Reorganization of Primary Motor Cortex in Adult Macaque Monkeys With Long-Standing Amputations

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Qi, Hui-Xin, Iwona Stepniewska, and Jon H. Kaas. Reorganization of primary motor cortex in adult macaque monkeys with long-standing amputations. J Neurophysiol 84: 2133–2147, 2000. The organization of primary motor cortex (M1) of adult macaque monkeys was examined years after therapeutic amputation of part of a limb or digits. For each case, a large number of sites in M1 were electrically stimulated with a penetrating microelectrode, and the evoked movements and levels of current needed to evoke the movements were recorded. Results from four monkeys with the loss of a forelimb near or above the elbow show that extensive regions of cortex formerly devoted to the missing hand evoked movements of the stump and the adjoining shoulder. Threshold current levels for stump movements were comparable to those for normal arm movements. Few or no sites in the estimated former territory of the hand evoked face movements. Similar patterns of reorganization were observed in all four cases, which included two monkeys injured as adults, one as a juvenile, and one as an infant. In a single monkey with a hindlimb amputation at the knee as an infant, stimulation of cortex in the region normally devoted to the foot moved the leg stump, again at thresholds in the range for normal movements. Finally, in a monkey that had lost digit 5 and the distal phalanges of digits 2–4 at 2 yr of age, much of the hand portion of M1 was devoted to movements of the digit stumps.

INTRODUCTION

The primary motor area (M1) of macaque monkeys is located in a mediolaterally elongated strip of cortex mostly buried in the central sulcus just rostral to somatosensory cortex. This cortex represents body movements in an orderly fashion from tail and foot medially, trunk and hand more laterally, and face and tongue movements most laterally. The general pattern of M1 organization in primates has been known for some time (e.g., Leyton and Sherrington 1917), since it was possible to generate effective stimulating currents long before it was possible to record the electrical responses of cortical neurons. Early modern studies of motor cortex in monkeys outlined the basic organization of M1 with electrodes placed on the brain surface (e.g., Woolsey et al. 1952), but more detailed results were obtained with microelectrodes that penetrated close to the corticospinal neurons in layer V of motor cortex (for results in macaque monkeys, see Andersen et al. 1975; Asanuma and Rosén 1972; Huntley and Jones 1991; Jankowska et al. 1975; Kwan et al. 1978; McGuiness et al. 1980; Sessle and Wiesendanger 1982; Wise and Tanji 1981). Our present understanding of the normal organization of M1 in monkeys and other primates allows us to ask if and how the organization of motor cortex is altered by injuries that remove part of the muscle targets of motor cortex, such as those caused by limb amputations and motor nerve injuries. Sensory maps in cortex clearly reorganize after a sensory loss so that the remaining sensory inputs are represented in the deprived portions of the cortex (for review, see Florence et al. 1997; Kaas 1999; Kaas and Florence, 2000). The mutability of sensory maps in cortex suggested that deprivations might change motor maps as well.

The question of the plasticity of motor maps in cortex of adult mammals was first addressed in rats where the loss of a forelimb or the section of a motor nerve to the moveable vibrissa of the face was followed by a reorganization of M1 so that electrical stimulation of locations that normally evoked movements of the forelimb or facial vibrissa evoked movements of remaining moveable body parts instead (Donoghue et al. 1990; Sanes et al. 1990). In primates, studies have been limited to individuals with amputations as a result of injury and therapeutic treatments. Although such cases are rare in captive primates, a number of humans with amputations have been studied with the noninvasive technique of transcranial magnetic stimulation (TMS). While this procedure does not provide a detailed estimate of the organization of the motor maps in M1, the results of TMS studies in humans nevertheless suggest that face and stump representations expand into the former territory of the amputated forelimb (Cohen et al. 1991; Dettmers et al. 1999; Fuhr et al. 1992; Hall et al. 1990; Kew et al. 1994; Pascual-Leone et al. 1996; Ridding and Rothwell 1995). Studies in nonhuman primates with amputations are limited to two recent reports. Schieber and Deuel (1997) were able to obtain a single macaque monkey that had undergone an arm amputation at about 2 yr of age and used microelectrodes to stimulate and map motor cortex 15 yr later. They found that movements of the stump of the amputated limb could be evoked throughout the normal territory of missing distal limb in M1. The threshold current levels for evoking movements for the stump were about the same or higher than those for the upper arm in cortex contralateral to the normal arm. Similar results were subsequently reported for squirrel monkeys and prosimian galagos with therapeutic limb amputations (Wu and Kaas 1999). In two infant squirrel monkeys and one infant galago, the forearm had been amputated near or at the shoulder joint. In one infant galago and one adult squirrel monkey, the hindlimb had been amputated near or at the hip joint. Motor
cortex contralateral to the amputation was mapped with microelectrodes 4–12 yr later. In all cases, stimulation of the deprived portion of M1 elicited movements of remaining muscles just proximal to the amputation. The minimal levels of current needed to evoke these movements ranged from normal to higher than normal.

