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Reorganization in Primary Motor Cortex of Primates with Long-Standing Therapeutic Amputations

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Intracortical microstimulation was used to investigate the organization of primate primary motor cortex (M1) in three squirrel monkeys and two galagos years after the therapeutic amputation of an injured forelimb or hindlimb. In two squirrel monkeys with forelimb amputation, physiological results were correlated with the distribution of corticospinal neurons after injections of tracers into the lower cervical segments of the spinal cord. Distributions of labeled corticospinal neurons helped identify the locations of the former forelimb cortex in M1. Evoked movements from M1 ipsilateral to the missing limb were not obviously different from M1 of normal controls. Stimulation in the deefferented part of M1 contralateral to the missing limb elicited movements of the remaining proximal muscles as well as movements from adjacent body representations in all cases. Stimulation in the deefferented forelimb cortex evoked shoulder stump, trunk, and orofacial movements, whereas stimulation in the deefferented hindlimb cortex evoked hip stump, trunk, and tail movements. Movements were evoked from all sites in the deprived cortex, so that there were no unresponsive zones. Minimal levels of current necessary to evoke these movements varied from those in the normal range to those of much higher levels, with the average threshold higher than normal. Finally, multiunit recording from the two galagos revealed that the deprived portions of S1 were responsive to touch or taps on the stump and neighboring body parts.

Key words: plasticity; microstimulation; frontal lobe; somatosensory cortex; monkeys; prosimians

Presently, we have only a limited understanding of what happens to motor cortex in adult mammals after the loss of some of the muscles this cortex controls. Much of what we know comes from early studies that examined changes in the primary motor cortex (M1) of rats after amputation of the forelimb or section of the facial motor nerve (Sanes et al., 1988, 1990; Donoghue et al., 1990). After these manipulations, the adjacent normal parts of the representation in M1 appeared to have expanded to create a reorganized field, and microstimulation in the deefferented portion of M1 evoked movements of remaining body part at normal or lower than normal levels of current. Findings consistent with this interpretation have been obtained by transcranial magnetic stimulation of motor cortex in humans with amputated limbs (Cohen et al., 1991; Pascual-Leone et al., 1996). Although the results from this approach are less precise and open to more interpretation than those from microstimulation, stump movements were evoked from stimulation sites over deefferented motor cortex. Similar but somewhat different results were obtained from a single adult macaque monkey long after the loss of an arm (Schieber and Deuel, 1997). Although movements of the remaining shoulder girdle and arm stump could be evoked by intracortical microstimulation (ICMS) throughout the presumed deprived forelimb region of the contralateral M1, higher stimulus currents were often needed than the opposite M1.

In the present study, we stimulated motor cortex in three squirrel monkeys and two galagos years after amputations of a forelimb or hindlimb. In each case, the animals had been injured to such an extent that a therapeutic amputation was necessary. Our studies of motor cortex with squirrel monkeys and galagos were aided by the fact that these primates have only a short, shallow central sulcus, and motor cortex is exposed on the dorsolateral surface of the cortex, which allows a thorough systematic mapping. In addition, we used injections of tracers into the cervical spinal cord of two squirrel monkeys with forelimb loss to label corticospinal neurons related to forelimb movements as an aid to identifying the forelimb portion of M1.

With these primates, we sought to determine the following questions. First, what happens in the deefferented M1 after the loss of a limb? Would there be a region of deefferented cortex where electrical stimulation failed to evoke movements, or would adjacent body representations invade the deefferented cortex? Second, would stimulation thresholds in the deefferented cortex be normal or changed? Third, would the nature of the reorganization vary with such factors as species, age, and site of amputation? Finally, reorganization of somatosensory cortex after amputation has been studied in monkeys (Florence and Kaas, 1995) but not in prosimian galagos. Thus, we also used microelectrode recording to determine whether primary somatosensory cortex (S1) in galagos is reorganized in a manner similar to the reorganization that occurs in monkeys.

MATERIALS AND METHODS

Microelectrodes were used to electrically stimulate many sites in motor cortex of three adult squirrel monkeys and two galagos with long-standing amputations of a limb. In an effort to determine the organization of motor cortex related to the missing limb, cortex from normal squirrel monkeys and galagos as well as cortex contralateral to the intact limb of the amputees were used as comparison. Injections of tracers in the lower cervical spinal cord of two forelimb-amputated squirrel mon-

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Table 1. Summary of information on the squirrel monkeys and galagos with long-lasting limb amputation used in this study

Amputee cases	Age of amputation	Extent of amputation	Survival duration (years)	ICMS	S1 recording	Spinal cord injection sites
Squirrel monkeys						
Case 98-61	2 months old	Near shoulder joint	8	+		C6–C7
Case 98-64	4 months old	Shoulder joint	5	+		C7–C8
Case 97-127	6 years old	Near hip joint	12	+		
Galagos						
Case 97-100	1.5 months old	Shoulder joint	4	+	+	
Case 97-134	1.5 months old	Hip joint	7	+	+	

keys labeled corticospinal neurons in M1, which helped identify the extent of cortex formerly devoted to the missing limb.

Animals. After an extensive search, we were able to obtain three adult squirrel monkeys (*Saimiri sciureus*) and two galagos (*Galago garnetti*) with long-standing therapeutic amputations of a limb. Each of these monkeys had previously received injury to a limb that was serious enough that the treatment was surgical amputation of the limb. Except for one animal, all of them received the amputation before they were adults. Each of these primates lived 4 or more years after the amputation (Table 1). Results were compared with those obtained from two normal adult squirrel monkeys and four normal adult galagos. All surgeries were performed under asceptic conditions, and animals were cared for in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the guidelines of the Vanderbilt Animal Care and Use Committee.

Injection of tracers. Each of the two squirrel monkeys that received spinal cord injections was premedicated with dexamethasone (2 mg/kg, i.m.) and Robinul (0.015 mg/kg, i.m.) and then anesthetized to surgical levels with isofluorane gas. Because the motoneurons that innervate hand muscles are exclusively located in the lower cervical segments (Kuypers, 1981; Jenny and Inukai, 1983), these segments were chosen for tracer injections. A short segment of the lower cervical spinal cord was exposed after identifying vertebrae and dorsal root entry zones (Hill, 1974; Stevens et al., 1981). The dura was opened on both sides, and wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP, 2% in saline) was injected into segments C6–C7 (case 98-61) or C7–C8 (case 98-64). Two bilaterally symmetrical 0.5 μl injections were placed at the depths of the intermediate zone and the ventral horn of the spinal cord gray matter of each side. The injections were placed with a fine-tipped glass micropipette attached to a 1 μl Hamilton syringe. The syringe entered the spinal cord at an angle just lateral to the dorsal columns to avoid damage to the dorsal column and the dorsolateral funiculus. The opening was closed, and muscles as well as skin were sutured. Anesthesia was discontinued, and recovery was rapid and uneventful. Normal movements of the hand were observed after recovery from anesthesia. Post-surgical care included treatment with antibiotics and analgesics.

Surgery and perfusion. In general, experimental procedures followed those used previously in the laboratory (Preuss et al., 1996). For the microstimulation and somatosensory recording sessions, each monkey was anesthetized to surgical levels with an initial injection of Telazol (Tiletamine HCl and Zolazepam HCl, Fort Dodge Laboratories Inc.) of 10–20 mg/kg, i.m. Anesthesia was maintained by subsequent injections of one-third of the initial dose as needed. The anesthetized animals were placed in a stereotaxic apparatus for mapping. Some of the body hair was clipped so that muscle movements could be easily observed.

For case 98-64, frontal cortex was exposed contralateral to the missing limb under sterile conditions, and the cortex was kept moist with sterile saline. After a microstimulation session, the cortex was covered with sterile, absorbable gelatin film, and the bone flap was replaced over cortex and cemented in place with dental acrylic. The skin and muscle were sutured. Anesthesia was discontinued, and antibiotics and analgesics were given as a precaution. One week later, the spinal cord was injected, and 5 d later, motor cortex contralateral to the intact limb was also stimulated as part of a terminal procedure. In case 98-61, the spinal cord injections were placed before cortical stimulation, and cortex was exposed and stimulated bilaterally 6 d later. One squirrel monkey and two galagos with amputation received no spinal cord injections, and motor cortex was stimulated unilaterally contralateral to the missing limb. In addition, microelectrode recordings were obtained from somatosensory cortex in the two galagos with amputations. The motor cortex of two normal squirrel monkeys and four galagos was stimulated unilaterally for comparison. During the terminal sessions, cortex was protected with silicone fluid to prevent dessication.

At the end of these stimulation sessions, reference lesion sites were marked in cortex by passing a 10 μA DC current for 10 sec at several depths in microelectrode penetrations. The animals were then given a lethal dose of sodium pentobarbital. When they became areflexive, they were perfused transcardially with PBS, followed first by a cold solution of 4% paraformaldehyde and next by a mixed solution of 4% paraformaldehyde and 10% sucrose. Blocks of brain and spinal cord were removed and stored overnight in 30% sucrose at 5°C before cutting.

Motor mapping. Low-impedance tungsten microelectrodes (0.9–1.1 MΩ at 1 kHz, Microprobe, Inc.) were used to stimulate cortex. For most of the M1 region, electrodes were lowered perpendicularly to the brain surface and with a hydraulic microdrive to a depth of ~1500 μm below the surface, approximating the level of cortical layer V, from which movements can be elicited with the lowest levels of current (Stoney et al., 1968; Asanuma and Rosen, 1972; Sato and Tanji, 1989). In addition, some stimulation sites were in cortex along the medial wall of the cerebral hemisphere, which was reached by deeper electrode penetrations that started on the dorsal surface ~1–1.5 mm from the midline. The microstimulation currents were delivered in 60 msec trains, with a pulse duration of 0.2 msec and a pulse frequency of 300 Hz. All penetration sites were first stimulated with a current level that was likely to be above threshold (10–30 μA). If movements were reliably elicited, the current was gradually reduced until movements no longer occurred. Threshold was defined as the current level at which the last just noticeable movements were observed. If a moderate level of stimulation failed to produce movements, current level was increased to as high as 400 μA. Unresponsive sites were defined as sites from which movements could not be evoked at the current level of 400 μA. However, most sites (>85%) in the explored cortex were responsive at levels well <60 μA. Because electrode displacements as small as 100 μm with currents <30 μA can produce entirely different movements and electromyographic responses (McGuinness et al., 1980; Strick and Preston, 1982), the effective spread of current in the present study was generally likely to be less than the distances between penetrations (see Stoney et al., 1968; Nudo et al., 1990).

Movements were detected visually by two observers. Each responsive site was characterized by visible body movements at the threshold current. For convenience, movements were grouped into categories involving major body parts (Table 2). In brief, the orofacial cortex included any movements involving the mouth and face. The forelimb movements involved the actions of the muscle groups and joints of the shoulder, arm, and hand. The trunk cortex included upper and middle torso movements. Finally, the hindlimb cortex comprised movements of the lower body, including the lower trunk, hip, leg, foot, and tail. Within each main body representation, movements were described using terminology commonly applied (Gould et al., 1986; Preuss et al., 1996). Hand movements were assigned to sites that included those for which wrist and digit movements were not clearly dissociable. Similarly, foot movements included ankle and toe movements that were not clearly dissociable. Arm and leg movement assignments were made when the movement involved several sections of the limb.

