% (45 of 297) after the forelimb amputation. Since shoulder sites are typically masked within the low threshold distal forelimb region of normal rats, that is, shoulder movements are found at higher stimulating currents at distal forelimb sites, the overall percentage of shoulder movements evoked at currents up to 60 μA was also considered. Shoulder movements were evoked at 10.1% of all sites from which movements were evoked in normal rats, whereas they were found at 30.6% of all positive sites in experimental rats.

By contrast, there was no significant increase in the size of the vibrissa representation in animals with a forelimb amputation (Fig. 4). The vibrissa area was 3.01 ± 0.46 mm^2 (range, 1.28–6.1 mm^2) in normal and 3.11 ± 0.55 mm^2 (range, 1.3–5.25 mm^2) in experimental rats. However, the vibrissa area of four of the six experimental animals was larger than the mean size of the normal vibrissa area. Additionally, two measures suggested that there may be redistribution of the vibrissa representation that we were not able to detect by simple areal measurements. First, vibrissa movements were evoked at a greater

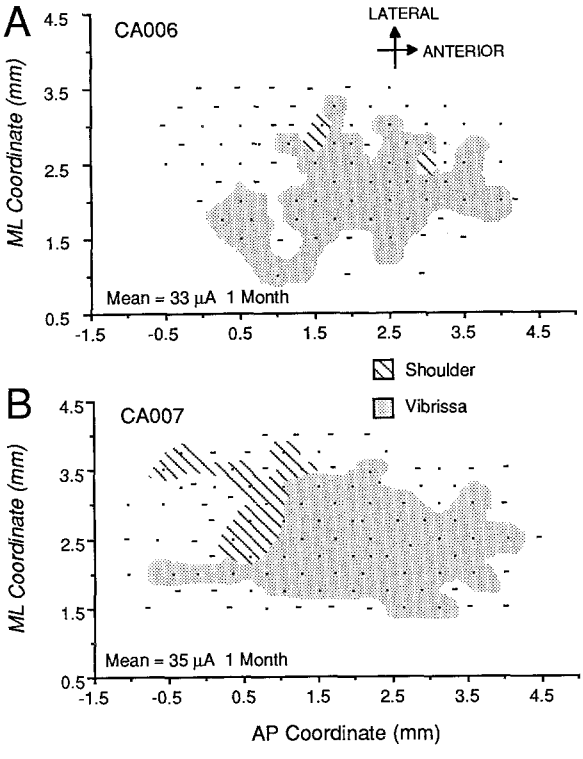

Fig. 3A,B. MI maps obtained after forelimb amputation. Amputations were performed 1 month prior to stimulation mapping. **A** Shoulder movements were evoked in two discontinuous and discrete areas in MI. **B** Shoulder movements were evoked from a relatively large and contiguous cortical area. In these maps, eye/eyelid movements were not differentiated from the negative points

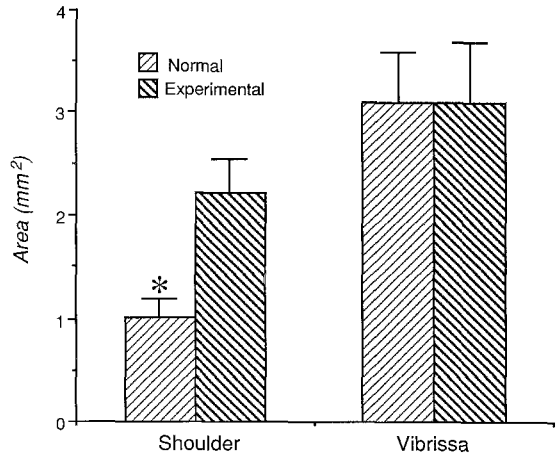

Fig. 4. Area of MI output zones in normal and experimental rats. The size of the shoulder region increased in animals with an experimental forelimb amputation. The vibrissa area was unchanged (*$P \leqslant 0.05$)

percentage of sites in the experimental (41.1%) than in the normal (28%) rats even though the mapping densities were similar in the two groups. Second, a greater percentage of the vibrissa sites were found more laterally in MI of experimental rats (Fig. 5).

