Rapid reorganization of adult rat motor cortex somatic representation patterns after motor nerve injury

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ABSTRACT The potential for peripheral nerve injury to reorganize motor cortical representations was investigated in adult rats. Maps reflecting functional connections between the motor cortex and somatic musculature were generated with intracortical electrical stimulation techniques. Comparison of cortical somatotopic maps obtained in normal rats with maps generated from rats with a facial nerve lesion indicated that the forelimb and eye/eyelid representations expanded into the normal vibrissa area. Repeated testing from an electrode placed chronically in the motor cortex showed a shift from vibrissa to forelimb within hours after facial nerve transection. These comparatively quick changes in motor cortex representation pattern suggest that synaptic relations between motor cortex and somatic musculature are continually reshaped in adult mammals.

The primary motor area of the cerebral neocortex (MI) is defined in a variety of mammals as the cerebral neocortical area in which movements can be evoked at the lowest levels of electrical stimulation (1). MI is involved, either directly or through subcortical linkages, in the flexible and skilled control of somatic musculature (2, 3). Lesions of MI or damage to MI inputs or outputs compromises skilled motor performance and other motor functions (4–7). In some cases functional deficits recover with time, but aberrations in motor control may persist or worsen (8, 9). Contributions to the control of related muscle groups appear to arise from a topographically localized region of MI. The stability of this topographical pattern in adult mammals is unclear, but alterations in the size, shape, and distribution of motor representations could alter the extent to which MI participates in motor control of various muscle groups over time.

Recently, we demonstrated that the development of MI representation patterns can be altered by peripheral nerve lesions (10). By use of electrical stimulation mapping methods, forelimb amputation on the day of birth was shown to result in representations of remaining body parts that were larger than normally observed. This finding suggests that the development of motor representation patterns is dynamic—one that is influenced by nerve injury and perhaps by selective forms of experience or deprivation. In developing sensory systems the potential for such factors to modify the normal organization of sensory representations is well-established. In some instances—namely, the development of ocular dominance of visual cortical neurons, modifications are restricted to a distinct “critical period” (11), whereas in other cases reorganization of intact sensory systems has been demonstrated following nerve lesions in immature and adult animals (12).

In the present experiments we were interested to know if the potential for MI to alter its topographic relationships

with muscles persists into adulthood, and if so, how rapidly these effects are expressed. In adult rats, the short- and long-term changes in MI organization were examined after a lesion was made to the motor innervation of the facial vibrissae.

MATERIALS AND METHODS

In a first experiment, organization of MI was studied in eight normal adult rats and in seven adult rats in which motor innervation of the right facial vibrissae was transected. After nerve transection, individual experimental animals survived for 8 days to 4 months. The buccal and marginal mandibular branches of the right facial nerve were ligated and cut surgically during ketamine hydrochloride anesthesia (100 mg/kg). These branches carry motor axons that innervate the muscles attached to the vibrissae, and no sensory fibers are carried in these branches of the facial nerve (13). After transection, distal and proximal ends of the nerve were ligated with silk suture; the wound was sutured closed, and after recovery from anesthesia the animal was returned to its home cage. In experimental and control animals the left MI and the adjacent cortex were mapped with intracortical stimulation techniques. With this technique, electric currents in the microampere range are applied through a micro-electrode inserted among cortical output cells in layer V to identify the muscles or movements that are activated from that cortical site (14, 15). The acute mapping procedures have been described previously (16). Briefly, animals were anesthetized with ketamine hydrochloride (i.p. injections, 100 mg/kg) and mounted in a stereotaxic frame. Microelectrodes (glass insulated, Pt/Ir, 0.5–2 M Ω impedance at 1 kHz) were lowered to a depth of 1.6–1.8 mm below the pial surface. Current trains (30-msec duration, 300 Hz, 200- μ sec monopolar cathodal pulses) of 5–60 μ A were passed through the electrode tip while we examined the body visually and by touch to determine which body parts moved or which muscles contracted. The distance between the electrode penetrations usually ranged between 0.1 mm to 0.5 mm. At the end of each experiment, marking lesions were made at selected cortical sites by passing 10 μ A of cathodal direct current through the electrode tip for 10 sec. The animals were perfused, and their brains were removed and processed for histological localization of penetration sites.