To describe the ICMS results from amputees that received entire limb removal up to the shoulder or hip joints, we further categorized the limb movement into two subgroups. Within the forelimb cortex of the normal control, movements elicited by activity of shoulder girdle were defined as

Table 2. Categories of body movements and abbreviations

Orofacial cortex	
Jaw	J
Check	Chk
Chest	Ch
Ear	
Eye blink	EB
Eyebrow	
Mouth	Mo
Neck	Ne
Nose	
Lower eyelid	l.eyelid
Throat	
Tongue	Ton
Upper lip	u.lip
Whiskers	
Forelimb cortex	
Arm	A
Digits	Digs
Forearm	fA
Hand	H
Shoulder	Sh
Upper arm	u.A
Trunk cortex	
Lower trunk	l.Tr
Mid trunk	m.Tr
Upper trunk	u.Tr
Hindlimb cortex	
Foot	Ft
Leg	L
Lower leg	l.L
Tail	
Toes	
Upper leg	u.L
Hip	

"shoulder" forelimb movements, and any movements involving the rest of the forelimb were defined as "nonshoulder" forelimb movements. The few sites (<8% in normal squirrel monkeys and <13% in normal galagos) in the forelimb cortex from which axial body movements were elicited, including neck, chest, or trunk, were referred to as "nonlimb" movement sites. In the amputated animals, contractions in both shoulder muscles and stump were observed in few sites. Movements involving muscles of shoulder, and/or stump were classified as "shoulder/stump" movements. Movements in hindlimb cortex were similarly classified into the "hip," "nonhip," and "tail" hindlimb movement categories for normal animals, as well as "hip/stump," "nonhip," and "tail" for hindlimb-amputated animals.

Somatosensory recording sessions. In two galagos microelectrode recordings were obtained from S1 in addition to the motor cortex mapping. Multiunit activity was recorded in the middle layers (1000–1200 μm) of area 3b in the two limb-amputated galagos by advancing a low-impedance tungsten microelectrode (0.9–1.1 MΩ at 1 kHz) perpendicular to the surface of the brain. The procedure for somatosensory recordings was similar to that described elsewhere for galagos (Sur et al., 1980). In brief, neuronal activity was recorded during cutaneous stimulation of the body using fine probes and camel hair brushes. Body parts were gently tapped, and joints were manipulated when cutaneous stimuli failed to evoke neuronal activity. At each recording site, the receptive field was defined as the skin area from which near-threshold stimuli effectively evoked responses. Neuronal responses were amplified, filtered, viewed on an oscilloscope, and heard through a loudspeaker.

Histology and anatomical analysis. Blocks of brain that contained frontal and adjoining parts of parietal cortex were cut on a freezing microtome at 40–50 μm in the coronal plane. Sets of one-in-six sections were stained for Nissl substance with cresyl violet or were processed with tetramethylbenzidine to reveal WGA-HRP (Gibson et al., 1984). Other sets of sections were processed for cytochrome oxidase (Wong-Riley, 1979) or acetylcholinesterase (Geneser-Jensen and Blackstad, 1971) to aid in the architectonic identification of motor cortex. The cervical spinal cord was cut at 60 μm in the coronal plane, and every one of six sections was processed for WGA-HRP or Nissl substance. Some tissue was also processed with other histochemical and immunocytochemical methods for subsequent analysis.

Injection sites in the spinal cord were defined as the zone that contained the densest reaction product where labeled cells were masked by the densely stained neuropil (Mesulam, 1978). The extents of the injection sites and the transported tracer were determined under dark- and bright-field illumination. Drawings of cortical sections were used to reconstruct surface views of the cortical distribution of labeled corticospinal neurons. These drawings included architectonic boundaries, microlesions placed for reference, blood vessels, sulci patterns, and other landmarks so that the electrophysiological results could be related to the brain sections and the reconstructed surface view.

RESULTS

The effects of ICMS in M1 within and around the portion formerly devoted to a missing limb were examined in three animals with forelimb amputations, including two squirrel monkeys and one galago, as well as two animals with hindlimb amputations, including one squirrel monkey and one galago. In addition, two normal squirrel monkeys and four galagos were used as a control group for comparison. We first describe the ICMS results from the squirrel monkeys, followed by the ICMS results from the galagos, and finally the somatosensory recording results from two galagos.

In two forelimb-amputated squirrel monkeys, results were obtained from cortex contralateral and ipsilateral to the missing limb. The portion of cortex in both hemispheres devoted to the normal forelimb or the missing limb was identified by ICMS results, by location relative to brain surface landmarks, and by the distribution of labeled corticospinal neurons projecting to the lower cervical spinal segments that control forelimb muscles. Thus, results from cortex ipsilateral to the missing limb served as one control. Another control was to use identical methods to stimulate and map M1 as well as parts of adjoining cortical fields in two normal squirrel monkeys. ICMS results from one forelimb-amputated galago and two hindlimb-amputated animals were compared with those from four normal animals of the same species.

ICMS mapping in squirrel monkeys

M1 of normal animals

M1 is located ~1–2 mm rostral to the short, shallow central sulcus in squirrel monkeys (Fig. 1, also see Figs. 2, 3, 6). Traditionally, M1 has been defined by its overall somatotopic pattern of evoked movements at low threshold of current and by its agranular cytoarchitectonic appearance with large pyramidal cells in layer V (Gould et al., 1986; Donoghue et al., 1992; Stepniewska et al., 1993; Preuss et al., 1996). In the present study, M1 was delineated by noting elevation of thresholds for evoked movements in cortex rostral and caudal to it. In addition, the cytoarchitecture of the region defined as M1 was examined in coronal brain sections in every case, and at least the bulk of the region physiologically defined as M1 was clearly agranular M1.

The major somatotopic organization of M1 is apparent from the representative map of one of the normal squirrel monkeys shown in Figure 1. The map corresponds to a surface view of cortex just rostral to the central sulcus, with electrode penetrations marked. Next to each penetration, the threshold level of current and the movement evoked from the site at threshold are given. Results from our second normal squirrel monkey (not

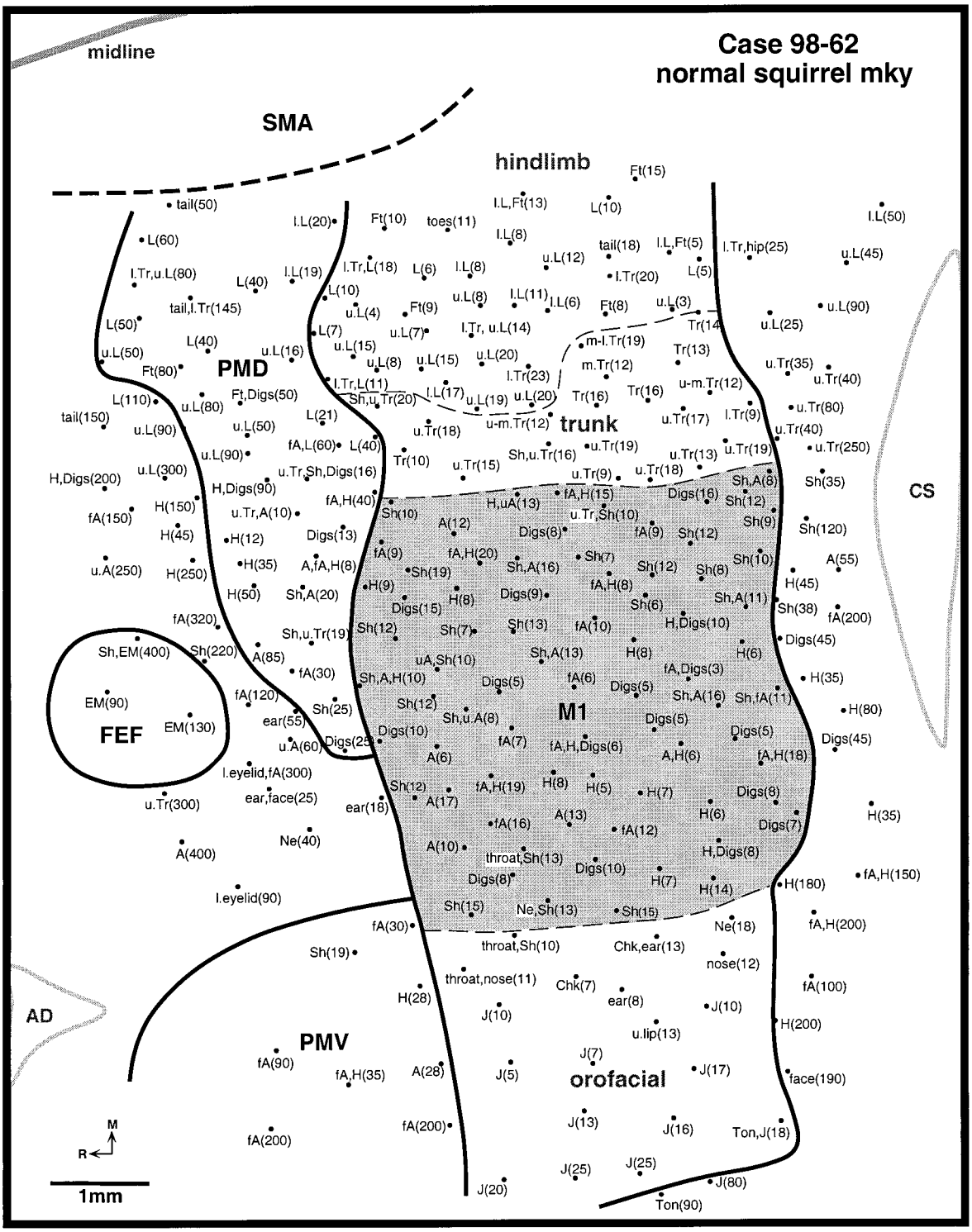

Figure 1. Organization of motor cortex in a normal squirrel monkey. Areas M1, PMD, and PMV are outlined on the dorsolateral surface of the frontal lobe. The shallow central sulcus (*CS*) is opened on the *right*, and the arcuate dimple (*AD*) is on the *bottom left*. The frontal eye field (*FEF*) and the estimated location of SMA are indicated. *Dots* mark electrode penetration sites. Next to each dot, the body movement evoked by electrical stimulation is abbreviated (see Table 1 for abbreviations). The *number* in *parentheses* next to each abbreviation indicates the current used to evoke movements at threshold. *Dotted lines* separate the forelimb region (*shaded*) from orofacial, trunk, and hindlimb regions of M1. In the forelimb cortex of M1, nonlimb movements could be elicited at few sites and are highlighted with a *white background*. *EM*, Eye movements.

shown) were similar to those shown in Figure 1 (Table 3). The borders and the estimated sizes of forelimb cortex [19.6 mm^2 (see Fig. 1) and 18.1 mm^2 (results not shown)] of M1 from these two normal animals were similar. The details of the internal organization within the specific body parts varied case by case, but the global order was the same. The average threshold for each major body movement from the two normal squirrel monkeys is shown in Table 3.