Stimulation thresholds. The overall mean threshold to evoke movements in MI was slightly, but non-significantly, higher in rats that had received a forelimb amputation (normal, 27.7 ± 1.2 μA; experimental, 31.2 ± 1.3 μA). In absolute terms, shoulder thresholds of the experimental rats were unchanged (normal, 37.9 ± 3.1 μA, experimental, 37.1 ± 1.1 μA). Because the mean overall threshold current was higher in experimental rats, the shoulder threshold for each group (control, experimental) was also calculated as a per cent of the mean threshold for that group (Fig. 6). This comparison showed that shoulder thresholds after forelimb amputation were about 50% higher than the overall mean to evoke any movement in normal rats. By contrast, the mean shoulder movement threshold in experimental rats was only 20% greater than the overall mean threshold for experimental rats; this value was significantly lower than in the normal ($p \leqslant 0.01$). Slightly higher absolute current intensities were required to evoke vibrissa movements in experimental rats than in normal rats (control, 26.3 ± 1.1 μA; experimental, 29.4 ± 1.3 μA, $p \leqslant 0.05$). This contributed to the overall higher current to evoke movement in experimental rats. However, vibrissa movements were evoked at the same relative stimulation intensity when compared to their respective group's mean threshold (Fig. 6).

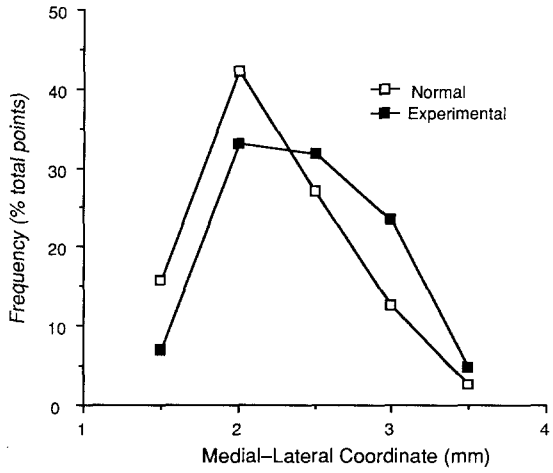

Fig. 5. Frequency distribution of sites from which vibrissa movements were evoked. This graph illustrates the medial-lateral distribution of vibrissa sites in normal and experimental animals. All vibrissa points were grouped into 0.5 mm wide bins within the medial-lateral dimension, irrespective of anterior-posterior coordinate. The experimental animals had a greater proportion of lateral points from which vibrissa movements were evoked

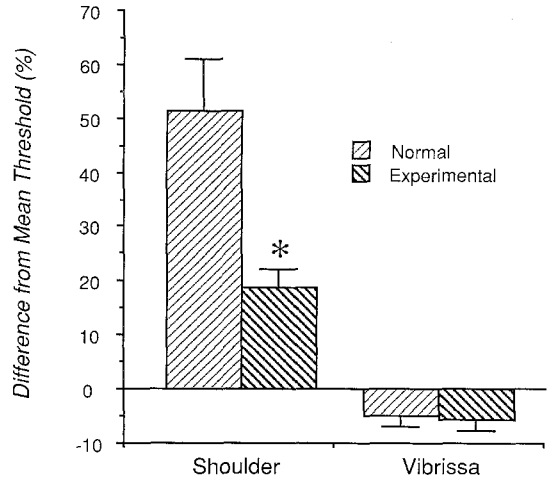

Fig. 6. Relative threshold for evoking shoulder movements decreases after forelimb amputation. In normal rats, the current required to evoke shoulder movements is about 50% higher than the overall mean threshold (horizontal line at 0% on the graph) to evoke any movement in controls. Following forelimb amputation, there was a significant decrease ($p \leqslant 0.05$) in the intensity to evoke shoulder movement compared to the overall threshold for this experimental group. By contrast, there was no difference in the relative thresholds to evoke vibrissa movements in the two groups of rats

MI organization following facial nerve section

General organization. The results of the forelimb amputation experiments indicated that the relationship between MI output networks and target muscles reorganized following limb amputation in adult rats. Since amputation destroys both sensory and motor nerves, we next tested the hypothesis that motor nerve lesions alone are sufficient to induce reorganization of MI output patterns.