The studies mentioned in the preceding text provide compelling evidence that the motor maps in M1 of primates are sometimes capable of reorganization after the loss of a limb. However, most of the results from monkeys were obtained from animals injured as infants or juveniles. Thus it is uncertain if the extent of reorganization is similar or different after injury in adult primates. The results from humans suggest that extensive reorganization occurs in the mature brain, but the more precise microstimulation results are limited to those from a single mature squirrel monkey with a hindlimb amputation (Wu and Kaas 1999). In addition, there is the question if the face representation expands into the deafferented forelimb representation after forelimb amputation. Studies in humans suggested that this might be the case (Ojemann and Silbergeld 1995), but the microstimulation studies in monkeys seem to indicate that there is little (Wu and Kaas 1999) or perhaps no (Schieber and Deuel 1997) invasion of the face into the forelimb territory. Furthermore there are questions about the levels of current needed to evoke movements in muscles proximal to the amputation. Some of the transcranial magnetic stimulation results in humans suggest that cortical neurons for these muscles may be more excitabile contralateral to the amputation than contralateral to the intact arm (Chen et al. 1998; Cohen et al. 1991; Kew et al. 1994). In contrast, Wu and Kaas (1999) found sites in reorganized motor cortex with normal stimulation thresholds and sites with higher than normal thresholds. Somewhat differently, Schieber and Deuel (1997) found no significant difference in stimulation threshold for the muscles in the amputated or intact arm in their macaque monkey. We also need to know if the reorganization that occurs after the loss of the hindlimb is similar to the reorganization that occurs after the loss of a forelimb. While the results appear to be similar, only one squirrel monkey and one galago have been studied after lower limb loss (Wu and Kaas 1999), and only a few humans with lower limb loss have been studied with TMS (Chen et al. 1998). Finally, little is known about possible species differences in results. In particular, little is known about motor cortex reorganization in macaque monkeys. Currently all the information on motor cortex reorganization in Old World monkeys comes from a single monkey with an amputation at an immature age.

To address some of these questions about motor cortex reorganization in primates, we were able to obtain four macaque monkeys with long-standing therapeutic amputations of the forelimb, one macaque monkey with a hindlimb amputation, and one with a partial loss of digits. Some of our recent results have been briefly reported elsewhere (Qi et al. 1999).

### METHODS

In this study, we used intracortical microstimulation procedures to evaluate the organization of M1 in adult macaque monkeys long after part of the forelimb or hindlimb had been amputated as a result of veterinary treatment for traumatic, accidental injuries. After an extensive search, we were able to obtain six such monkeys (4 Macaca mulatta and 2 M. nemestrina) from other facilities. The injuries had occurred at ages ranging from 4 mo to 7 yr. Survivals ranged from 4 to 15 additional years (Table 1). Amputations included parts of limbs or digits. The individual variability in the histories of the animals is a consequence of the unintended injuries. In addition, because of the rare opportunity to study such monkeys, these monkeys were used to determine the effects of the deafferentations on somatosensory cortex (see Florence et al. 1998, 2000). Microstimulation of motor cortex usually followed somatosensory cortex recording, thus the time available for microstimulation was sometimes limited. Results from the injured monkeys were compared with those obtained under the same stimulation conditions in three normal adult monkeys (M. mulatta). Procedures were in accordance with those outlined by the National Institutes of Health, and approved by the Animal Care Committee of Vanderbilt University.

### Surgery

In preparation for surgery for the recording and microstimulation, the monkeys were given ketamine hydrochloride (10–20 mg/kg im) and xylazine (0.4 mg/kg im) as a sedative. For somatosensory mapping, a surgical level of anesthesia was achieved using isoflurane gas in O2. Isoflurane concentrations ranged from 4% during induction to 1% after a surgical level of anesthesia was attained (Florence et al. 2000). For the motor mapping, anesthesia was continued with either a combination of ketamine and xylazine or urethane. Supplemental injections of ketamine and xylazine (20% of initial dosage) or urethan (20% of 0.25g/kg ip) were given as needed to maintain a surgical level of anesthesia during stimulation sessions. Dexamethasone (2 mg/kg im) was given to prevent brain swelling. Fluids were continuously given intravenously, body temperature was maintained with a water-circulated heating pad, and heart rate and respiration were monitored. The anesthetized monkeys were secured in a stereotaxic apparatus, the skull was exposed, and an opening was made over much of the dorsolateral extent of the central sulcus. The opening in the skull was surrounded by a dam of acrylic plastic, the dura was retracted, and the exposed brain surface was covered with silicon fluid to prevent dessication. Recordings were first made from somatosensory cortex with microelectrodes for a period of 6–12 h as described elsewhere (see Florence et al. 1998, 2000). After this time, sites in motor cortex were stimulated and evoked movements were recorded for another