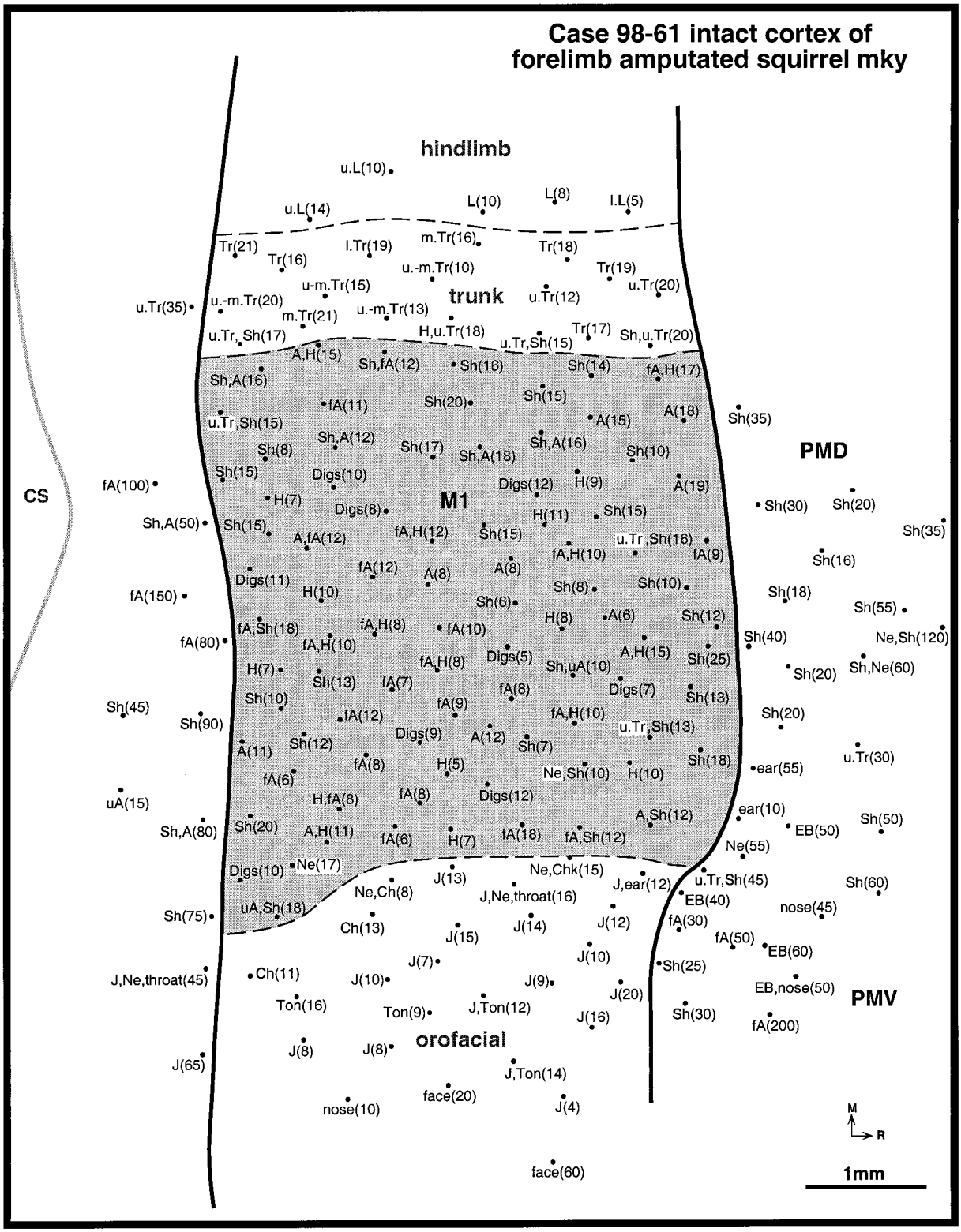

Figure 2. Organization of M1 ipsilateral to an amputated forelimb in a squirrel monkey (98-61). Conventions are as in Figure 1. See Figure 6 for the position of M1 on a dorsolateral view of the cerebral hemisphere.

The first point to stress is that threshold levels in cortex caudal and rostral to the region we define as M1 are higher than in M1. The M1 we define in this manner corresponds closely to that identified by electrical stimulation in other studies (Donoghue et al., 1992; Nudo et al., 1992; Sanes and Donoghue, 1992). The second point is that the characteristic global organization of M1 is apparent, with a zone of cortex related to the leg and tail most medial, cortex involving the trunk next, followed by a large, more

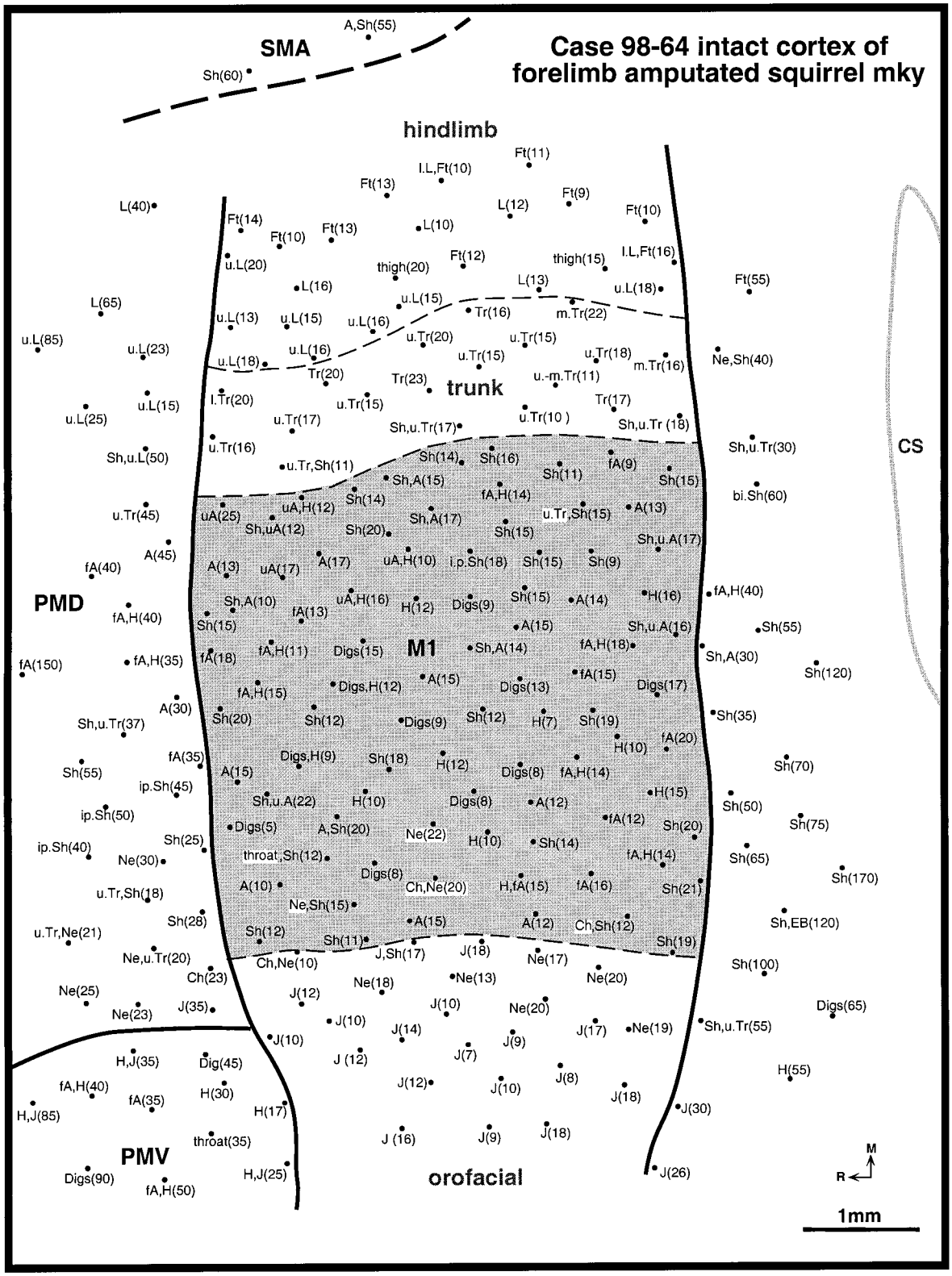

Figure 3. Organization of M1 in cortex ipsilateral to an amputated forelimb in a second squirrel monkey (98-64). Conventions are as in Figure 1. See Figure 6 for the position of M1 on a dorsolateral view of the cerebral hemisphere. *Bi*, Bilateral; *ip*, ipsilateral.

lateral zone representing the forelimb and digits, and finally the most lateral zone devoted to the face and the mouth. There was no attempt to fully define the medial and lateral borders of M1 physiologically. However, these borders were delineated cytoarchitectonically in coronal brain sections. Third, movements were evoked in a parallel topography at higher thresholds from area 3a, as expected, and in different topography and at higher thresholds from regions rostral to M1 that correspond to the dorsal and

Table 3. Average current thresholds and frequencies for evoked movements in M1 of control and amputated squirrel monkeys

		Forelimb cortex				Hindlimb cortex		
	Orofacial cortex	sh/sh-stump,	non-sh,	nonlimb	Trunk cortex	hip/hip-stump,	nonhip,	tail
Normal control								
Case 98-62								
Threshold (μA)	13.6	11.4	9.9	12.0	14.9		(11.8)	
Frequency (%)		38.7	72.0	4.0		14.7	91.2	3.0
Case 98-63								
Threshold (μA)	12.8	15.0	13.3	14.8	17.7		(18.9)	
Frequency (%)		34.8	71.0	7.2		25.0	82.5	10.0
Average of cases 98-62 and 98-63								
Threshold (μA)	13.2	13.2	11.6	13.4	16.3		(15.4)	
Frequency (%)		36.8	71.5	5.6		19.9	86.9	6.5
Forelimb amputee								
Case 98-61								
M1 contralateral to intact limb								
Threshold (μA)	12.1	13.8	10.7	14.2	17.1			
Frequency (%)		41.6	67.4	5.6				
M1 contralateral to amputated limb								
Threshold (μA)	10.3	29.9		26.5	17.4			
Frequency (%)		80.0	0	32.6				
Case 98-64								
M1 contralateral to intact limb								
Threshold (μA)	13.8	15.3	13.5	16.0	16.7			
Frequency (%)		41.4	66.7	6.9				
M1 contralateral to amputated limb								
Threshold (μA)	14.5	28.8		48.7	17.6			
Frequency (%)		86.5	0	22.5				
Hindlimb amputee								
Case 97-127								
Threshold (μA)			(12.9)		17.2		(40.7)	
Frequency (%)						70.8	0	31.3

Within each main body representation of M1, movements can be further subcategorized. In the control and normal cortex, movements from shoulder (sh), arm and hand (non-sh), as well as trunk and face (nonlimb) make up the forelimb cortex. Similarly, movements from hip, leg, and foot (nonhip), as well as tail make up the hindlimb cortex. In contrast, the deefferented cortex produced movements of the shoulder and stump (sh-stump) as well as nonlimb movements in the forelimb amputees or hip and stump (hip-stump) as well as tail movements in the hindlimb amputee. The average threshold and the frequency are shown for each subcategory of movement in forelimb and hindlimb cortex except when the subcategorization is not shown and, instead, the average threshold for the main body representation is given in parentheses. Note that because some sites elicited more than one type of movement, the sum of the frequencies can be >100%.

ventral premotor areas (PMD and PMV, respectively) (Preuss et al., 1996; Wu et al., 1997), as well as the frontal eye field (Huerta et al., 1986). Fourth, the results indicate that a given movement, of digits, for example, often can be evoked from several discontinuous locations in M1. The mosaic pattern of local organization of M1, first stressed for M1 of owl monkeys (Gould et al., 1986), has been repeatedly confirmed in squirrel monkeys (Donoghue et al., 1992; Nudo et al., 1992, 1996; Sanes and Donoghue, 1992). Fifth, as it has been described in earlier studies, suprathreshold levels of current stimulation typically evoked movements in addition to those revealed by low threshold levels of stimulation, and these additional movement patterns were not always related to the movements of neighboring sites (Sanes et al., 1990; Huntley and Jones, 1991; Nudo et al., 1996). Finally, the internal organization of the forelimb cortex and hindlimb cortex of M1 in our two normal squirrel monkeys did not differ notably from previous descriptions of forelimb or hindlimb cortex (Gould et al., 1986; Donoghue et al., 1992; Nudo et al., 1992; Preuss et al., 1996) in New World monkeys, although some details differed, possibly because of individual differences in monkeys or experimental differences in procedures and movement detection. Additionally, details of somatotopic organization and thresholds for evoked movements appear to be modifiable by experience and motor training (Nudo et al., 1992; Sanes and Donoghue, 1992) and even changes in limb position (Sanes et al., 1992). Thus, normal organization is somewhat broadly defined, and normal monkey studies under the same experimental conditions probably serve as the best comparison group for monkeys with amputations.