Facial nerve lesions produced a number of changes in MI organization, and in general these changes were qualitatively more noticeable than those seen after forelimb amputation. Electrical stimulation effects were examined at 635 sites in 10 rats that had received a facial nerve transection 1 week to 4 months prior to mapping. Movements were observed at 76.9% of these sites. In nine unoperated normal rats, 667 penetrations were made in the region of MI, and movements were evoked at 83% of these sites. Three representative maps obtained at different times after facial nerve transection are illustrated in Fig. 7. As found following forelimb amputation, it was possible to evoke movements throughout MI after the facial nerve

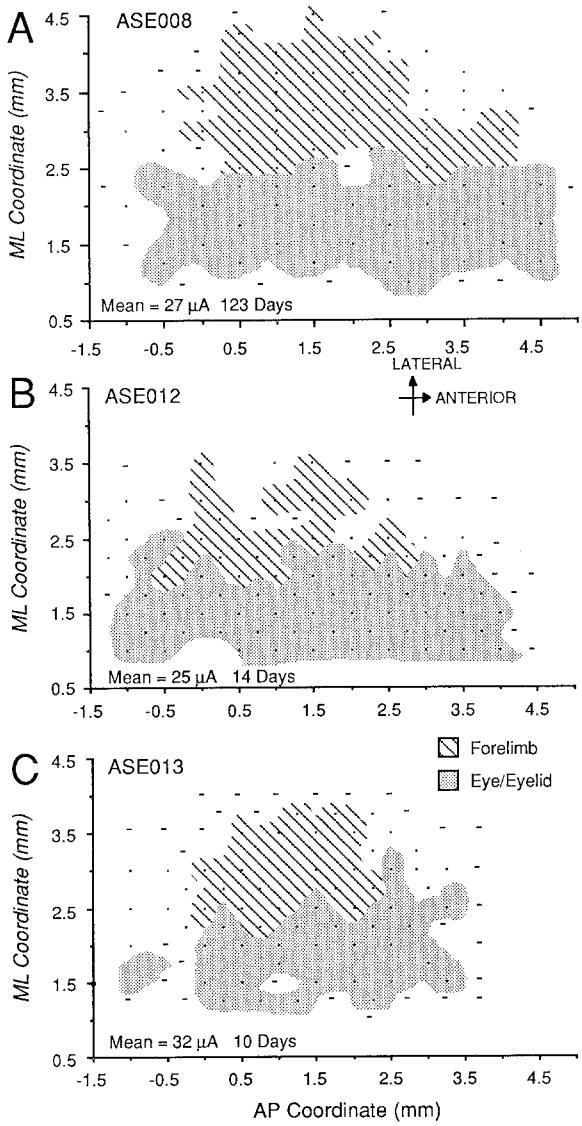

Fig. 7A-C. Motor output maps from rats that had the facial nerve transected unilaterally as adults. These maps can be compared with those of normal animals shown in Fig. 2. The animals survived for different amounts of time after the nerve section, as noted in days on each plot. There was never a large silent area in the region that normally contains the contralateral vibrissa representation (between 1.5 to 2.5 mm lateral). The forelimb, and especially the eye/eyelid representation, appear to have expanded medially and laterally into the former vibrissa representation. Also note the long, contiguous border between the eye/eyelid and forelimb areas, not present in normal rats

transection. The most notable alterations in the MI representation pattern were the larger size and the more lateral extent of the eye/eyelid area, suggesting that the former vibrissa territory was occupied primarily by an enlarged eye/eyelid area. A modest medial expansion of the forelimb output zone also appeared to be present. Although some areas were identified from which ipsilateral vibrissa movements were evoked, these movements were not commonly the threshold movement evoked within the region normally containing the vibrissa representation. Another unusual feature of the MI maps of these rats was the long common boundary of the eye/eyelid and forelimb areas. Normally these areas have, at most, a short border apposition. The types of eye/eyelid or forelimb movements evoked in experimental rats were typical of those found throughout these representations in normal rats. Similar maps were observed at all survival times.

Size of eye/eyelid and forelimb representations. Both the forelimb and eye/eyelid area were significantly larger following facial nerve transection (Fig. 8). The area of the eye/eyelid representation of the experimental rats (2.39 ± 0.47 mm^2, range, 0.51–3.35 mm^2) increased to 217% above normal (5.18 ± 0.51 mm^2, range, 3.4–6.65 mm^2, $p \leqslant 0.005$). The size of the eye/eyelid representation of individual experimental rats exceeded that of all normal animals. The forelimb representation increased in size an average of 49.5%. The forelimb area in normal rats was 2.48 ± 0.26 mm^2 (range, 1.7–3.36 mm^2), whereas in experimental rats it was 3.71 ± 0.46 mm^2 (range, 1.96–5.35 mm^2, $p \leqslant 0.05$). The forelimb region of only one of the experimental rats was smaller than the mean size of the normal forelimb area. The total area occupied by the eye/eyelid and forelimb representations in the experimental rats (mean = 8.89 mm^2) was larger than the combined area of the forelimb, vibrissa, and eye/eyelid representations of the normal rats