In normal rats, we defined MI as the region of frontoparietal cortex in which low-intensity (≤ 60 μ A) electrical stimulation evoked movement. A similar cortical expanse was mapped in the nerve-transected animals. At each electrode penetration site the stimulation threshold map for each body part that was activated at ≤ 60 μ A was determined. Maps were constructed by plotting penetration sites on a surface view of the cortex. Each body part representation was defined as the region that enclosed the cortical area

where movement of that body part was evoked at the lowest current intensity. Map borders were defined as the midpoint between penetrations evoking movements of two separate body parts (e.g., vibrissa and forelimb) at the lowest current tested at that site. However, boundaries were drawn through points where movement of two body parts was evoked at similarly low current (within $\pm 1 \mu\text{A}$ of the lowest-threshold movement at that site), unless the points were separated by $>1 \text{ mm}$. In that case, boundaries were drawn $250 \mu\text{m}$ from the data point. Total representation for a body part often extends beyond the map of low-threshold representation, but in this extended zone, which is most often contiguous with the low-threshold zone for that body part, movement of other body parts is evoked at lower currents. Maps from normal rats show that the areas from which forelimb, vibrissae, or eye/eyelid movements are elicited as the lowest-threshold movement compose the bulk of MI. These three areas form roughly anterior-posterior strips with the forelimb representation located most laterally and the eye/eyelid representation most medially. This topographic pattern has been repeatedly demonstrated in other studies (15–17).

In a second experiment, changes in representation pattern were followed over time in the same rat. Six normal rats were anesthetized, and the vibrissa and forelimb zones were identified as described above. Then, the stimulating electrode was implanted at a test site within the vibrissa area from which electrical stimulation failed to evoke forelimb movements. This site was located at least $500 \mu\text{m}$ medially from the mapped border of the forelimb and vibrissa representations. The lack of forelimb activation at this site prior to nerve section was documented by recording the electromyogram (EMG) from the wrist extensor and the biceps muscles and by direct observation. Previous experiments have suggested that in rats these muscles are those most commonly activated from the forelimb area of MI (16–19). Fine wires ($100 \mu\text{m}$ stranded, stainless steel) were implanted into the muscles. The muscle activity was amplified, rectified, and low-pass filtered with conventional techniques, and the digitized EMG (sampled at 2 kHz) was stored on a computer. The movements and EMG evoked by stimulating at $60 \mu\text{A}$ at this implanted brain site were monitored before and up to 10 hr after transection of the vibrissae motor nerves at 15- or 30-min intervals.

RESULTS

Low-threshold stimulation maps of MI from a normal and an experimental rat are shown in Fig. 1. In the eight normal rats, a total of 126 of 406 penetrations were within low-threshold vibrissa representations. Maximal stimulation at 32% ($n = 40$) of these sites yielded only vibrissa movements; these sites were typically located in a central core of the low-threshold vibrissa region. At the remainder of the sites, additional movements were evoked in conjunction with vibrissa movements when higher current intensities were used (up to $60 \mu\text{A}$); eye/eyelid movements were found at 50 of the vibrissa sites, and forelimb movements were observed at 25 sites. These coincident sites were most typically located near the borders between the low-threshold zones of two different representations. Frequently, the current required to evoke additional movements at these coincident sites was higher than that required to evoke movements in the low-threshold representation of that body part. This could mean that two adjacent areas overlap to some extent or that current spreading from the microelectrode is able to activate neurons located in two different representations. Because movement of the electrodes as little as $100 \mu\text{m}$ could produce completely different movements at $60 \mu\text{A}$, the explanation of current spread seems not to account for overlaps greater than $200 \mu\text{m}$.

FIG. 1. Representation pattern in MI of a control and an experimental (nerve-transected) rat. In each section, a dot marks a stimulation site from which movements were evoked and a dash marks a site from which no movements could be evoked at currents up to $60 \mu\text{A}$. (A) Pattern of representation at the lowest current intensities showing the location of the forelimb (hatched area), vibrissa (stippled area), and eyelid/eye (cross-hatched area) representations in MI of a normal rat. (B) Low-threshold map from an experimental rat. Illustrated are zones from which movements of the forelimb (hatched area), eyelid/eye (cross-hatched area), and ipsilateral vibrissa (clear region between the forelimb and eyelid/eye areas) are evoked at the lowest currents at each site. Note that the forelimb area and eyelid/eye area are separated by the vibrissa representation in the normal rat but are contiguous throughout most of the anterior-posterior extent of MI in the experimental animal.