### TABLE 1. Details about the studied monkeys with limb amputation

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Species</th>
<th>Sex</th>
<th>Weight, kg</th>
<th>Age at ICMS, yr</th>
<th>Amputation Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>97-96</td>
<td>Macaca mulatta</td>
<td>Female</td>
<td>5.7</td>
<td>17</td>
<td>Left forelimb at mid-upper arm and right leg above knee</td>
</tr>
<tr>
<td>98-81</td>
<td>M. mulatta</td>
<td>Male</td>
<td>11</td>
<td>7</td>
<td>Right forelimb at mid-upper arm</td>
</tr>
<tr>
<td>98-95</td>
<td>M. mulatta</td>
<td>Female</td>
<td>8</td>
<td>17</td>
<td>Right leg at knee</td>
</tr>
<tr>
<td>98-110</td>
<td>M. nemestrina</td>
<td>Male</td>
<td>22</td>
<td>14</td>
<td>Left forearm just above elbow</td>
</tr>
<tr>
<td>99-2</td>
<td>M. nemestrina</td>
<td>Female</td>
<td>4.7</td>
<td>19</td>
<td>Digits and toes</td>
</tr>
<tr>
<td>99-26</td>
<td>M. mulatta</td>
<td>Female</td>
<td>4.5</td>
<td>5</td>
<td>Right forearm just below elbow</td>
</tr>
</tbody>
</table>

* Parentheses enclose age in months.
10–20 h. The stimulation sessions were terminated by giving a lethal dose of pentobarbital sodium.

**Motor mapping**

Low-impedance tungsten microelectrodes (0.9–1.2 MΩ at 1 kHz, Microprobe) were used to stimulate cortex. The stimuli consisted of 60-ms trains of 0.2-ms monophasic cathodal pulses of DC current at 300 Hz ranging from less than 10 to 80 μA. Trains were repeated as needed with gaps of at least 1–2 s. The microelectrodes were held in a micromanipulator with a hydraulic microdrive and lowered perpendicularly to the exposed surface of the brain. The angle of the electrode was adjusted as sites became more medial or lateral so that groups of penetrations were roughly perpendicular to the pial surface. However, penetrations into the depths of the central sulcus were parallel to each other along an angle that was appropriate for most penetrations. The goal was to stimulate sites throughout the portions of M1 likely to be altered by the amputations. This included mediolateral extents of M1 along the rostral bank of the central sulcus and as much as 7 mm rostral to the central sulcus. For penetrations along the exposed dorsolateral portion of M1, the electrode was lowered in increments to depths of 1.5 mm or more to where stimulation produced threshold movements at the lowest levels of current (near or within layer V). Penetrations in the cortex along the rostral bank of the central sulcus were placed 1–3 mm rostral to the sulcus and progressively advanced to depths of up to 9 mm in an effort to stimulate neurons in layer V throughout the portion of M1 that is buried in the sulcus. Because of variations in the curvature of the sulcus, several rostrocaudally aligned penetrations were often needed to place recording sites in or near layer V at all depths. Stimulations were made at sites from 1.5 or 1.8 mm below the surface and subsequently every 0.5 mm until movements were no longer evoked or depths of 9 mm were reached. Electrode penetrations were placed in a grid with 0.5–1.0 mm or more between penetrations.

Current levels for stimulation were typically started at a level above the expected threshold for a detectable movement and then reduced until a movement was no longer detected. Threshold current was considered to be the current level where a just-detectable movement occurred on at least half of the stimulations. At these levels, the movement could involve a muscle twitch without obvious movement of a body part. On the occasions when no movement was initially detected, current levels were gradually raised until a movement was observed or until the current of 80 μA was reached. Sometimes the electrode was replaced to reevaluate a site to see if the threshold and movement remained stable. Most of the movements were detected visually by lowest threshold currents, a muscle twitch could be sometimes felt but not seen. Movements were classified according to type (e.g., flexion, extension, for a detailed description see Gould et al. 1986) and the body part where the movement occurred (e.g., jaw, shoulder, arm stump).

**Histology**

At the end of the stimulation session, the electrode was returned to critical locations in M1, such as topographical boundaries, and reference electrolytic microlesions were placed by passing a DC current at 10 μA for 10 s (Fig. 1). These lesions allowed us to later relate the stimulation results to cortical histology. The animals were given lethal injections (30 mg/kg) of pentobarbital. When areflexive, they were perfused through the heart with phosphate-buffered saline (PBS, pH 7.4), followed by 2–4% paraformaldehyde in PBS as a fixative and then followed by 4% paraformaldehyde with 10% sucrose in PBS. The brain was removed, immersed in 30% sucrose in PBS, and refrigerated until the next day when it was blocked and photographed. The motor cortex was cut at 50 μm on a freezing microtome in a plane between parasagittal and horizontal such that the brain was cut along the plane of the most relevant electrode penetrations. A one-in-five series of sections was stained for Nissl substance with cresyl violet, and additional sets of sections were processed for cytochrome oxidase (Wong-Riley 1979) or acetylcholinesterase (Geneser-Jensen and Blackstad 1971) to aid in the identification of architectural boundaries.