M1 contralateral to the intact limb of amputees

Although we used cortex contralateral to the intact limb as a control for comparison to cortex contralateral to a missing limb, this cortex may not be completely normal, and altered use of the intact limb may have changed the organization of motor cortex (Nudo et al., 1992). Yet, our physiological results from ipsilateral cortex did not reveal any obvious difference from our normal controls. In both cases, cortex contralateral and ipsilateral to the missing limb was mapped with large numbers of closely spaced stimulation sites (Figs. 2–5). As for the M1 of normal squirrel monkeys, the global organization of M1 contralateral to the intact limb of the two forelimb-amputated squirrel monkeys proceeded from hindlimb to trunk to forelimb to orofacial (Figs. 2, 3). The

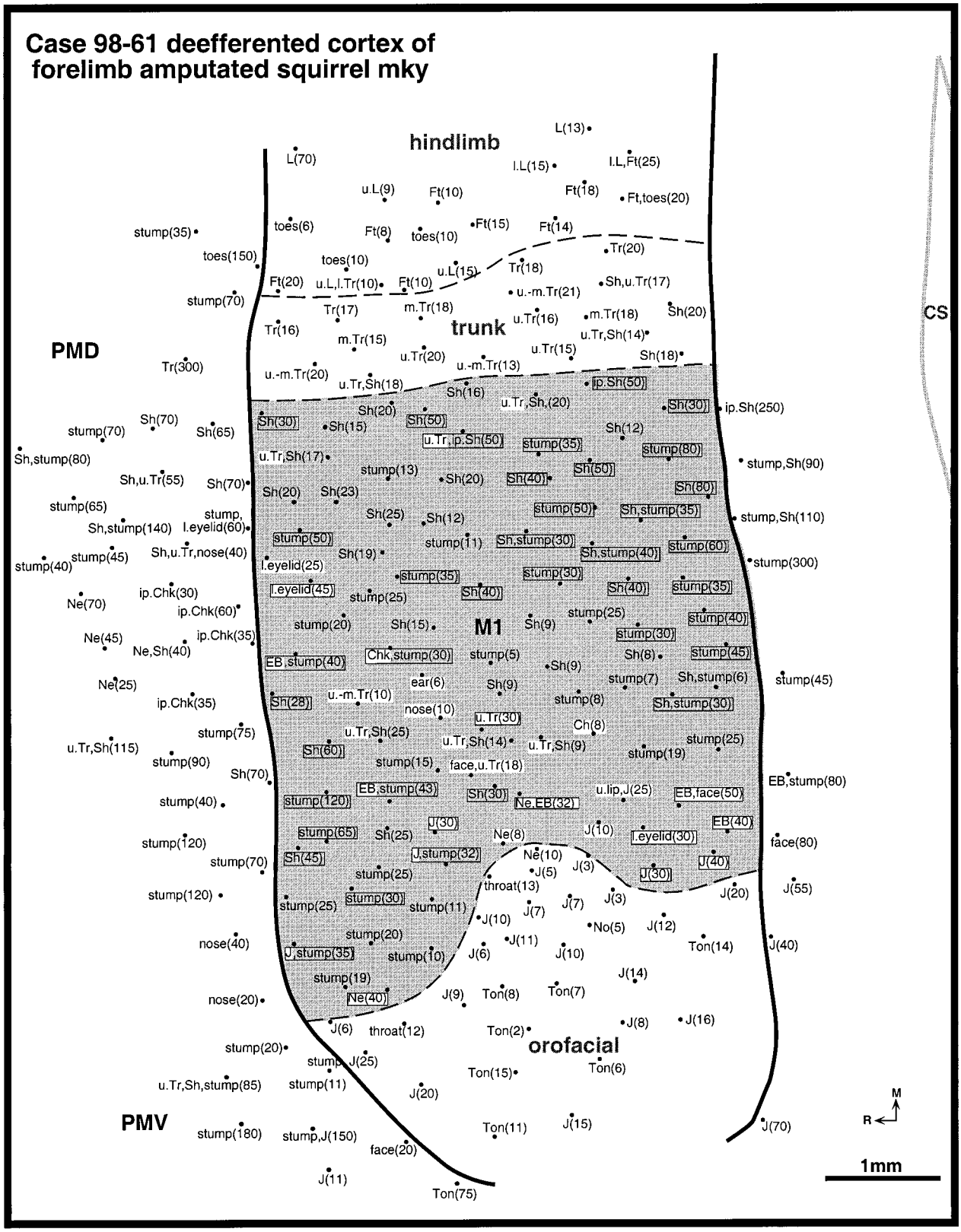

Figure 4. Organization of motor cortex contralateral to the amputation of a limb at the level of the upper arm near the shoulder joint in squirrel monkey case 98-61. Electrical stimulation evoked movements throughout the forelimb cortex (*gray shading*), with much of the cortex devoted to stump and shoulder muscles. Moreover, sites evoking nonlimb movements (highlighted by a *white background*) were significantly increased. In the reorganized forelimb cortex, many sites required threshold currents greater than the highest current threshold (25 μA) from the opposite hemisphere; these high-threshold sites are outlined. Conventions are as in Figures 1 and 3. See Figure 6 for the position of M1 on a dorsolateral view of the cerebral hemisphere.

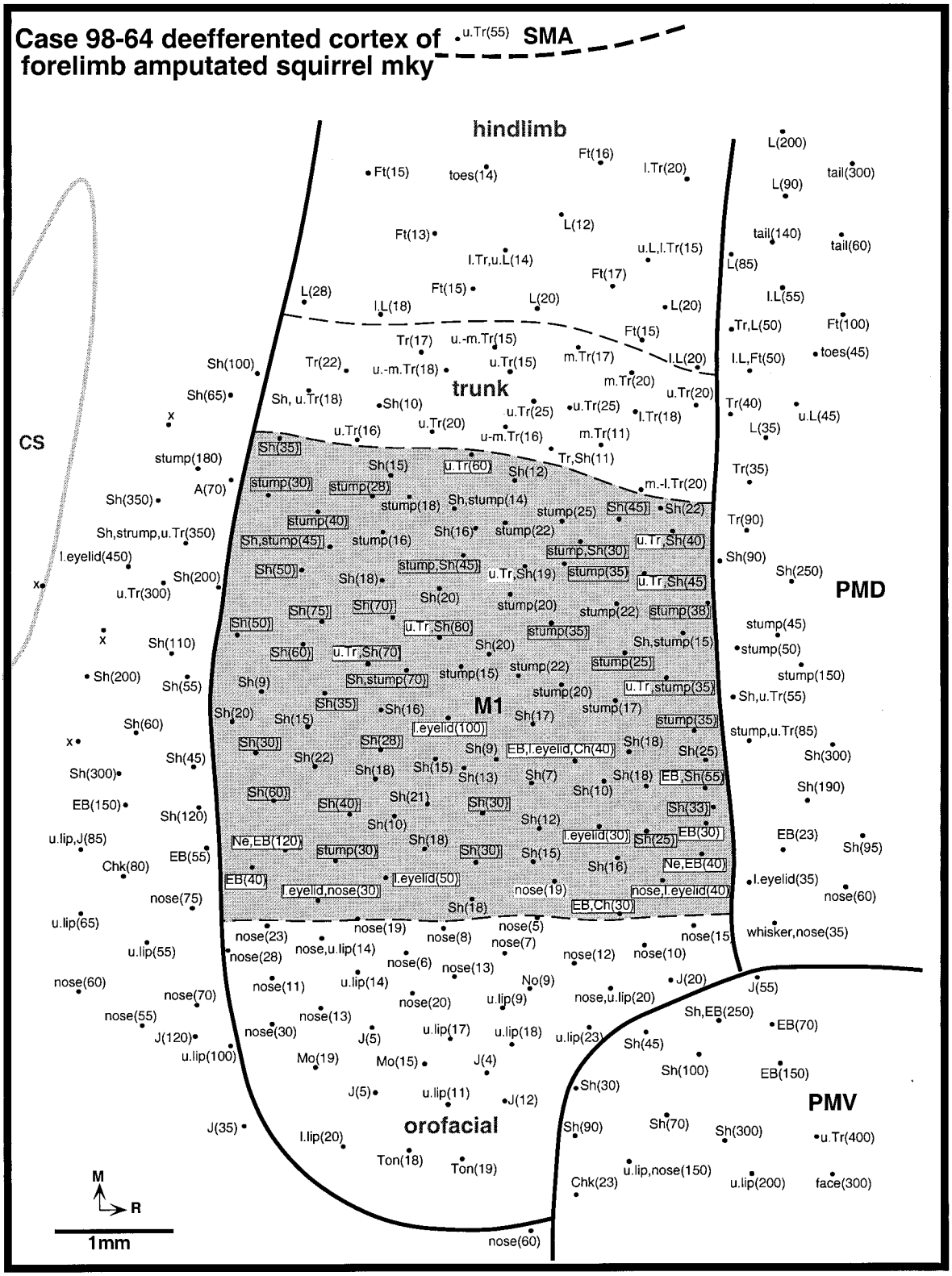

Figure 5. Organization of motor cortex contralateral to an amputated limb at the level of the shoulder joint in squirrel monkey 98-64. Conventions are as in Figures 1, 3, and 4. See Figure 6 for the position of M1 on a dorsolateral view of the cerebral hemisphere.

forelimb cortex appears to be normal in size (19.8 and 17.9 mm^2 for cases 98-61 and 98-64, respectively) as well as in internal organization. Sites evoking digit movements were scattered among those evoking hand, arm, and shoulder movements. There was no significant difference in the percentage of sites evoking hand, shoulder, and nonlimb movements in the forelimb cortex

between hemispheres from the normal animals and the hemispheres contralateral to the intact forelimb (Table 3; χ^2 tests, $p > 0.05$). Most importantly, the thresholds for evoking these forelimb movements were similar between M1 contralateral to the intact limb and M1 of two normal controls (Table 3; t tests, $p > 0.05$). The same was true of the thresholds for the movements in trunk and orofacial cortex. Thus, these similarities indicate that M1 contralateral to the intact limb can be used as an important comparison with M1 contralateral to a missing limb.

In cortex contralateral to the missing limb, we wanted another way to define the forelimb region of M1 in addition to electrical stimulation of cortex, because the amputation would obviously alter the electrophysiological results, changing somatotopy and possibly stimulation thresholds. Thus, we injected the anatomical tracer WGA-HRP bilaterally into the lower cervical spinal segments where corticospinal afferents from the forelimb portion of M1 terminate (Kuypers, 1981; Bortoff and Strick 1993; He et al., 1993; Maier et al., 1997; Wu and Kaas, 1998) and motoneurons that innervate the forelimb muscles are located (Jenny and Inukai, 1983; Rouiller et al., 1996). We were, however, concerned that the spinal cord injections might impair spinal cord function and reduce the effectiveness of motor cortex in evoking movements. Thus, we looked for threshold and somatotopic changes in cortex contralateral to the intact limb in the monkeys with amputations, because both sides of the spinal cord had injections. As described above, thresholds in the cortex contralateral to the intact side were not significantly different from those in normal control cases (Table 3). Therefore, cortex contralateral to the intact limb appeared to be normal, although we had injected tracers into the spinal cord.