MI Representation Pattern After Nerve Lesion. At 369 sites tested in the seven rats that had received a facial nerve transection, cortical stimulation within the region of MI evoked movements; no large negative zones were evident. Stimulation within the region of the expected vibrissa area most often resulted in eye/eyelid, forelimb, or, less frequently, ipsilateral vibrissa movements. The threshold currents required to evoke movements were not elevated in this area. In normal rats, the mean ($\pm \text{SEM}$) low-threshold current to evoke eyelid/eye movements was $29.63 \pm 1.33 \mu\text{A}$ ($n = 102$ sites). In experimental animals, the low-threshold representation for eyelid/eye movements had a mean current of $24.83 \pm 1.05 \mu\text{A}$ ($n = 128$), which was significantly lower than for normal animals ($P \leq 0.005$, Student's *t* test). In normal rats, the low-threshold forelimb zone had a mean current of $24.35 \pm 1.03 \mu\text{A}$ ($n = 82$), and in animals with a facial nerve transection, the low-threshold forelimb zone had a mean current of $25.07 \pm 1.08 \mu\text{A}$ ($n = 122$). The average minimal currents required to evoke move-

ments in the low-threshold forelimb and eye/eyelid zones were relatively uniform (Fig. 2).

Maps in the experimental rats had larger than normal low-threshold eye/eyelid and forelimb representations (Fig. 1B). Unlike normal rats, the eye and forelimb representations in these animals shared a long, anterior-posterior boundary. In addition, forelimb sites were found in areas more medial and eye/eyelid sites were found more laterally than any corresponding sites in the normal rats. Another indication of the expansion of representations in MI was the greater probability of finding eye/eyelid and forelimb movements at single sites after the facial nerve transection. Pairing of these movements, even by stimulating at the maximum current at a single site, was rare in normal rats (8 of 242 penetrations, or 3.3%), but occurred at 12.4% of all sites tested in experimental rats. At least one site was found in each of the eight experimental rats (4.86 ± 1.12 sites, mean $\pm$ SEM). Similar forms of reorganization were seen at all postlesion survival times examined.

Time Course of MI Reorganization. The second experiment investigated the time course of shifts from the vibrissa to the forelimb representation in individual rats. In each of the six animals, stimulation at a single MI test site showed a change from vibrissa to forelimb movements within 45–180

min after the facial nerve transection (Figs. 3 and 4). In individual rats, the maximal forelimb EMG amplitude evoked from the test site occurred from 1–7 hr after facial nerve transection. Peak EMG evoked from the test site was 30–80% of the maximum EMG evoked from direct stimulation within the normal forelimb area of the same animal. After nerve section, the amount of forelimb muscle activity obtained fluctuated over time; the reason for these oscillations was not self-evident; they did not correlate with anesthetic administration or with changes in body temperature. The relatively independent fluctuations of the two forelimb EMGs recorded in the same rat (Fig. 4A) suggest that these variations were not related to the overall metabolic condition of the rat.

Two control experiments were done to show that these rapid shifts in representation patterns were directly related to the nerve section. First, the cortex was mapped in three normal rats, and then the stimulating electrode was placed exactly as in the previous experiment, but the facial nerve was not transected. In none of these controls did stimulation within the vibrissa region evoke forelimb EMG during the 10-hr examination period (data not shown). Second, an array of three stimulating electrodes was implanted so as to straddle the MI forelimb and vibrissa regions of a normal rat. In this animal, electrically evoked movements and forelimb EMG were observed for 10 hr after the facial nerve was transected (Fig. 4B Lower). The types of movements evoked from this forelimb area site remained constant over the 10 hr. In contrast, and consistent with the results shown in Figs. 3

FIG. 2. Comparisons of the stimulation thresholds required to evoke eyelid/eye or forelimb movements in normal and experimental (nerve-transected) rats. The graphs were constructed by taking the low-threshold current required to evoke eyelid/eye (Upper) or forelimb (Lower) movements across all control and experimental rats. The sites were grouped, averaged, and plotted (mean $\pm$ SEM) according to distance, in 0.5-mm bins, lateral from the midline, irrespective of the anterior-posterior location. Within a body representation, the mean low-threshold current remained roughly constant. Note that the current required to evoke movements is similar throughout the entire representation in experimental animals.

FIG. 3. Muscle activity patterns in a normal and an experimental (nerve-transected) rat. *Pretransection.* Muscle activity evoked in biceps (upper trace) and wrist extensor (lower trace) muscles by stimulating at a single site in the forelimb area of a normal rat. *Posttransection.* Stimulation at a site where vibrissa movements alone were strongly evoked before facial nerve transection. Immediately following transection of the facial nerve, the biceps EMG (upper trace) could not be evoked from this site. The wrist extensor EMG (data not shown) was also not evoked. The lower trace illustrates the biceps EMG evoked by stimulation at the same brain site 1 hr after the facial nerve transection. All EMGs in this illustration are at identical amplification, and stimulating current was fixed at 60 μ A. All trials are the average of 30 stimulus presentations.