**Data analysis and cortical maps**

To reconstruct a map of M1, stimulation sites were plotted on a three-dimensional grid such that sites on the dorsolateral surface of the brain were represented on the horizontal plane and sites along the depth of the central sulcus on the vertical plane (Figs. 2–8). All sites are illustrated at a depth of 1.8 mm from the cortical surface (about layer V) for both planes, although many sites were at slightly different depths. Stimulation sites at different depths within the same column of cortex evoked nearly identical movements, although thresholds varied. All thresholds were included in the calculations of the threshold means. However, in the summary maps, only the responses evoked by the lowest threshold currents were plotted for those sites located on dorsolateral surface rostral to central sulcus. Therefore the numbers of mapping sites in the mean threshold calculations are greater than those in the summary maps. In anterior bank of central sulcus, all the mapping sites were plotted.

Means of the threshold currents for evoked movements were calculated for each body part and compared with means for different body movements and reported as the means ± SE. We used a nonparametric one-way ANOVA (Kruskal-Wallis) followed by a post hoc test for statistical comparisons of mean thresholds across body representations. A probability value of less than 0.05 was considered statistically significant.

**RESULTS**

Microelectrodes were used to stimulate sites in M1 of monkeys with long-standing amputations and normal monkeys, and the types of movements and current thresholds for evoking these movements were compared. In every studied monkey, sites normally devoted to the missing body part evoked movements of more proximal remaining parts. While each injured case was unique, basic features of cortical reorganization were similar across animals and thus there appeared to be consistent consequences of injury.
Organization of M1 in normal monkeys

Data were obtained from three normal macaque monkeys. Our most extensive map of M1 in a normal monkey (Fig. 2) included large portions of M1 buried in the central sulcus as well as exposed on the precentral gyrus rostral to central sulcus. Movements were evoked from most sites that were histologically confirmed to be in M1. At few sites, no movement was evoked with current up to 80 μA. For these sites, the electrode tip may have been too far from layer V to effectively activate layer V neurons. Alternatively, it is possible that evoked movements were sometimes not detected. Whatever the reason, the scattering of a few ineffective sites in motor cortex of normal monkeys indicates that such sites in a recorded monkey need not always reflect a consequence of injury.

Movements were evoked from 600 effective microstimulation sites in three control animals. The overall pattern of evoked movements for sites across M1 in the normal monkeys closely reflected the basic pattern expected from previous studies of M1 organization in macaque monkeys (see Discussion). Thus most laterally in the explored cortex, a large representation of the tongue was seen. Successively more medial locations were devoted to the face, digits, wrist, elbow, and shoulder (Fig. 2). Even more medial locations were devoted to trunk, hindlimb, and tail movements (not shown). Within this overall pattern, the details of somatotopic organization varied across cases. Sites where specific digit movements were evoked, for example, varied somewhat in relation to other sites (see Gould et al. 1986). The levels of current needed to evoke movements varied for different types of movements and locations in the map (Table 2). Face, hand, and arm movements were often evoked by currents under 20 μA, and the majority of such movements were evoked by currents under 40 μA (Figs. 2C and 9A and B). Shoulder movements needed higher currents, most often in the 21- to 40-μA range, while trunk movements usually required currents in an even higher range of 21–80 μA.

Additional information about the normal M1 organization was obtained from portions of the M1 maps that related to intact parts of the body in the amputee monkeys. For example, the face and forelimb portions of M1 appear to be normal in the monkey with a loss of part of the hindlimb (Fig. 8A).

Organization of M1 after arm amputation

Movements were evoked from a total of 1,344 microstimulation sites in four monkeys, each with a long-standing loss of much of an arm (Table 1). In each of these cases, parts of the cortex normally devoted to digit and wrist movements became devoted to movements of the remaining stump of the arm and shoulder. Currents needed to evoke movements of the stump and shoulder in the reorganized cortex were often in the normal range for arm movements, although higher than typical for digit movements in normal monkeys. Results from each case differed somewhat from the others.

Among four arm amputees, the case with most extensive reorganization in the motor map was 99-26. This was a monkey that had amputation of the forelimb just below the elbow, and the loss was early in life at the age of 5 mo. The recovery time was 4.5 yr. The stump was large enough to be useful, and it was used in locomotion and to help manipulate food. The reorganization of deprived cortex was extensive (Fig. 3), and movements of the stump occupied a mediolateral extent of 11.1 mm of cortex. Sites lateral to the stump-movement region where the face is typically represented evoked face and tongue movements. Sites medial to the stump-movement region of cortex evoked upper arm, shoulder, and trunk movements. Additionally, three small regions where shoulder movements could be evoked were lateral to the expanded stump representation just next to the face region. Thresholds for face, shoulder, and stump movements were all highly comparable (Figs. 3C and 9C) with most movements being evoked at current of 40 μA or less. Unresponsive sites tended to be along the rostral lip of the central sulcus or deep in the central sulcus, locations where the electrode was likely superficial or deep to layer V neurons. The representation of the stump was most extensive in this case, and thresholds were comparable to those for face movements, which generally have the lowest threshold in normal animals (Fig. 2).