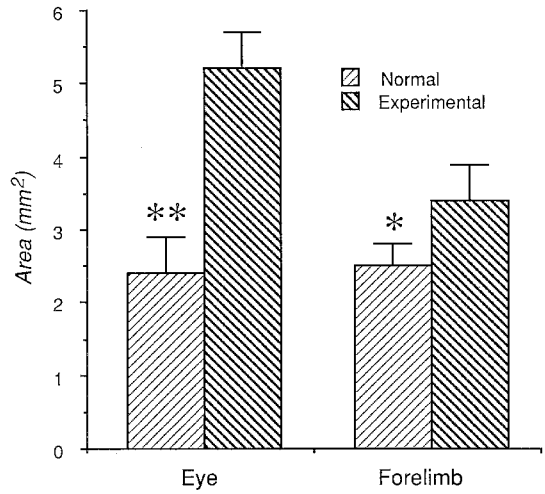

Fig. 8. Area of the eye/eyelid and forelimb zones in normal and facial nerve transected rats. There was a significant increase in the size of both eye/eyelid and the forelimb output regions. * $p \leqslant 0.05$, ** $p \leqslant 0.005$

($7.97\,mm^2$), suggesting that the forelimb and eye/eyelid regions probably accounted for all of the expansion into the former MI vibrissa representation.

Another indication of the expansion of the eye/eyelid and forelimb output zones was their considerable overlap only in the experimental animals (Fig. 9). The eye/eyelid and forelimb MI representations are largely separated in normal animals.

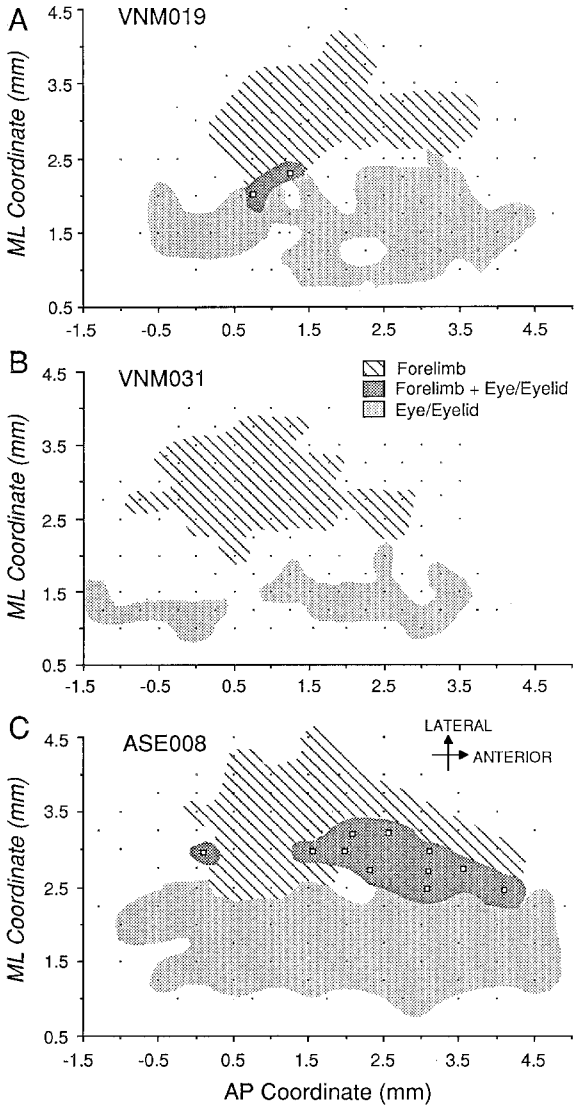

Fig. 9A–C. Overlap of the eye/eyelid and forelimb regions in MI of normal and facial nerve transected rats. In these maps, all sites from which eye/eyelid or forelimb movements were evoked at 60 μA are enclosed by shading, with areas of overlap indicated by the darkest shading (see key). **A,B** Maps from normal rats, showing that there is little overlap of the representations even at the highest current intensities measured (see Fig. 2 for the low threshold maps of these animals). **C** Map from an experimental rat showing the large area of overlap after section of the facial nerve (see Fig. 6A for the low threshold map of the same animal)

Both movements were evoked at only 8 of the 513 (1.56%) positive sites, and they were never evoked at the same low threshold current at any individual site. In the experimental rats, eye/eyelid and forelimb movements were both evoked from many sites (78 of 527 sites, 14.8%) in the central parts of frontal agranular cortex from which contralateral vibrissa movements are evoked normally. Thus, the occurrence of forelimb and eye/eyelid movements at the same site was encountered nearly 10 times as often in MI following facial nerve transection.