The spinal cord injection sites were confined to the gray matter covering a large portion of the intermediate zone and ventral horn throughout lower cervical segments C6–C7 or C7–C8. The spread of tracer involved the dorsal horn along the insertion track of the micropipette tip. The cortical locations of neurons labeled by the spinal cord injection in the two monkeys with forelimb loss were similar in both hemispheres (Fig. 6). The densest zones of label were found in the M1 forelimb cortex, and they covered the entire subdivision. In agreement with early reports, the labeled corticospinal neurons were found exclusively in cortical layer V, where they were distributed in an uneven pattern and constituted pyramidal neurons of various sizes (Murray and Coulter, 1981; Nudo and Masterton, 1990; Dum and Strick, 1991a; Wu and Kaas, 1998). Because the motoneuron pools that influence the control of distal forelimb muscles are most heavily represented in the lower cervical segments where tracer injections were placed, the uneven distribution of corticospinal neurons was consistent with the mosaic patterns of organization observed in the electrophysiological data. Labeled neurons were also observed in the border zones medial and lateral to the forelimb cortex, possibly from tracer injections involving the motoneurons of the medial cell column throughout the lower cervical enlargement that innervate the axial musculatures for shoulder and neck (Jenny et al., 1988; Ueyama et al., 1990; our unpublished observations) and possibly other motoneurons. However, the distribution extent of labeled neurons was symmetrical in the two hemispheres, and the densest region of labeled cortical neurons closely coincided with the forelimb cortex (He et al., 1993; Wu and Kaas, 1998). Thus, this labeled region appears to be a suitable way of defining the forelimb cortex in monkeys with a missing forelimb.

The extents of the forelimb cortex of M1 was judged to be comparable in both hemispheres (19.8 and 17.9 mm^2 for intact cortex compared with 19.2 and 16.8 mm^2 for deefferented cortex in cases 98-61 and 98-64, respectively; also see Fig. 6), and the same was true for the face and trunk cortex. Also, as expected, the corticospinal neurons retrogradely labeled by the lower cervical spinal cord injections were similarly distributed in the forelimb portion of M1 in both hemispheres. Note also that in both hemispheres injections equally labeled smaller numbers of neurons in areas 3a and 3b of somatosensory cortex and in premotor cortex. The great similarity in the location and extent of the main focus of labeled neurons in all four hemispheres effectively indicates that the procedure usefully identified the forelimb cortex, even after long-standing amputation of the forelimb. There was no evidence from these injections that long-standing forelimb loss alters the nature of the corticospinal projections, although some changes, not revealed by these methods, might have occurred.

M1 contralateral to the amputated limb

Overall, movements were evoked by ICMS throughout M1 contralateral to the missing limbs in three limb-amputated squirrel monkeys. M1 contralateral to the amputated limb had a global organization similar to M1 of normal control animals, so that mediolateral organization proceeded from hindlimb to trunk to forelimb to face. However, the patterns of movements and current thresholds for evoked movements in the deefferented cortex were quite different from normal. In contrast, ICMS sites located medial or lateral to deefferented cortex produced normal patterns of movements at low current thresholds as typical of M1 cortex and were comparable with those observed in the normal cases as well as the opposite hemisphere (compare Figs. 4, 5 with 1–3; also see Table 3).

The portions of M1 cortex normally corresponding to the forelimb cortex were revealed by the locations of dense concentration of labeled corticospinal neurons. The densely labeled zones contralateral to the amputated limbs were consistent with the forelimb cortex expected by either relative location in M1 or position in the opposite hemisphere. Within these regions, the internal organization of M1 was grossly abnormal. However, movements were evoked from all sites, and no unresponsive zones were found in the deeffferented cortex. A mixture of shoulder, stump, trunk, and orofacial movements were elicited throughout the regions at threshold levels ranging from normal to much higher than normal currents (5–120 μA; see Figs. 4, 5). For convenience, in Figures 4 and 5, we outlined the abnormally high threshold values for sites that exceed the maximum threshold current of any site in forelimb cortex of the opposite hemispheres.

Within the deefferented forelimb cortex, stimulation at ~80% of the sites resulted in movements of the shoulder or stump, or both (referred to as shoulder/stump movements), whereas stimulation at ~20% of the sites resulted in movements of the trunk or face (referred to as nonlimb movements). The deefferented portion of M1 had significantly more shoulder/stump movement sites than shoulder movement sites in the forelimb cortex of opposite hemispheres (case 98-61, $\chi^2 = 27.0$; $p < 0.001$; case 98-64, $\chi^2 = 37.1$; $p < 0.001$) and significantly more nonlimb movements sites involving trunk and orofacial muscles in the forelimb cortex than those in the forelimb cortex of opposite hemispheres (case 98-61, $\chi^2 = 19.6$; $p < 0.001$; case 98-64, $\chi^2 = 7.3$; $p < 0.01$). Moreover, the current thresholds for shoulder/stump movements in the deefferented hemisphere were significantly higher than those for shoulder movements in the opposite hemispheres (case 98-61, $t_{87} = 6.9$; $p < 0.001$; case 98-64, $t_{88} = 6.7$; $p < 0.001$; see Table 3, Fig. 7). The current thresholds for nonlimb movements within the

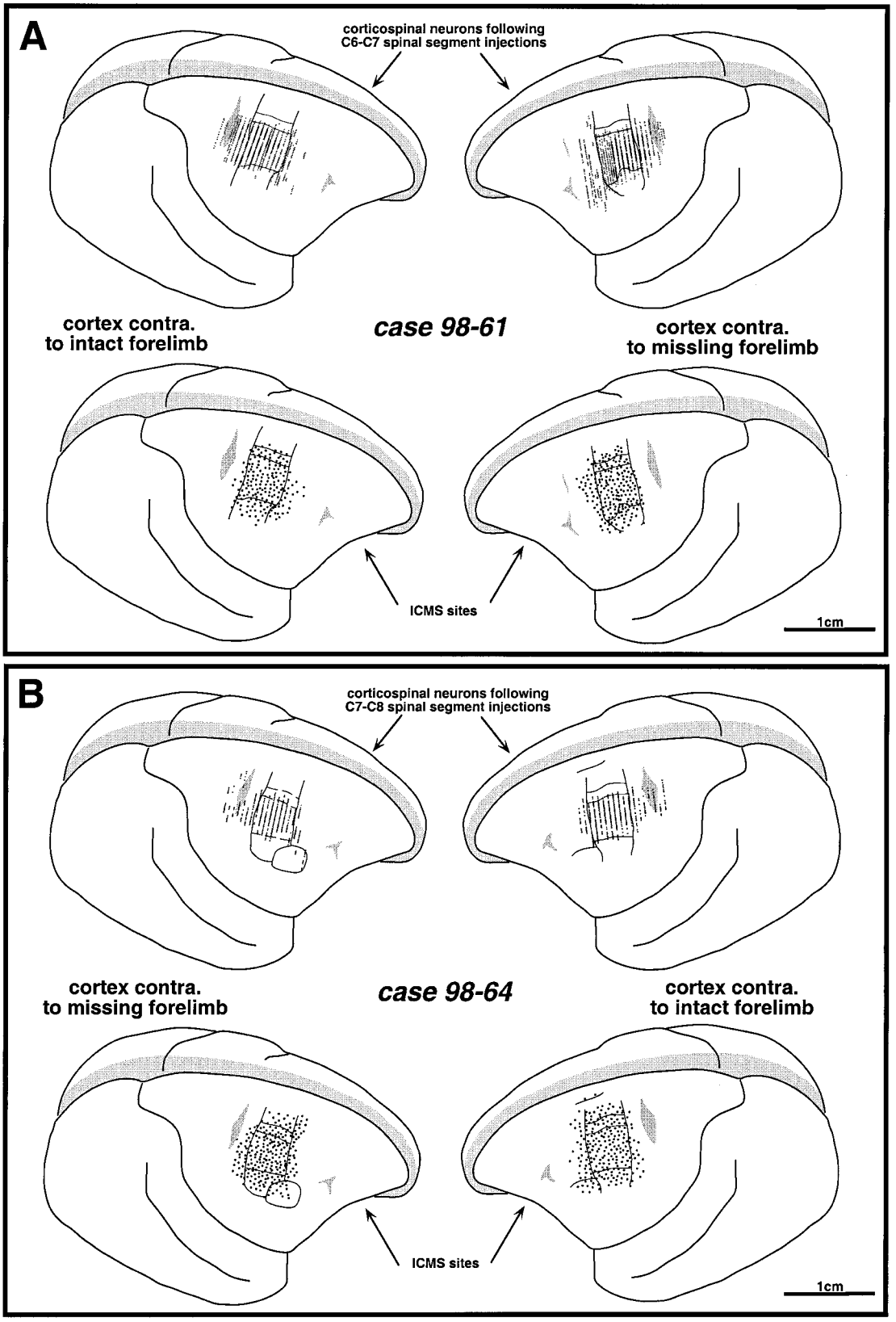

Figure 6. Locations of ICMS sites and labeled corticospinal neurons after tracer WGA-HRP was placed in the lower cervical spinal cord ipsilateral and contralateral to an amputated forelimb in two squirrel monkeys. The large, dense focus of labeled neurons in each hemisphere effectively indicates the forelimb region of M1 and is confirmed by ICMS (see Figs. 2–5 for detailed mapping results). Smaller foci of labeled neurons also mark forelimb regions of subdivisions of somatosensory and premotor cortex. *A*, Deefferented cortex is on the *right*. *B*, Deefferented cortex is on the *left*.

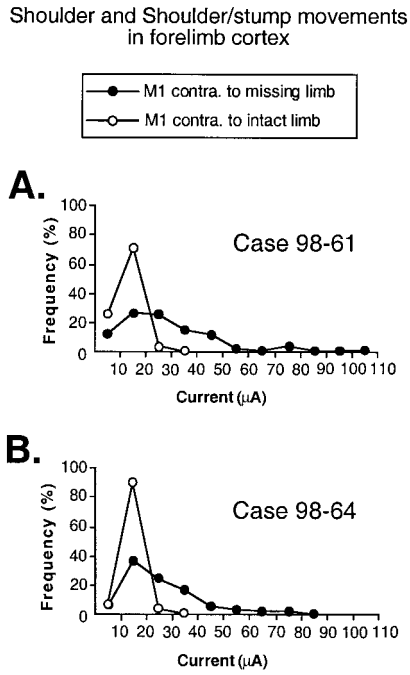

Figure 7. Frequency distribution of current thresholds for evoked shoulder/stump movements from sites in M1 forelimb cortex contralateral to the missing limb (*filled circles*) and shoulder movements contralateral to the intact limb (*open circles*) in monkeys 98-61 (*A*) and 98-64 (*B*). The deefferented cortex exhibits a wide range of current thresholds that was not observed in the opposite hemisphere.