Another case with a similar injury was case 99-110. This monkey lost the forelimb just above the elbow at 2 yr and was studied 13 yr later. A large representation of stump movements extended across the deprived hand region of M1 all the way to the face representation, which was in the expected location (Fig. 4). Sites evoking trunk and shoulder movements were mixed in more medial cortex. More lateral sites evoked shoulder movement at high-threshold currents. Overall thresholds for stump and shoulder movements tended to be somewhat higher than expected, with most in the 21- to 60-μA range (Figs. 4C and 9D). As the thresholds for face movements paralleled those for stump movements, the somewhat higher thresholds for stump and shoulder movements appeared to reflect the overall condition of M1 cortex rather than reorganized cortex only. A number of unresponsive sites were found, but they occurred in face and trunk regions of cortex as well as in the reorganized cortex.

Case 98-81 had an amputation of the forelimb at the level just below the humeral head at age of 14 mo and was studied 6 yr later. Only a portion of the humerus remained attached to the shoulder. Yet the representation of movements and muscle contractions in the stump extended from the normal region for shoulder and upper arm movements laterally all the way to the border of face representation, roughly a 8.3 mm mediolateral extent of cortex excluding the medial island of motor cortex on precentral gyrus for stump movements (Fig. 5). Additionally, the region for shoulder movements appeared to be somewhat, but not dramatically expanded. There was no clear evidence that the face representation had expanded into the deprived hand cortex, although this possibility cannot be ruled out given that the landmarks for the border of the face representation (e.g., about 6 mm medial to the lateral end of central fissure; Fig. 2) (McGuinness et al. 1980) are approximate. Threshold currents for face movements were comparable to those in normal monkeys, most threshold current levels were under 40 μA (Figs. 5C and 9F). Most shoulder, trunk, and stump movements were evoked by currents ranged from 21 to 40 μA, while a few occurred with currents of 20 μA or less. Thus these movements were evoked by currents in the normal range for proximal limb and trunk movements. However, no movements were evoked by stimulation at a number of sites in the deprived cortex.
Case 97-96 was a monkey that lost its left arm at the level just below the humeral head and also lost its right leg above the knee. The amputations had occurred at the age of 7, and the cortex was studied 10 yr later. Only the cortex contralateral to the missing forelimb was studied. Again sites evoking shoulder, stump, and even trunk movements extended across the deprived region of the M1 forelimb representation (Fig. 6A), which by its relation to the central sulcus appeared to be in the normal location (Fig. 6B). The main stump movement region extended 7.4 mm mediolaterally and even further if more medial isolated stump sites located in the precentral gyrus are included. Sites evoking stump and stump muscle movements
as well as trunk movements were immediately adjacent to those evoking lip and chin movements. While the threshold currents for face movements were the lowest, 40% of the stump movements were evoked by current of 20 μA or less. The lowest thresholds for trunk movements were for lateral sites next to the face representation (Figs. 6C and 9G). The results indicated that the sites in the deprived hand region of M1 evoked stump, shoulder, and trunk movements at current threshold in the normal range for such movements for all but a small number of sites where no movements could be evoked.

**Organization of M1 after loss of digits**

We were able to study motor cortex in one monkey that had amputations of fingers and toes at age of 2, and cortex was studied 17 yr later (Fig. 7). Cortex in the hand portion of M1 was stimulated contralateral to the right hand, which was missing portions of digits and had a fusion of the stumps of digits 2 and 3 (see drawing of hand in Fig. 7A). Movements were evoked from a total of 333 effective microstimulation sites in M1. As expected, digit stump movements were evoked over at least part of the former territory of the hand representation. Sites related to digit movements were concentrated next to the face representation laterally and in a more medial island of cortex surrounded by a large region of cortex related to wrist movements. Wrist and digit movements were evoked over a 9 mm mediolateral expanse of cortex, a distance comparable to the 9.5 mm mediolateral extent of the wrist-digit territory in our most extensively mapped normal animal (Fig. 2). Most of the threshold currents for digit stump movements were 20 μA or less, roughly comparable to those for normal digit movements (Figs. 7C and 9H). Few unresponsive sites were present.

**Organization of M1 after loss of hindlimb**

We also stimulated motor cortex in a 15-yr-old monkey that had an amputation of right leg at the knee at 4 mo. The monkey did use the stump to support weight and assist locomotion. Movements were evoked from 424 microstimulation sites in M1 (Fig. 8). A large (5 mm) region of cortex in the region expected to represent the toes and foot in normal monkeys (not

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**TABLE 2. Statistical comparison of mean threshold current**