Stimulation thresholds. After facial nerve lesions, thresholds to evoke movements from MI were either not different from or were lower than thresholds in normal rats (Fig. 10). The overall mean threshold to evoke movements after facial nerve lesion was slightly, but significantly, lower than in normal rats (normal, 27.5 ± 0.5 μA, experimental, 25.5 ± 0.5 μA, $p \leqslant 0.005$). Eye/eyelid movement thresholds were also significantly lower in experimental rats (25.5 ± 0.07 μA vs. 30.3 ± 1.1 μA, $p \leqslant 0.0001$). In normal rats, eye/eyelid movements were typically evoked at currents that were about 10% higher than the overall mean, whereas in experimental rats the overall and eye/eyelid mean thresholds were indistinguishable. Stimulation currents to evoke forelimb movements were similar in normal and experimental rats (Fig. 10).

Thresholds to evoke either eye/eyelid or forelimb movements around the region of the former vibrissa representation were examined by plotting the mean

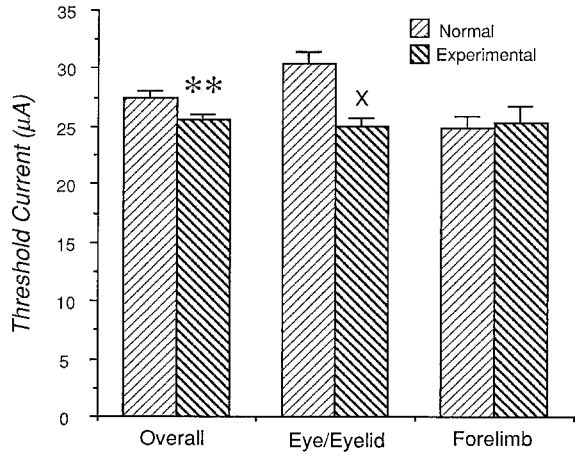

Fig. 10. Electrical stimulation thresholds for all movements and for eye/eyelid and forelimb movements in normal and facial nerve transected rats. After motor nerve lesion, the overall and eye/eyelid mean thresholds were decreased. Thresholds were unchanged for activating the forelimb musculature. ** $p \leqslant 0.005$, $^{x}p \leqslant 0.0001$

current required to evoke these movements with respect to distance from the midline, irrespective of the anterior-posterior coordinate (Fig. 11). We collapsed the data in this manner on the assumption that the three major MI representations approximate elongated anterior-posterior strips (Figs. 1, 2 and 7). The current required to evoke eye/eyelid movements in experimental animals is similar to or below the current required in normal rats at all medial-lateral positions (Fig. 11A). Forelimb movements are evoked at low thresholds throughout the extent of the MI forelimb representation (Fig. 11B). The lateral expansion of the eye/eyelid output zone can also be illustrated by plotting the relative frequency for evoking eye/eyelid movements with respect to distance from the midline (Fig. 12). This shows that a greater percentage of the eye/eyelid