FIG. 4. Time course of EMG changes after facial nerve transection. These graphs show the integrated EMG obtained by stimulating at a single site before and after nerve transection. Each open square represents a single average of 30 trials. Plots are scaled as a percentage of the largest EMG activity evoked at that site. (A) Rapid increase in activity in biceps after nerve section. Note that activity was high for several hours, then dropped to near zero before returning to a moderate level. Waning and waxing of the EMG obtained from biceps (*Upper*) and wrist extensor (*Lower*) muscles at a single site. Note that peaks and valleys of the two muscle responses do not covary over time. (B) EMG from an animal in which an array of electrodes was implanted in MI. (*Upper*) Wrist extensor EMG evoked by stimulating at a site in the normal vibrissa area. (*Lower*) Averaged amount of wrist extensor EMG evoked from a forelimb area site over 10 hr.

and 4A, stimulation in the former vibrissa site evoked forelimb muscle activity subsequent to facial nerve transection.

DISCUSSION

These results show that the amount of cortex involved in the control of muscle groups is altered rapidly after nerve lesions. Within hours of nerve section the former MI vibrissa territory has shifted its descending relationships to the new muscle groups. At the level of our analysis, this shift appears to produce a new, stable organization in MI representation patterns, because it can be observed months after the lesion.

The newly expanded representations are not produced by uncovering of existing high-threshold representations; they are likely to result from changes in the strength of one or more of the set of synaptic connections between MI and the muscles. After nerve section current thresholds necessary to evoke these postlesion movements were comparable to those seen in the normal representations for these body parts. Further, no EMG activity could be evoked in the "abnormal" forelimb muscle area until after the nerve was sectioned. Otherwise, the type of movements evoked from the test site remained stable before nerve section and over long periods of time in sham controls, although fluctuations in EMG evoked from single sites were clearly evident. These fluctuations could reflect ongoing subtle modifications in synaptic strength.

The rapid time course of shifts in MI representation patterns suggests that, initially, the representation shift is not likely to involve the formation of new synaptic contacts by axon sprouting, at least not over very long distances. Previous studies have shown that functional recovery prob-

ably does not occur until at least 12 days following facial nerve crush near the stylomastoid foramen (20). In addition, nerve section and ligation would be expected to delay functional reinnervation for at least 2 months (21). Peripheral axon sprouting as a mechanism for the MI plasticity seen here would also require that circumorbital muscle fibers become polyinnervated. Within the central nervous system, local sprouting has been seen, but such sprouting appears to require a much longer time than the shifts in representation we have seen (22–24). However, sprouting might be involved in some longer-term consolidation processes.

More probably the rapid shift in MI representation patterns is related to mechanisms that regulate strength of existing synapses. Ordinarily, these "unmasked" synapses must be relatively weak, or perhaps even totally ineffective, because in the acute experiments we saw a shift from no evoked EMG to relatively strong activation from a former portion of the vibrissa representation. Unmasking-like phenomena have been shown to exist and act in the central nervous system over fairly rapid time courses and have been suggested as one mechanism for reorganization of central sensory representations (25–27). An interpretation of unmasking would mean that routes from the MI vibrissa area to the forelimb musculature, for example, must already exist in the adult animal. We do not know the site of these connections. They could be any of the following: (i) within MI, where afferent and intrinsic axons have extensive arbors (28), or (ii) at any of a number of subcortical sites where changes could occur in the strength of connections made by those pyramidal tract neurons that have widely distributed collaterals (29–33); or (iii) by neurons within subcortical structures themselves. Nor do we know the cue that initiates the change in synaptic effectiveness. Nerve section causes a

number of reactions in motor neurons (34), although the speed at which these changes occur makes it difficult to identify a simple mechanism for the axotomized motor neurons to signal remote parts of the motor system. Another possibility is that facial nerve section alters the pattern of sensory information relayed from the vibrissa to the central nervous system; this change in activity might then serve as a stimulus for reorganization.

Results of this study suggest that a flexible relationship exists between the MI cortex and muscles. This finding complements reports that adult sensory representations can reorganize after nerve injury (12, 35–39). Thus, it appears that cortical input–output relationships may be continually reshaped throughout life, although it remains to be shown that outputs of cortical areas other than MI are capable of reorganization. The functional consequence of shifts in MI representation pattern remains unclear. However, the ability for rapid shifts to occur in the size of MI muscle representations suggests similar mechanisms operate during the learning of skilled voluntary movements or during the recovery period after central or peripheral nervous system damage.

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