<table>
<thead>
<tr>
<th>Case</th>
<th>Representation Mean, μA</th>
<th>n</th>
<th>Significant Level</th>
<th>Pair of Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>94-73</td>
<td>Shoulder 44.43 ± 9.98</td>
<td>7</td>
<td>P &lt; 0.01</td>
<td>Trunk vs. lower leg</td>
</tr>
<tr>
<td>Control</td>
<td>Trunk 59.16 ± 3.57</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H = 14.34</td>
<td>Upper Leg 42.56 ± 7.33</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.0025</td>
<td>Lower Leg 38.17 ± 2.59</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>97-122</td>
<td>Face 24.93 ± 1.47</td>
<td>136</td>
<td>P &lt; 0.001</td>
<td>Face vs. wrist; face vs. trunk</td>
</tr>
<tr>
<td>Control</td>
<td>Digits 26.73 ± 1.97</td>
<td>93</td>
<td>P &lt; 0.001</td>
<td>Digits vs. wrist;</td>
</tr>
<tr>
<td>H = 55.01</td>
<td>Digits 24.10 ± 1.97</td>
<td>93</td>
<td>P &lt; 0.001</td>
<td>Digits vs. elbow;</td>
</tr>
<tr>
<td>P &lt; 0.0001</td>
<td>Digits 26.32 ± 1.97</td>
<td>93</td>
<td>P &lt; 0.001</td>
<td>Digits vs. elbow;</td>
</tr>
<tr>
<td>99-26</td>
<td>Face 29.33 ± 1.51</td>
<td>116</td>
<td>P &lt; 0.001</td>
<td>Face vs. stump; face vs. trunk</td>
</tr>
<tr>
<td>Loss arm</td>
<td>Stump 37.79 ± 1.06</td>
<td>260</td>
<td>P &lt; 0.001</td>
<td>Stump vs. trunk</td>
</tr>
<tr>
<td>H = 54.61</td>
<td>Shoulder 33.98 ± 1.78</td>
<td>114</td>
<td>P &lt; 0.001</td>
<td>Shoulder vs. trunk</td>
</tr>
<tr>
<td>P &lt; 0.0001</td>
<td>Trunk 55.83 ± 2.21</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98-110</td>
<td>Face 37.75 ± 1.61</td>
<td>99</td>
<td>P &lt; 0.001</td>
<td>Face vs. stump</td>
</tr>
<tr>
<td>H = 17.40</td>
<td>Stump 47.52 ± 1.52</td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.0006</td>
<td>Shoulder 41.29 ± 2.64</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99-81</td>
<td>Face 29.49 ± 1.47</td>
<td>189</td>
<td>P &lt; 0.001</td>
<td>Face vs. stump, shoulder, trunk</td>
</tr>
<tr>
<td>Loss arm</td>
<td>Stump 43.52 ± 1.99</td>
<td>83</td>
<td></td>
<td></td>
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<tr>
<td>H = 55.84</td>
<td>Shoulder 41.24 ± 1.86</td>
<td>63</td>
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<tr>
<td>P &lt; 0.0001</td>
<td>Trunk 46.32 ± 3.16</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>97-96</td>
<td>Face 25.77 ± 2.29</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss arm</td>
<td>Stump 37.83 ± 2.74</td>
<td>59</td>
<td>P &lt; 0.01</td>
<td>Face vs. stump</td>
</tr>
<tr>
<td>H = 25.52</td>
<td>Shoulder 44.11 ± 3.75</td>
<td>27</td>
<td>P &lt; 0.01</td>
<td>Face vs. shoulder</td>
</tr>
<tr>
<td>P &lt; 0.0001</td>
<td>Trunk 51.44 ± 9.01</td>
<td>9</td>
<td>P &lt; 0.05</td>
<td>Face vs. trunk</td>
</tr>
<tr>
<td>99-2</td>
<td>Face 24.67 ± 2.23</td>
<td>68</td>
<td>P &lt; 0.01</td>
<td>Face vs. elbow</td>
</tr>
<tr>
<td>Loss digits and toes</td>
<td>Stump 24.1 ± 4.41</td>
<td>28</td>
<td>P &lt; 0.01</td>
<td>Stump vs. elbow</td>
</tr>
<tr>
<td>H = 36.31</td>
<td>Wrist 21.85 ± 2.12</td>
<td>67</td>
<td>P &lt; 0.001</td>
<td>Wrist vs. elbow</td>
</tr>
<tr>
<td>P &lt; 0.0001</td>
<td>Elbow 36.23 ± 1.96</td>
<td>70</td>
<td>P &lt; 0.05</td>
<td>Elbow vs. leg</td>
</tr>
<tr>
<td></td>
<td>Shoulder 30.36 ± 2.27</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trunk 35.83 ± 9.8</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leg 21.31 ± 3.44</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98-95</td>
<td>Face 36.92 ± 2.52</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss leg</td>
<td>Digits 31.71 ± 2.22</td>
<td>47</td>
<td>P &lt; 0.05</td>
<td>Digits vs. trunk</td>
</tr>
<tr>
<td>H = 29.91</td>
<td>Wrist 31.11 ± 1.6</td>
<td>110</td>
<td>P &lt; 0.01</td>
<td>Wrist vs. stump; wrist vs. trunk</td>
</tr>
<tr>
<td>P &lt; 0.0001</td>
<td>Elbow 36.43 ± 2.37</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shoulder 35.88 ± 1.96</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trunk 61.33 ± 6.57</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stump 40.25 ± 2.01</td>
<td>87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.
FIG. 3. Organization of motor cortex in a macaque monkey with a long-standing amputation of a forelimb. A: the partial motor output map includes the representation of face to upper trunk. Microstimulation sites are marked by dots. For each mapping site, we have noted the response threshold in μA and the type of response elicited at threshold. The abbreviations indicate the types of evoked body movement. The letters in subscript indicate actions of the limb. Abbreviations for this and other figures as in Fig. 2. B: a lateral view of the brain with central sulcus open and the region investigated with microstimulation marked. C: distributions of threshold currents for different body representations. The number in parentheses indicates the number of mapping sites for movement of that body part.
shown) (but see Woolsey 1964) was devoted to movements of the leg stump in this monkey. Only three sites were found where stimulation evoked trunk movements. However, multiple sites where no apparent movements could be evoked were found in the expected location of the trunk representation.