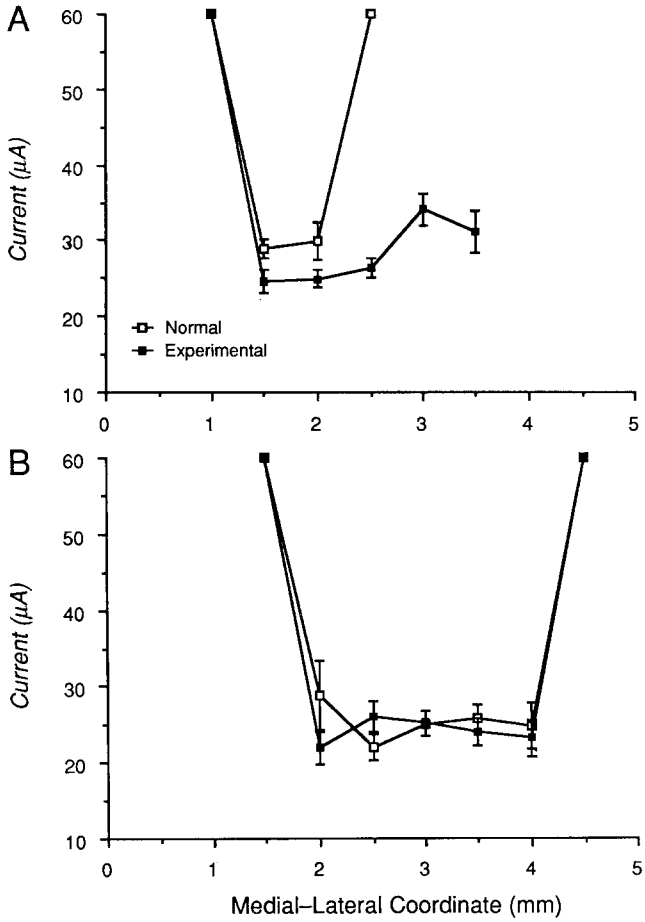

Fig. 11A,B. Medial-lateral distribution of currents required to evoke eye/eyelid (**A**) and forelimb (**B**) movements in normal and facial nerve transected rats. Threshold values were grouped into 0.5 mm wide bins according to their distance from the midline and irrespective of the anterior-posterior coordinate. Thresholds to evoke movement after the nerve lesion are as low as or lower than controls throughout the medial-lateral extent of MI

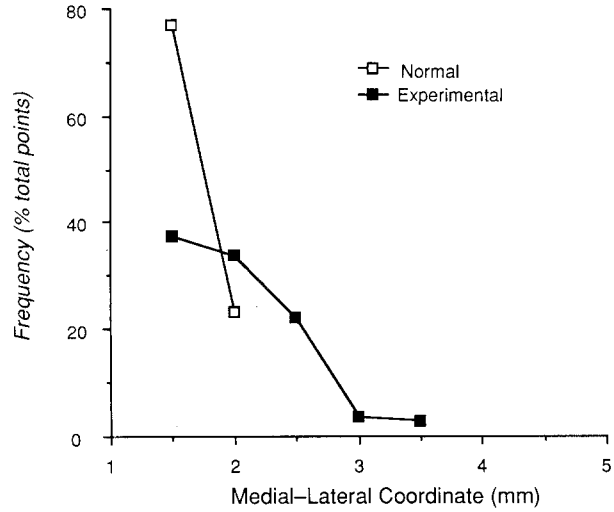

Fig. 12. Frequency of eye/eyelid points as a function of distance from the midline. The number of stimulation sites (expressed as a percent of total) were grouped into 0.5 mm wide bins based on their distance lateral from the midline. Note that eye/eyelid points were encountered more laterally in experimental rats

sites was found at a more lateral position in rats with the facial nerve transection.

Discussion

The results of these electrical stimulation mapping studies indicate that MI output relationships with target muscles reorganize in response to nerve injury in adult animals. Maps obtained after either of the two manipulations show an enlargement of adjacent representations so that affected areas of MI associate with new muscle groups. Thresholds obtained in MI after nerve injury are consistent with the conclusion that a strong connectional relationship is established between the reorganized part of MI and target muscles. For the forelimb amputation the clearest change was the enlargement of the shoulder representation. Threshold adjustments were less clear-cut, since a decrease in shoulder threshold was only evident when measured on a relative basis. Facial nerve transection resulted in an increase in eye/eyelid and forelimb areas and a marked decrease in eye/eyelid movement thresholds. The results also show that a motor nerve injury is a sufficient stimulus to restructure MI output. These types of changes indicate that the amount of cortex dedicated to the control of a particular muscle group and the physiological strength of the pathway between a group of MI neurons and a set of muscles is adjustable in adult mammals. The diversity of effects observed in the two experiments suggests that the form of the reorganization may depend on the type of injury and the muscle groups involved.

Use of intracortical electrical stimulation to identify reorganization of MI representation patterns

Intracortical or surface electrical stimulation has been used to assess the normal relationship between MI and somatic musculature for more than a century, and a plethora of experiments has been done with various modifications of this methodology. The present study has relied on the technique of delivering a train of intracortical electrical stimuli with low current intensities (Stoney et al. 1968; Asanuma and Rosén 1973). It is believed that this technique excites directly "a constant number of corticofugal units" (Andersen et al. 1975) and produces repetitive neuronal discharges that result in summation of excitatory synaptic potentials in motor neurons (and likely in many other cell groups) that in turn evoke muscle activity. Thus, intracortical electrical stimulation activates muscles from a cortical site via a set of cortical output neurons through various subcortical pathways to the muscles. In addition, cortical stimulation activities intracortical pathways. The fact that MI stimulation effects remain, but are different, after pyramidotomy (Woolsey et al. 1972; Asanuma et al. 1981; Mitz and Humphrey 1986) demonstrates that this stimulation also accesses muscles through pathways other than the corticospinal tract.