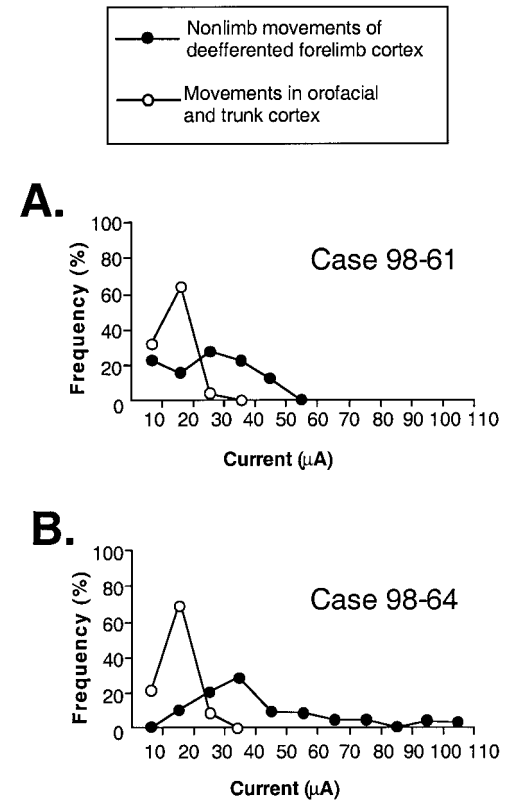

Figure 8. Frequency distribution of current thresholds for orofacial and trunk evoked movements in deefferent M1 forelimb cortex (*filled circles*) and movements in face and trunk cortex in M1 of the same and opposite hemispheres (*open circles*) for squirrel monkey 98-61 (*A*) and 98-64 (*B*). Current thresholds for the orofacial and trunk cortex in the two hemispheres were not significantly different; thus, data from the two hemispheres were combined. Although the distribution of current thresholds for the deefferented cortex overlap those for orofacial and trunk cortex; many sites required much higher threshold levels.

deefferented forelimb cortex were also significantly higher than those for nonlimb movements within the forelimb cortex of the opposite hemisphere (case 98-61, $t_{31} = 4.5$; $p < 0.001$; case 98-64, $t_{22} = 5.4$; $p < 0.001$; see Table 3) or higher than movements in trunk and orofacial cortex of the opposite hemisphere (case 98-61, $t_{35} = 5.0$; $p < 0.001$; case 98-64, $t_{19} = 5.7$; $p < 0.001$; see Table 3, Fig. 8), as well as movements in trunk and orofacial cortex medial and lateral to the deefferented forelimb cortex (case 98-61, $t_{37} = 5.3$; $p < 0.001$; case 98-64, $t_{20} = 5.6$; $p < 0.001$; see Table 3, Fig. 8). Except for forelimb cortex, the threshold levels for movements from the deefferented hemispheres were not significantly different from those in the opposite hemispheres or in controls (t tests, $p > 0.05$).

When the distributions of current thresholds for shoulder/stump movements within deefferented cortex and normal shoulder movements in opposite hemispheres were compared, it was clear that the distributions overlapped, but higher levels of current were required only in the deefferented cortex (Fig. 7). In fact, the percentage of sites for shoulder/stump movements with normal thresholds ($\leq$25 μA) in the deefferented hemisphere was not significantly different from the percentage of sites with normal thresholds for shoulder movements in the opposite hemisphere (case 98-61, $\chi^2 = 0.01$; $p = 0.94$; case 98-64, $\chi^2 = 0.85$; $p = 0.36$). It is likely that the sites eliciting shoulder/stump movement with normal threshold in the deefferented cortex correspond to sites that evoked shoulder movements before amputation (as is the case for opposite hemisphere or control cases). On the other hand, the high-threshold shoulder/stump movement sites likely reflect sites that formerly evoked movements from the missing limb.

The loss of a hindlimb appears to result in changes in deefferented hindlimb cortex that are comparable with those induced in deefferented forelimb cortex by forelimb loss. In brief, throughout the deefferented hindlimb cortex, no unresponsive sites were found, and electrical stimulation resulted in movements of remaining body parts (Fig. 9). Usually, these movements were of the stump or the tail but sometimes also of the lower trunk. Consequently, there was a significantly higher percentage of tail movement sites in the deefferented hindlimb cortex than that in the normal animals ($\chi^2 = 11.0$; $p < 0.001$). Also, as in the forelimb-amputated cases, current thresholds for evoking these movements ranged from the normal range to much higher, with a significantly higher average threshold level than in normal animals ($t_{52} = 7.7$; $p < 0.001$).

Effects of amputation on premotor cortex and area 3a

Area 3a is generally considered to be a somatosensory field within the anterior parietal cortex. This area is activated by a relay of muscle spindle afferents from the thalamus and is interconnected with other fields in somatosensory cortex and primary motor cortex (for review, see Kaas and Pons, 1988). Premotor area includes the PMD and PMV, as well as the supplementary motor area (SMA). Movements can be evoked from all of these fields (Preuss et al., 1996; Wu and Kaas, 1998) (for review, see Dum and Strick, 1991b). In addition to stimulating M1, in the present cases, many electrode sites were in premotor cortex and somatosensory area 3a, where movements were evoked at somewhat higher current levels than in M1. In normal squirrel monkeys (Fig. 1) and

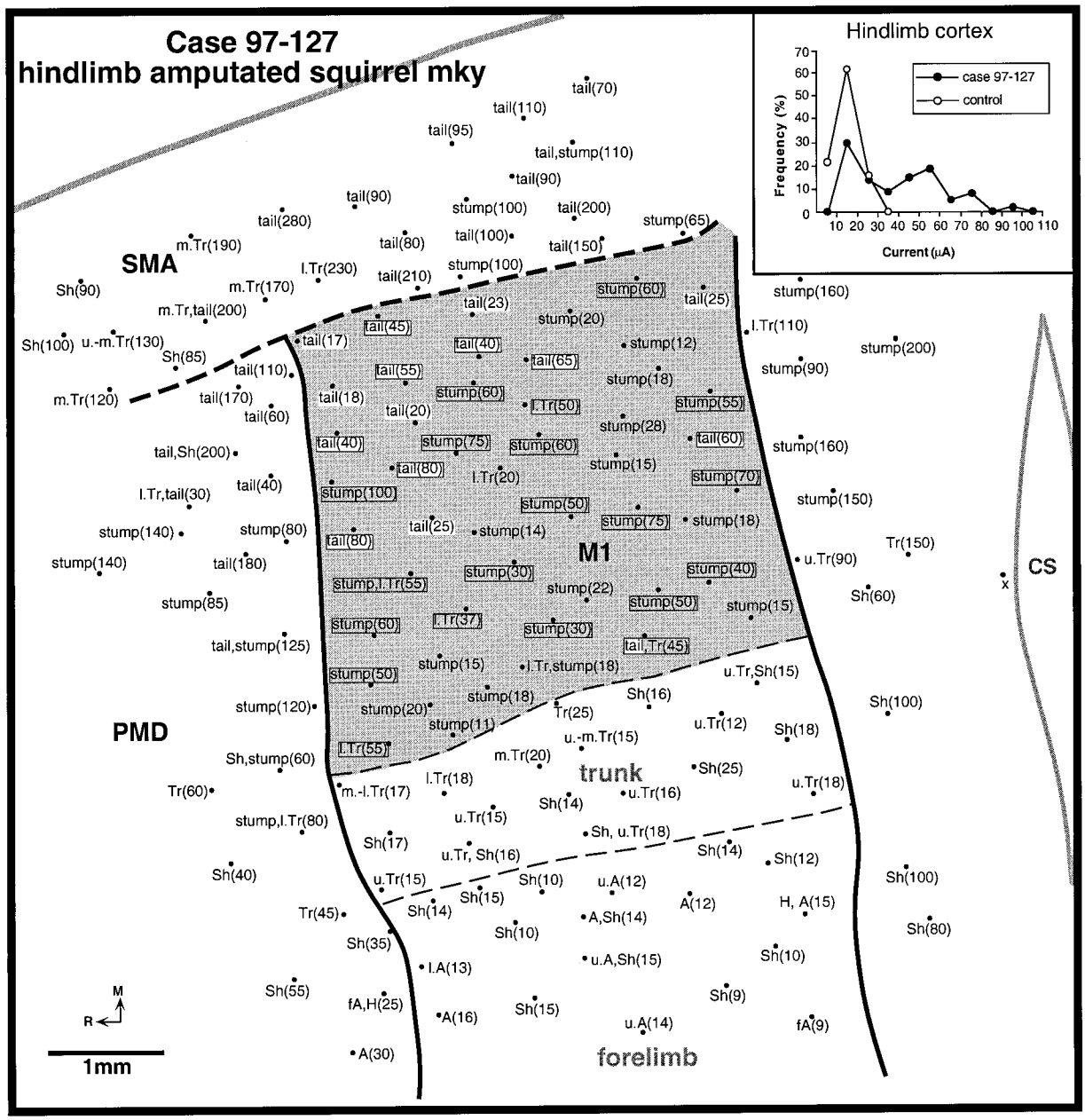

Figure 9. Organization of motor cortex contralateral to a hindlimb amputated at the hip joint in squirrel monkey (case 97-127). Movements of the stump of the amputated limb, lower trunk, or tail were evoked throughout the hindlimb portion of M1 (*gray shading*). Current thresholds levels exceeding the maximum current threshold in the normal control animals are *outlined*. Sites eliciting tail movements are highlighted by a *white background*. Stump movements were also evoked from PMD and medial cortex judged to be SMA. The *line* at the *top left* marks the medial wall of the cerebral hemisphere, whereas the central sulcus (*CS*) is on the *right*. Conventions are as in Figures 1 and 4. *Top right inset*, Frequency distribution of current threshold in the hindlimb amputee (*filled circles*) compared with controls (*open circles*). A wider range of current thresholds occurred in deefferented cortex.

in cortex contralateral to the intact limb in two forelimb-amputated squirrel monkeys (Figs. 2, 3), parts of areas PMD and PMV produced forelimb movements, as did the part of area 3a just caudal to the forelimb region of M1. In cortex contralateral to the missing forelimb, stump or shoulder movements were produced from many stimulation sites in PMD, PMV, and the forelimb portion of area 3a (Figs. 7, 8). Current levels needed to evoke these movements were higher than those for stump and shoulder sites in M1, but they were often comparable with corresponding premotor and somatosensory sites in normal cortex. However, some sites required much higher than normal levels of current for evoked shoulder and stump movements in these fields. Thus, this limited evidence from PMV, PMD, and area 3a suggests that the changes that occurred in these fields were very comparable with those in M1.

ICMS mapping in galagos

M1 of normal animals

The location and organization of motor cortex in galago is known from recent studies using ICMS mapping, cytoarchitectonic features, and patterns of connections with area M1 and the spinal cord (Fogassi et al., 1994; Wu et al., 1997; Wu and Kaas, 1998). In summary, galago M1 is situated between anterior and posterior parts of frontal sulcus (FSa and FSp, respectively), with orofacial

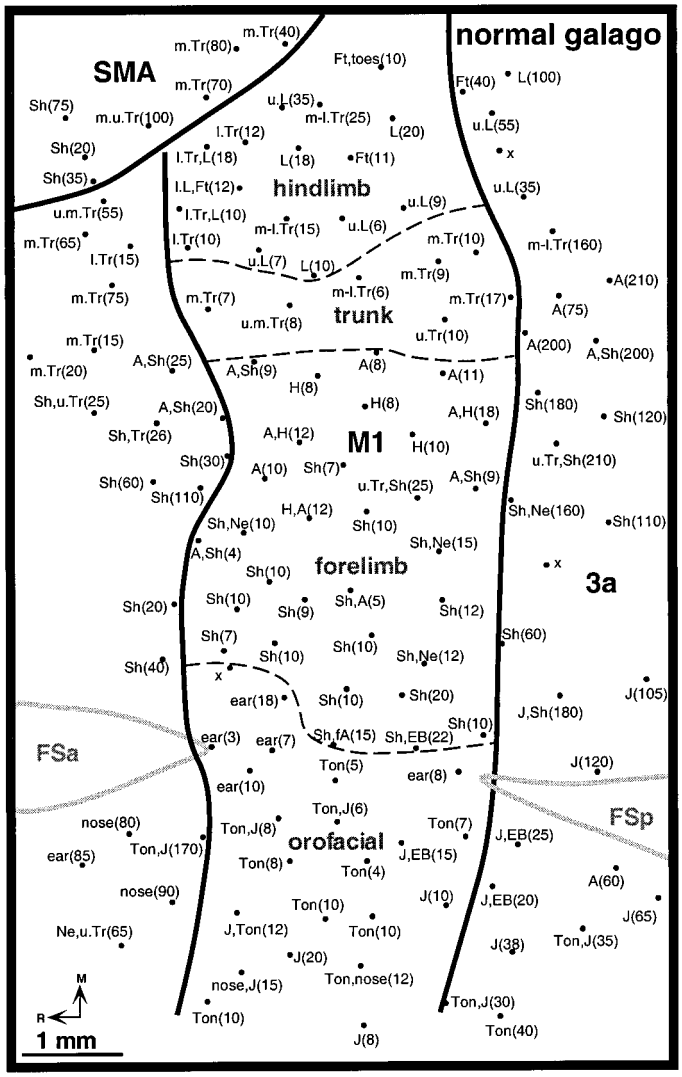

Figure 10. Example of ICMS mapping result and motor cortex organization in a normal galago. Area M1 of galagos is located between two frontal sulci, FSa and FSp. Note that the forelimb cortex is located above FSa and FSp. Conventions are as in Figure 1. Compare with Figures 11 and 12.

movements represented most laterally and hindlimb movements most medially, extending deep into the medial wall (Fig. 10). The indentation between these two frontal sulci caused by a large blood vessel separates the orofacial from forelimb cortex. To serve as a control group, data from four normal galagos were used to compare with the organization of M1 in the limb-amputated galagos. Detailed maps obtained from these normal galagos will be published elsewhere. The size of the forelimb cortex in these galagos ranged from 5.6 to 7.9 mm^2, with an average of 6.4 mm^2. As in squirrel monkeys, movements evoked from galago M1 were restricted to small muscle groups or joints contralateral to the stimulated hemisphere, and M1 contained a complete body motor representation. However, the movements evoked in galagos appeared to be less precise, involving more muscle groups at some ICMS sites. For example, a single-digit or toe movement was evoked only in few sites in galagos, and movements involving single muscles were rarely observed. Such a difference in the motor organization of M1 forelimb cortex in these two primate species probably reflects their behavioral differences, because galagos have less precise hand movements than squirrel monkeys (Torigoe, 1985; Costello and Fragaszy, 1988; Larson et al., 1989).