Thresholds for leg stump, arm-wrist-digits, and face movements were all similar (Figs. 8C and 9E). The evidence suggests that cortex normally devoted to the missing foot became devoted to the leg stump and that stimulation thresholds for leg stump movements are in the normal range.
DISCUSSION

The monkeys in this study provided an unusual opportunity to investigate the consequences of long-standing limb amputation and amputation at different ages on the detailed organization of primary motor cortex. The major finding is that, regardless of the age at which the injury occurs, the deprived cortex does not remain nonfunctional. Instead this cortex takes on new roles and triggers movement in new target muscles. There is variability in the specific patterns of reorganization across cases, reflecting differences in the level of injuries and injuries to different limbs; however, the overall patterns of reorganization are highly similar.

FIG. 5. Motor cortex organization in a macaque monkey with a long-standing amputation of a forelimb. A: the partial motor output map includes the representation of face to upper trunk. B: a lateral view of the brain with central sulcus open and the region investigated with microstimulation marked. C: distributions of threshold currents for given body representations. The number in parentheses indicates the number of mapping sites for movement of that body part.
Reorganization of M1 in macaque monkeys

In all cases, the majority of neurons in the large expanse of deafferented cortex came to activate muscles that control body parts just proximal to the amputation. For example, after a forelimb loss, electrical stimulation of sites through the deprived forelimb cortex resulted in stump and shoulder movements. After a hindlimb loss, stimulation in deprived cortex evoked movements of the remaining hip muscles. Sometimes, the reorganization involved not only the stump but also more proximal portions of the limb or the adjacent trunk. For example, the wrist expanded into digit cortex after partial digit amputations.

There was little evidence for sectors of M1 without muscle targets after large-scale deafferentations. Ineffective sites, where current levels up to 80 µA did not evoke movements, were found, but they occurred nearly as frequently in the normal monkeys as in the amputees. Many of ineffective sites seem to relate to locations where the electrode tip was superficial or deep to layer V, where stimulation would be less effective, but the reasons for other ineffective sites are uncertain. However, the proportions of such sites varied somewhat across cases. In particular, the ineffective sites were fewest in case 99-26, a monkey injured as an infant and with the upper arm intact. Thus it is tempting to propose that there is greater...
FIG. 7. Organization of motor cortex in a macaque monkey with a long-standing amputation of digits and toes. A: the partial motor output map includes the representation of face to upper trunk. B: a lateral view of the brain with central sulcus open and the region investigated with microstimulation marked. C: distributions of threshold currents for given body representations. The number in parentheses indicates the number of mapping sites for movement of that body part.
FIG. 8. Organization of motor cortex in a macaque monkey with a long-standing amputation of a hindlimb. A: the partial motor output map includes the representation of face to hindlimb. B: a lateral view of the brain with central sulcus open and the region investigated with microstimulation marked. C: distributions of threshold currents for given body representations. The number in parentheses indicates the number of mapping sites for movement of that body part.
recovery and reorganization in monkeys with early injury and a more limited amputation (also see case 99-2 with digit loss only), but further observations from additional cases are needed.

There was no clear evidence that stimulation of sites in the deafferented forelimb-cortex evoked face movements. This finding was of considerable interest because studies of the effects of forelimb amputation on somatosensory cortex report that the face representation often expands into the deafferented forelimb cortex (Florence and Kaas 1995; Florence et al. 1998; Jain et al. 1997; Pons et al. 1991). However, the present results did not reveal an obvious shift in hand-face border. Because the expected location of the hand-face border in M1 was estimated from surface landmarks and compared with the physiological border in all five monkeys, it is possible that some minor, undetected shift in that border occurred but it would be minimal.

One of the strengths of the present study is that we were able to obtain numerous and very precise estimates of the threshold currents needed to evoke movements from sites in both normal and reorganized cortex. Such estimates are particularly critical for understanding the mechanisms that might underlie the massive changes in cortical organization produced by limb amputation. Threshold current levels for evoking minimal movements were in the normal range. This suggests that the evoked movements did not simply represent an “iceberg effect” where high levels of current evoke limb movements that were always present but previously masked by the presence of

![Graphs and diagrams showing mean threshold currents for movements of major body parts for each studied case.](http://jn.physiology.org/DownloadedFrom)
other low-threshold movements. For example, high-stimulation currents at sites in a distal limb representation may evoke proximal limb movement even in normal animals (Donoghue et al. 1992; Huntley and Jones 1991; Sessle and Wiesendanger 1982; Strick and Preston 1982). Some sites in M1 may relate to both distal and proximal forelimb muscles (Hill Karrer et al. 1995). Thus after amputation of distal portion of a limb, the activation of proximal muscles by high levels of current at sites in deprived cortex may reflect the normal potential of that cortex. Our finding that proximal movements can be evoked by low-threshold currents indicates that changes in the motor system have occurred.