One way that representations could show an apparent enlargement is if nerve transections altered the excitability of cells in adjacent representations so that they could be activated directly by distant stimulation. We have assumed that MI neurons in both the normal and experimental rats have similar biophysical properties so that a similar number of MI neurons are directly activated by electrical stimulation. The similarity of thresholds in the two groups supports the conclusion that *overall* cortical excitability is not changed by peripheral nerve lesions, although there may be local excitability differences. The currents required to evoke movements after our manipulation would not appear to be sufficient to spread directly to adjacent areas, though the area within which neurons are directly affected by electrical stimulation is difficult to calculate and estimates of this area vary. Such estimates are complicated by a generally anisotropic medium produced by the presence of cells and axons of varied diameters and by the action of intracortical circuits which may block or enhance effects at a distance (Asanuma and Rosén 1973). It has been estimated that a 5 μA current spreads from 60 μm (Stoney et al. 1968) to more than 200 μm (Jankowska et al. 1975). Andersen et al. (1975) have estimated that a 60 μA current would directly affect about 11,000 pyramidal cells in a 900 μm diameter cylinder, while a 20 μA curent would affect about 3,800 pyramidal cells in a 500 μm cylinder. Electrical stimulation, especially with stimulus trains, does not activate cells only by direct electrical activation but also produces synaptic activation of elements over a large cortical area (Stoney et al. 1968; Jankowska et al. 1975) and in a variety of subcortical structures. Such a wide pattern of activation might preclude the identification of a topographic arrangement or assessment of reorganization using this method. However, small displacements of the stimulating electrode appear to engage different cortical circuits since electrode displacements as small as 100 μm can produce entirely different movements and electromyographic responses (McGuinness et al. 1980; Strick and Preston 1982). Thus, we conclude that increases in the size of cortical output zones combined with low movement thresholds reflect the activation of restructured neuronal circuits in MI and not the direct electrical activation of neurons in adjacent MI output zones distant from the stimulating electrode.

Another explanation for the apparent increase in the size of motor representations in MI is that extant, higher threshold output circuits are simply unmasked when the pathway to a lower threshold representation is eliminated by nerve section. As exemplified by the shoulder representation in normal animals, additional movements can be revealed when suprathreshold currents are employed. Current thresholds to evoke these movements would be generally higher in experimental animals. However, the threshold data do not support this interpretation, since at least some expanded representations had thresholds that were as low as or lower than those found in normal rats. In the forelimb-amputated animals, the shoulder musculature was activated at lower than normal currents, but only when measured on a relative basis. A more convincing demonstration of a lowering of shoulder threshold would require chronic monitoring of thresholds in individual animals. The results of the facial nerve transection experiments more definitively support a lowering of movement thresholds. Group thresholds were actually lower after facial nerve transection and equally low thresholds were found across the medial-lateral extent of MI, which would include an expanded zone. From these data we conclude that the reorganized cortex established as strong a connection to its new target muscles as had cells in the original output zone. The strength of this new relationship between MI and somatic musculature is established within days and persists for long periods following nerve injury. By contrast, vibrissa

thresholds increased after forelimb amputation, though they were not markedly outside of the range of currents that are effective in evoking movements from motor cortex. The different directions of threshold changes for different muscle groups may reflect a variability in the form of reorganization that depends on the type of lesion, the specific muscles affected, and events that occur in the post-lesion period.

Changes in the size of motor representations

Following peripheral nerve injury, the size of some remaining representations increased. For animals with a forelimb amputation, only the shoulder representation enlarged. Although the vibrissa area did not increase in size, vibrissa movements could be evoked more laterally than in normal rats, and a greater proportion of vibrissa relative to the total number of sites was encountered in MI of amputated rats. These findings would suggest that the vibrissa region also enlarged after forelimb amputation. Expansion of the vibrissa area might be missed because of large individual variations in representation pattern. After disruption of the motor innervation of the vibrissa, both the MI eye/eyelid and forelimb regions were larger. The enlargements of these output zones occupied the MI region occupied normally by the vibrissa representation. The increase in the size of the eye/eyelid area was apparent even with subjective comparisons of individual maps: large eye/eyelid areas were never seen in normal rats. Areal measurements confirmed that the eye/eyelid region more than doubled in size. The expansion of the eye/eyelid area into the former vibrissa cortex is further supported by the finding that eye/eyelid movements were obtained at lateral coordinates where vibrissa movements are typically found and eye/eyelid movements are never found normally. The expansion of the forelimb and eye/eyelid areas into the normal vibrissa region of MI is also supported by the observation that the forelimb and eye/eyelid representations share a long, contiguous border in the animals with facial nerve section but not in the normal rats. The adjacency of these regions is further reflected in the more frequent occurrence of both eye/eyelid and forelimb movements at single sites in the rats with facial nerve section. This could mean that the two output zones overlap at single sites or that both movements are activated when stimulating along the border between the two areas. Because both movements could be evoked up to 500 μm from the apparent borders of these two areas (see Fig. 9C), it is possible that control of these quite widely separated muscle groups overlaps within a small region of MI as a consequence of the facial nerve lesion.