The average thresholds for evoked movements from different body cortex in control galagos are summarized in Table 4. Although part of M1 hindlimb cortex in galago extends into the medial wall (Wu and Kaas, 1998), we did not include values from these sites because of the difficulty in ensuring that the electrode was in layer V where the lowest thresholds are obtained. Because the sites buried in the medial wall were not included in the analysis, the total size of the hindlimb cortex was not determined.

M1 contralateral to amputated limbs

The general topography in M1 of the forelimb-amputated galago was remarkably similar to our observations in squirrel monkeys with forelimb amputation (Fig. 11). The orofacial, hindlimb, and trunk representations were not different from those in controls, either in current thresholds (t tests, $p > 0.05$) or patterns of evoked movements, and they were found in locations predicted from control cases. Similar to squirrel monkeys, there were no unresponsive sites in the deefferented cortex, and the range of thresholds for evoked movements (5–80 μA) was broader than in controls (2–25 μA). Within the expected location of former forelimb cortex (i.e., cortex between FSa and FSp but above the indention of the two sulci), there was a significant increase in the number of sites from which nonlimb movements could be elicited ($\chi^2 = 52.2$; $p < 0.001$). However, the percentage of sites from which shoulder/stump movements could be evoked was not different from that of normal shoulder movements in the control cases ($\chi^2 = 0.0$; $p = 0.99$). As in amputated squirrel monkeys, the current threshold for movements were abnormally high at some sites compared with normal cortex for both shoulder/stump movements ($t_{36} = 5.1$; $p < 0.001$) and nonlimb movements (compared with those in the forelimb cortex of controls, $t_{54} = 4.8$; $p < 0.001$; compared with trunk and orofacial cortex of controls: $t_{52} = 8.3$; $p < 0.001$; compared with trunk and orofacial cortex medial and lateral to the deefferented forelimb cortex, $t_{48} = 9.1$; $p < 0.001$), whereas the threshold in others are comparable with control cases (t tests, $p > 0.05$; Tables 3, 4; Figs. 10, 11).

The results of ICMS mapping in M1 of the galago with hindlimb amputation exhibited remarkable similarity to the squirrel monkey hindlimb amputee as described above. The orofacial, trunk, and forelimb cortex was not different from in controls either in current thresholds or patterns of evoked movements. Moreover, there was an increase in the percentage of sites from which hip/stump and tail movements could be elicited, and there was a wider distribution and a significantly higher average threshold for these movements (Table 4; Figs. 10, 12; $t_{58} = 4.5$; $p < 0.001$).

Somatosensory recording in limb-amputated galagos

In galagos, cortex located in FSp and intraparietal sulcus (IPS) is vigorously responsive to cutaneous stimulation, exhibits typical somatosensory koniocortex organization (Carlson and Welt, 1980; Sur et al., 1980; Wu et al., 1995), and is therefore defined as a subdivision of primary somatosensory cortex, area 3b. The somatosensory cortex contralateral to the missing limb was mapped in two limb-amputated galagos. In the region of area 3b where the forelimb and hindlimb cortex is normally located, neurons at some recording sites responded to the cutaneous stimulation. Sites where neurons did not respond to light cutaneous stimulation often could be activated by more intense stimulation produced by strokes or taps. In the deprived forelimb cortex, neurons were largely activated by shoulder and stump

Table 4. Average current thresholds for evoked movements in M1 of the control and amputated galagos (conventions as in Table 3)

		Forelimb cortex				Hindlimb cortex		
	Orofacial cortex	sh/sh-stump,	non-sh,	nonlimb	Trunk cortex	hip/hip-stump,	nonhip,	tail
Normal control								
Average for cases 97-17, 97-34, 97-59, 97-91								
Threshold (μA)	12.2	9.7	10.2	12.0	11.0		(12.7)	
Frequency (%)		48.7	56.5	12.2		31.6	59.5	0
Forelimb amputee								
Case 97-100								
Threshold (μA)	10.6	24.1		30.9	9.7			
Frequency (%)		50.0	0	65.6				
Hindlimb amputee								
Case 97-134								
Threshold (μA)			(12.9)		9.3		(23.1)	
Frequency (%)						92.5	0	17.5

stimulation, with the majority clearly having cutaneous receptive fields. However, some sites required more intense stimuli on the stump. In addition, receptive fields from chest, neck, or the lower portion of the face were observed in a few penetrations (Fig. 11). Similar observations were made in the deprived hindlimb cortex (Fig. 12). In short, stump and the lower body parts took over the majority of the deprived cortex, and both normal and higher levels of stimulation were required for activating these neurons. In both limb amputees, neurons in somatosensory cortex lateral or medial to the deprived region responded normally to cutaneous stimuli and had normal sizes of receptive fields.

DISCUSSION

The amputation of an injured limb directly severs most of the afferents and efferents of that limb and undoubtedly produces a host of related changes in the spinal cord, brainstem, and cortex (Calford and Tweedale 1988; Florence and Kaas, 1995; Jones and Pons, 1998). Because mammals with therapeutic amputations are rare and studies of humans with amputations are limited, the motor and sensory effects of limb amputations have not been extensively described. In the present study, we addressed the issue of what happens to motor cortex after the loss of a limb. More specifically, what are the consequences of electrical stimulation at sites in motor cortex that would normally move that limb? The answer was clear. Most of the sites throughout the forelimb cortex of M1 were still related to that limb, in that they excited muscles in the stump and shoulder. For some of these sites, the levels of current needed to evoke movements were similar to those needed to evoke arm movements from normal cortex. However, many of the sites required higher levels of current. Yet, there were no sites in the deprived cortex where movements could not be evoked. In addition, the small number of sites where movements of trunk or face could be evoked increased significantly in deprived forelimb cortex. The results were similar in both squirrel monkeys and prosimian galagos, and they closely correspond to those reported for a single investigated macaque monkey with a forelimb loss as a juvenile (Schieher and Deuel, 1997). Results were also quite similar after the loss of a hindlimb in both a squirrel monkey and a galago. Findings did not differ among individuals with a limb loss as an adult or a juvenile. Finally, limited results from deprived portions of premotor cortex suggest that this cortex largely becomes devoted to stump and shoulder movements as well.

Although the results seem robust and general, they raise several questions. Most notably, does the motor cortex of humans and other mammals such as rats change in a comparable manner after limb loss? What are the mechanisms of change?

M1 organization after limb amputation in humans and rats

The organization of motor cortex after long-standing limb amputations in young and mature mammals has also been studied in humans and rats. In humans, the results most comparable with those obtained in the present experiments came from the direct stimulation of motor cortex with surface electrodes in a patient 24 years after the amputation of an arm as an adult (Ojemann and Silbergeld, 1995). Stimulation of the portion of M1 that is normally devoted to hand and finger movements evoked movements from the proximal muscles of the missing limb (such as shoulder), except for a small region where no movements were evoked with the current levels used. In an earlier study of a patient 13 years after amputation of a leg above the knee (Woolsey et al., 1979), stimulation of three sites thought to be in or near the midline portion of M1 normally devoted to the leg produced stump movement (one site) or hip movements (two sites). These limited results suggest that the deprived cortex had become devoted to the remaining muscles proximal to the missing limbs in these patients and that current stimulation thresholds had increased for some of this cortex.

More extensive investigations of motor cortex in humans with amputations have used the noninvasive technique of transcranial magnetic stimulation. By pulsing a small magnetic coil over different portions of the skull, current flow can be induced in different populations of neurons in motor cortex. In individuals studied after long-standing limb amputations as adults, the general finding has been that remaining muscles proximal to the stump can be activated from more coil sites than the same muscles on the intact side (Hall et al., 1990; Cohen et al., 1991; Chen et al., 1998). A reasonable interpretation of this result is that movements from the remaining muscles can be evoked from a larger than normal portion of M1, perhaps most or all of the forelimb region.

According to this interpretation, M1 in humans reorganizes much as in other primates. However, there was no evidence from the transcranial magnetic stimulation experiments that sites within reorganized forelimb cortex required higher than normal