Does M1 reorganize in a similar way in all primates?

The present findings are extremely similar to those obtained from a single macaque monkey with a long-standing arm amputation that was studied under quite different stimulation conditions (chronic recording chamber; ketamine sedation) (Schieber and Deuel 1997). As for the present cases, stump movements were evoked at normal current levels from sites throughout the forelimb portion of M1. The major difference is that in this previous study there was evidence for stimulation sites in reorganized cortex where higher current or longer stimulation trains were needed to evoke movements, while for the present study current levels for threshold movements from reorganized cortex were in the normal range. The results obtained from both studies on macaque monkeys are also quite similar to those obtained from New World squirrel monkeys and prosimian galagos with forelimb amputations (Wu and Kaas 1999). In two squirrel monkeys and one galago with forelimb amputations, the deprived forelimb cortex became devoted to the stump and shoulder, and the threshold current levels needed to evoke minimal movements were in the normal range for many sites, although thresholds were higher than normal for many other sites. In these primates, motor cortex is exposed on the dorsolateral surface of the brain, so it was likely that all stimulation sites were in or close to layer V. Thus cortical reorganization appeared to be extensive in all studied primates, while stimulation thresholds for cortical sites varied from those in the normal range to those requiring higher levels of current. In the present study, we should allow for the possibility that the extensive reorganization was incomplete since some sites did not evoke movements. As with the macaque monkeys, the hand-face border in the squirrel monkeys and galago did not show a detectable shift in location.

After a hindlimb amputation in a macaque monkey, hip movements were evoked throughout the deprived cortex. Similar results were obtained after a hindlimb loss in a squirrel monkey and a galago (Wu and Kaas 1999). Thus across these primate species, there are three cases with comparable results. Hindlimb cortex appears to reorganize similar to forelimb cortex after amputations.

Our results from microelectrode stimulation maps in macaque monkeys also compare well with those from TMS in humans. The majority of early TMS studies in humans involved forelimb amputees. The overall finding is that more stimulation sites on the scalp contralateral to the amputation activated remaining muscles proximal to the stump than sites on the scalp ipsilateral to the amputation activated the corresponding muscles in the normal limb (Chen et al. 1998; Cohen et al. 1991; Dettmers et al. 1999; Fuhr et al. 1992; Hall et al. 1990; Kew et al. 1994; Ridding and Rothwell 1995). This suggests that more cortex contralateral to the amputations is devoted to the stump and shoulder muscles. Similar overall patterns of reorganization in motor cortex have been described in humans after a lower limb amputation (Fuhr et al. 1992; also see Woolsey et al. 1979; Chen et al. 1998). Also, the data from TMS studies in humans suggest that neurons in reorganized cortex are more responsive than normal since greater responses in stump muscles could be evoked by the same levels of TMS than in the same muscles in the normal limb. Alternatively, the greater responses produced in stump muscles by TMS may reflect the additive effects of activating a greater number of M1 neurons devoted to upper arm and shoulder movements in reorganized cortex (see Wu and Kaas 1999).

Because of the long recovery periods after amputation, the present results do not provide much information about the time course of reorganization in M1. It is likely that the removal of afferent drive by a limb amputation causes an immediate or rapid reweighting of synaptic effectiveness in the somatosensory system and in the motor system as the result of altered and reduced inhibition (see Kaas and Florence 2000). Some of the results suggesting immediate changes in the organization of motor cortex in rats (Huntley 1997; Jacobs and Donoghue 1991) and in humans (Brasil-Neto et al. 1993; Ziemann et al. 1998) after forelimb amputation or ischemic deafferentation provide support for the possibility that this mechanism accounts for some of the reorganization seen in motor cortex of monkeys. Other changes involving activity-dependent gene expression and alterations in neuromodulators and transmitter, receptor sites, and neurotrophic factors can take minutes to days to occur (see Kaas and Florence 2000), and they may be important as well. However, extensive reorganizations of somatosensory cortex after massive sensory loss can take months to emerge (Jain et al. 1997). Arm amputations and spinal cord injuries have been shown to result in substantial new growth of intact sensory afferent terminations in the brain stem (Florence and Kaas 1995; Jain et al. 2000) and considerable growth of horizontal axons in deprived somatosensory cortex (Florence et al. 1998). Corticospinal projections from deprived cortex remain intact (Wu and Kaas 1999) and perhaps even grow. Spinal cord deprived motor neurons even appear to target remaining proximal muscles (C. W. H. Wu and J. H. Kaas, unpublished data). Given that the monkeys in the present study were studied years after injury, it would seem unlikely that only short-term mechanisms of reorganization and recovery were involved.

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REFERENCES


REORGANIZATION OF PRIMARY MOTOR CORTEX