Site and mechanism of representation changes

The results of these mapping data show that nerve lesions alter the group of muscles that are activated by stimulation at focal cortical regions. Intracortical electrical stimulation presumably activates cortical axons and neurons, and movement thresholds are a measure of the connection strength between the cortical site stimulated and the muscles that are activated. This connectional strength may depend on such variables as the number and location of synapses and the effectiveness of individual contacts at any of a variety of locations. These sorts of changes could occur anywhere along the pathway from MI to the motor neurons: among the intracortical connections of MI neurons, at the subcortical targets of MI output neurons or at synaptic interactions on the motor neuron directly (Endo et al 1973; Fetz et al. 1976; Shinoda et al 1979, 1986). The present data cannot be used to distinguish among cortical or subcortical sites where this new pathway is established. The fact that reorganization following peripheral nerve section seems to occur between output zones that are adjacent in MI, but which may be widely separated subcortically, could suggest that it occurs within the cortex. None of these sites can be eliminated from consideration since it appears that forms of motor reorganization are possible even in the spinal cord (Nelson et al. 1979).

When it was demonstrated that neonatal nerve lesions altered MI organization (Donoghue and Sanes 1987, 1988), we proposed that anatomical changes could account for shifts in MI representations. In developing animals, shifts in MI output patterns might be evident from the preservation of "exuberant" connections of cortical neurons following nerve lesions (Innocenti and Frost 1979; Rhoades and Dellacroce 1980). This is not a possible source of MI reorganization in adult animals, since these projections are lost relatively early in postnatal development (Easter et al 1985). In the following paper (Donoghue et al. 1989) we show, and discuss, that reorganization occurs over a span of time that favors synaptic strength changes rather than axonal growth as the mechanism by which the relationship between MI and muscles reorganize.

Comparison with nerve lesions in neonatal animals

We have previously investigated the MI output pattern that develops after forelimb amputation in neonatal rats (Donoghue and Sanes 1987; 1988).

The MI output organization of these animals, when mapped as adults, is strikingly similar to that of animals that were given similar lesions when they were mature. However, in the neonatally amputated animals, both the vibrissa and shoulder areas showed marked increases in size. In addition, there was a marked reduction in threshold currents needed to evoke shoulder movements. The somewhat larger magnitude of these changes in neonatally injured animals could reflect a greater potential for modification of developing systems (Kennard 1942; Movshon and Van Sluyters 1981). Nevertheless, the extensive alterations in MI output patterns of animals injured as adults suggests that the ability for reorganization of the relationship between MI and target muscles persists into adulthood. The similarities in MI representations between neonatal and adult animals after nerve lesions could indicate that similar processes account for the pattern of organization produced by changes in MI inputs or outputs early or later in life.

Adequacy of motor nerve lesion to reorganize MI

Using facial nerve transections, we have demonstrated that selective motor nerve lesions, which effectively de-efferent portions of MI, are a sufficient stimulus to reorganize MI. However, we do not know which consequence of the nerve lesion triggers and then mediates the change. It is possible that the motor nerve lesion leads to the production of a trophic factor that results in reorganization. This hypothesis is supported by findings of Nelson et al. (1979), which indicate that changes in synaptic strength can be produced in parts of the spinal cord that are disconnected from the site of nerve injury. Another possibility is that transection of the motor nerve modifies afferent discharge patterns, which could signal reorganization. There are tonically active sensory fibers within the infraorbital nerve (Renehan et al. 1986; Jacquin et al. 1986). Since the facial nerve lesion disrupts the motor tone of the vibrissa, there is likely to be an alteration of the normal sensory inputs transmitted along the infraorbital nerve. At present we cannot distinguish between these hypotheses, but both seem to be testable.

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