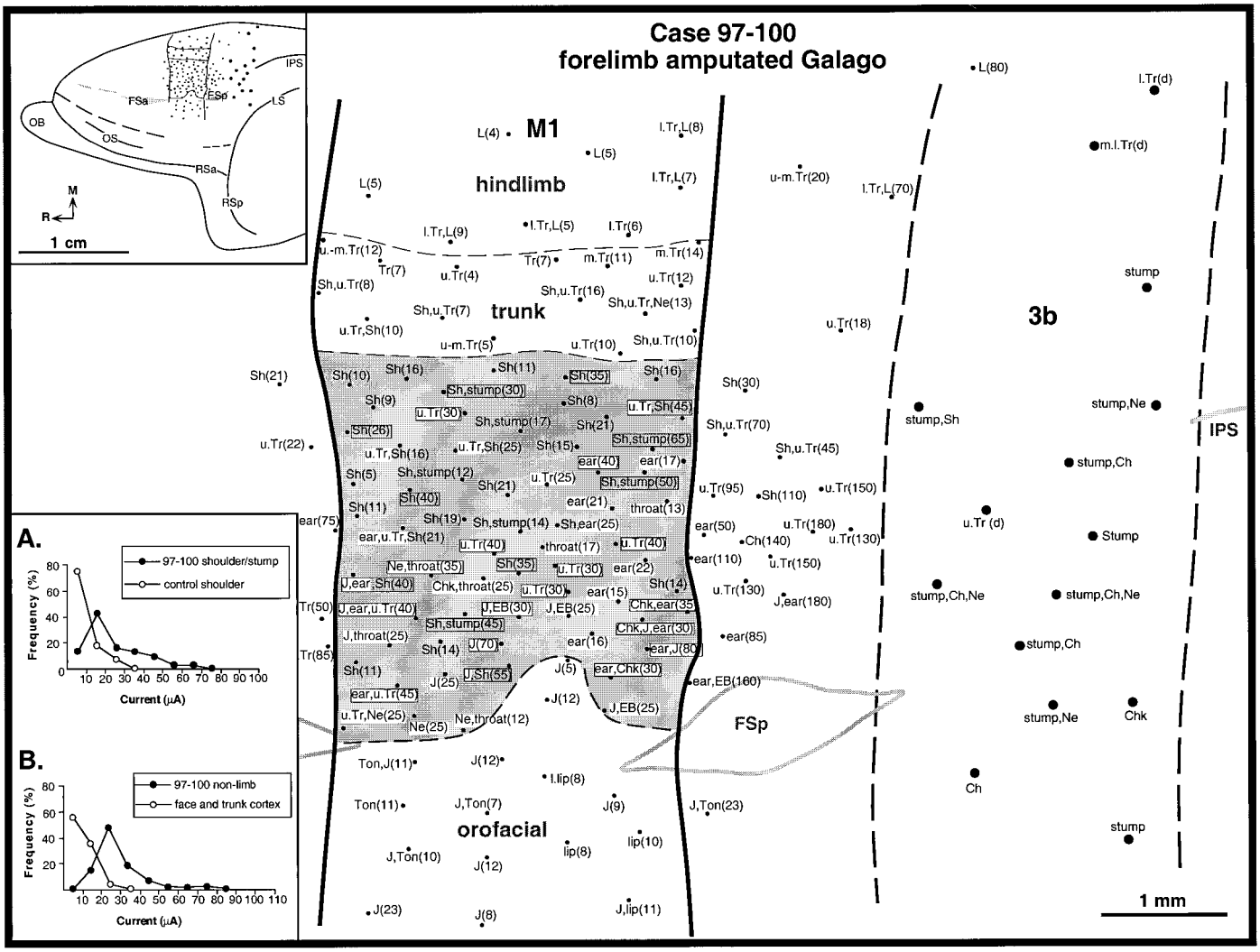

Figure 11. ICMS and somatosensory recording maps from the hemisphere contralateral to the missing limb of the forelimb-amputated galago (case 97-100). *Inset* at *top left*, Portion of mapped cortex on a dorsolateral view of the cerebral hemisphere. ICMS sites (*smaller dots*) were concentrated in M1, whereas somatosensory recording sites (*larger dots*) were concentrated between FSp and IPS where S1 is normally located. *Bottom left inset*, Histograms comparing the frequency distributions of current thresholds for shoulder/stump (*A*) and nonlimb (*B*) movements in deefferented forelimb cortex to the shoulder movements in the forelimb cortex of control cases (*A*) and average of face and trunk cortex in the same hemisphere and control cases (*B*). Sites in the deefferented cortex with current threshold exceeding the maximum current threshold in control cases are outlined. Conventions for ICMS are as in Figure 4. The receptive field of each somatosensory recording site is indicated using the conventions described in Table 1. Responses from the dorsal side of the body are indicated by (*d*).

levels of current to evoke movements. Instead, movements and muscle potentials were evoked in remaining muscles proximal to the stump, compared with the same muscles in the normal side, at similar or lower stimulus intensities, and stimulation at a fixed suprathreshold level evoked larger muscle potentials in these muscles (Cohen et al., 1991; Kew et al., 1994; Chen et al., 1998). Although these results might indicate that sites in reorganized motor cortex had normal or lower than normal thresholds, this may not be the case. Stimulation with magnetic coils likely involved larger populations of neurons than intracortical stimulation with microelectrodes (Day et al., 1987, 1989; Topka et al., 1991), and the convergence of many more active corticospinal projections in the spinal cord motoneuron pools may produce the larger muscle response, even if cortical sites of normal to higher than normal thresholds are involved.

The effects of forelimb amputation on the organization of motor cortex have also been studied in developing (Donoghue and Sanes, 1987, 1988) and mature rats (Sanes et al., 1990). One week to four months after forelimb amputation in adult rats, shoulder movements were evoked by ICMS over much of the forelimb region of cortex (Sanes et al., 1990). As a result, the shoulder representation more than doubled in size. Mean current thresholds for evoking shoulder movements in normal motor cortex or altered motor cortex were not significantly different. Thus, forelimb amputation in rats resulted in a reorganization of forelimb cortex so that sites throughout evoked shoulder and stump movements. Unlike monkeys and galagos, thresholds were normal rather than normal to elevated.

We conclude that the major consequence of limb amputation for motor cortex, regardless of species, is that sites throughout limb cortex come to evoke shoulder and stump movements. Current thresholds for evoking these movements from deprived portions of M1 clearly range from normal to above normal in monkeys and galagos. The few unresponsive sites obtained from

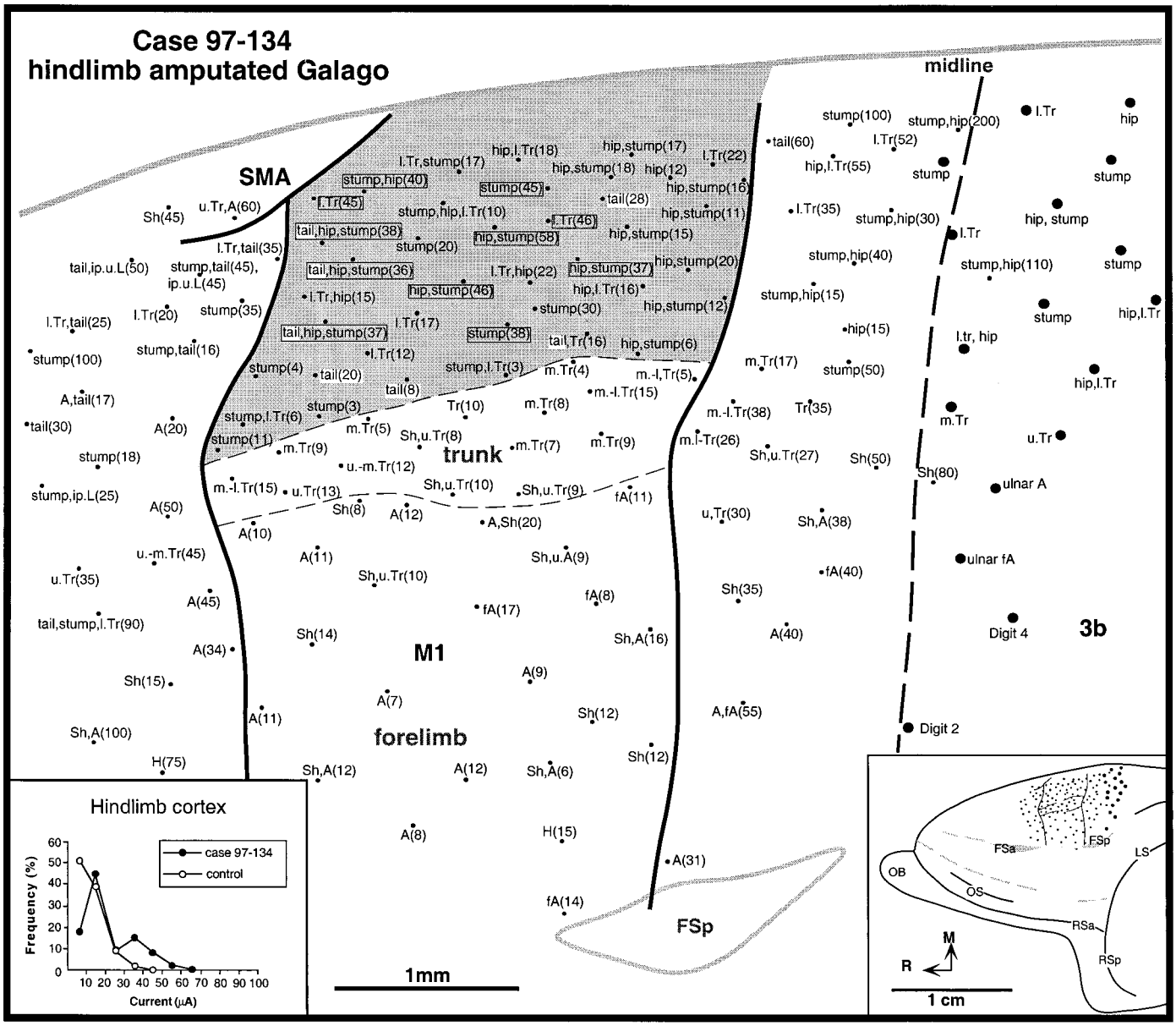

Figure 12. ICMS and somatosensory recording maps from the hemisphere contralateral to the missing limb of the hindlimb-amputated galago (case 97-134). *Bottom left inset*, Histogram showing the frequency distribution of current thresholds from deefferented hindlimb cortex (*filled circles*) compared with those from controls (*open circles*). See Figures 9 and 11 for conventions.

directly stimulating motor cortex in one patient suggest that thresholds may be raised in humans as well, but this observation is countered by evidence for lower thresholds with magnetic coil stimulation. In rats, thresholds appear to be normal. The time course for this reorganization of M1 is uncertain, because stimulation occurred at various but long times after amputation. The only exception is that a single patient was stimulated with a magnetic coil both before and three times within 11 months after an arm amputation (Pascual-Leone et al., 1996). The results suggest that the representation of muscles proximal to the stump enlarged slowly over weeks to months. Nevertheless, changes in motor cortex detected by magnetic coil stimulation during ischemic deefferentation of a forelimb (Brasil-Neto et al., 1993; Ziemann et al., 1998a) suggest that some reorganization can be very rapid.

The nature of the reorganization of M1

Given the consistency of the results across species, one might ask how cortex has changed. Here we consider several possibilities. First, the results could reflect residual upper arm and shoulder movements at digit and wrist movement sites. Increasing the levels of electrical stimulation at microelectrode sites in cortex does increase the magnitude of the response and the involvement of more muscles (Sanes et al., 1990; Huntley and Jones, 1991; Nudo et al., 1996). Possibly, some or many of the sites in the present experiments where higher than normal levels of current were used to evoke stump movements were sites where such movements could be evoked at higher levels of current in normal animals. Such residual effects have been described for the responses of neurons in the cochlear nucleus of cats after partial cochlear lesions (Rajan and Irvine, 1998). However, residual

responses would not account for movements evoked at normal or near-normal levels of stimulation or the observed increase in face and trunk movement sites within forelimb cortex. Second, some or all of the results could reflect potentiation of horizontal connections within motor cortex (Huntley and Jones, 1991; Keller, 1993; Weiss and Keller, 1994; Huntley, 1997). A reduction of inhibition in motor cortex could unmask the effects of excitatory horizontal connections so that stimulation at some sites activates patches of neurons at more distant sites. Changes in the sensory activation of the cortex attributable to loss of sensory afferents (Calford and Tweedale, 1988; Sanes et al., 1992) or the reorganization in somatosensory cortex (this study; Merzenich et al., 1984; Calford and Tweedale, 1988; Florence and Kaas, 1995; also see Jain et al., 1997) could alter the effectiveness of such connections. The horizontal connections in M1 also can be strengthened through long-term potentiation (Nudo et al., 1990; Hess and Donoghue, 1994; Hess et al., 1996; Rioult- Pedotti et al., 1998) or activity-dependent reduction of GABA-related inhibition (Jacobs and Donoghue, 1991; Ziemann et al., 1998b) (also see Dykes et al., 1984; Hendry and Jones, 1986; Welker et al., 1989; Akhtar and Land, 1991; Garraghty et al., 1991; Jones, 1993; Huntsman et al., 1994). Finally, amputations may produce structural changes in the motor system, including the possibility of the growth of new horizontal connections, as has been shown in sensory systems (Darian-Smith and Gilbert, 1994; Das and Gilbert, 1995; Florence et al., 1998), the expansion of terminal arbors of corticospinal axons, and the sprouting of damaged peripheral nerves to innervate new muscle targets. We have started to investigate some of these possibilities.

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