Early and Late Changes in the Distal Forelimb Representation of the Supplementary Motor Area After Injury to Frontal Motor Areas in the Squirrel Monkey


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Eisner-Janowicz I, Barbay S, Hoover E, Stowe A, Frost S, Plautz E, Nudo R.J. Early and late changes in the distal forelimb representation of the supplementary motor area after injury to frontal motor areas in the squirrel monkey. J Neurophysiol 100: 1498–1512, 2008. First published July 2, 2008; doi:10.1152/jn.90447.2008. Neuroimaging studies in stroke survivors have suggested that adaptive plasticity occurs following stroke. However, the complex temporal dynamics of neural reorganization after injury make the interpretation of functional imaging studies equivocal. In the present study in adult squirrel monkeys, intracortical microstimulation (ICMS) techniques were used to monitor changes in representational maps of the distal forelimb in the supplementary motor area (SMA) after a unilateral ischemic infarct of primary motor (M1) and premotor distal forelimb representations (DFLs). In each animal, ICMS maps were delineated using early (3 wk) and late (13 wk) postinfarct stages. Lesions resulted in severe deficits in motor abilities on a reach and retrieval task. Limited behavioral recovery occurred and plateaued at 3 wk postinfarct. At both early and late postinfarct stages, distal forelimb movements could still be evoked by ICMS in SMA at low current levels. However, the size of the SMA DFL changed after the infarct. In particular, wrist-forearm representations enlarged significantly between early and late stages, attaining a size substantially larger than the preinfarct area. At the late postinfarct stage, the expansion in the SMA DFL area was directly proportional to the absolute size of the lesion. The motor performance scores were positively correlated to the absolute size of the SMA DFL at the late postinfarct stage. Together, these data suggest that, at least in squirrel monkeys, descending output from M1 and dorsal and ventral premotor cortices is not necessary for SMA representations to be maintained and that SMA motor output maps undergo delayed increases in representational area after damage to other motor areas. Finally, the role of SMA in recovery of function after such lesions remains unclear because behavioral recovery appears to precede neurophysiological map changes.

INTRODUCTION

There are extensive data supporting the notion that recovery of function lost due to cortical injury may be attributable to adaptive plasticity in the remaining cortical motor network. For example, in earlier studies in squirrel monkeys, the distal forelimb representation (DFL) of the primary motor cortex (M1) was delineated using intracortical microstimulation (ICMS) mapping techniques. After small, subtotal ischemic lesions in a portion of the DFL, the remaining ICMS-defined M1 DFL was reduced in size, giving way to expanded proximal representations when the animals were allowed to recover spontaneously (i.e., without the benefit of rehabilitative training) for several weeks. However, in animals that underwent rehabilitative training with the impaired limb, the DFL was preserved or expanded (Nudo et al. 1996). Comparable studies in human stroke survivors suggest that the intact, peri-infarct cortex may play a role in neurological recovery (Cramer et al. 1997; Jaillard et al. 2005; Teasell et al. 2005). Using TMS after stroke, a reduced excitability has been shown in the motor cortex near the injury with a decreased cortical representation of the affected muscles (Buteifisch et al. 2006; Traversa et al. 1997). It has been suggested that this effect occurs from a combination of diaschisis-like phenomena and disuse of the affected limb (Liepert et al. 2000). After several weeks of rehabilitation, motor representations in the injured hemisphere typically are enlarged relative to the initial postinjury map (Carey et al. 2002; Traversa et al. 1997). Also, when goal-directed movements with the impaired hand are encouraged, a significant enlargement of the representation of the paretic limb results (Liepert et al. 1998), closely paralleling findings in non-human primates.

The notion that neurophysiological properties of neurons in cortical motor areas more remote from the site of injury are altered was given credence by results of a study in the supplementary motor area (SMA) in macaque monkeys (Aizawa et al. 1991). It is well known that neurons in SMA are active prior to the onset of movement (Wiesendanger et al. 1996). Aizawa et al. found that overlearning of a motor task resulted in substantial reduction in SMA premovement activity. However, after a lesion in M1, premovement activity in SMA returned, indicating that the functional organization of remote cortical areas is altered after a focal cortical lesion.

Evidence for remote ipsilesional plasticity was supported further by studies in squirrel monkeys showing topographic changes in motor representations of the ventral premotor cortex (PMv) following experimental ischemic lesions in the M1 DFL (Frost et al. 2003). Several months after the infarct, the size of the PMv DFL increased, and this increase was proportional to the size of the M1 lesion with larger lesions resulting in larger PMv DFL expansions (also see Dancause et al. 2006). Extensive intracortical rewiring also occurred after such lesions.
area was induced. After the infarct, animals were returned to their individual cages, where they underwent spontaneous recovery (i.e., no specific pharmacologic or rehabilitative intervention) for the subsequent 13 wk. Weekly assessment of hand preference and manual dexterity was conducted. Three to 13 wk after the infarct, a second and third set (respectively) of representational maps of the SMA forelimb representation were derived in each animal. At the end of the experiment, animals were humanely killed and perfused for histological examination of the lesions. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center.

Behavioral methods

Monkeys were trained to retrieve small, banana-flavored food pellets (45 mg), delivered one at a time, from wells drilled into a Plexiglas board (Klüver board) attached to each monkey’s home cage. The board was attached to the cage so that the monkey had unrestricted access to each food well. Five wells were distributed across the Klüver board. All wells were 5 mm deep but differed in diameter (well 1 = 25 mm, well 2 = 19.5 mm, well 3 = 13.5 mm, well 4 = 11.5 mm, and well 5 = 9.5 mm) and thus varied in task difficulty. To remove food pellets from the smaller wells (wells 3–5), the monkeys had to learn to use their digits differentially, whereas on the larger wells (wells 1 and 2), synergistic flexion of the digits and a prehensile grip were adequate to remove the pellets (Nudo et al. 1992).

Prior to preinfarct training, each monkey was tested for hand preference on the Klüver board task over two consecutive days. In this procedure, food pellets were successively placed into one of the five wells in random order for a total of 25 trials (5 trials per well) with the board in one position (e.g., well 1 to the right of the monkey) and 25 trials with the board reversed to counterbalance any possible position bias (well 1 repositioned to the left of the monkey). One session was conducted per day, over two consecutive days (a total of 100 trials). This will be referred to as the “open board” task. On each trial, the monkeys could use the hand of their choice to retrieve the pellet. The preferred hand was defined as the one used for the majority of the trials (i.e., >50%).

During the preinfarct motor-skill training period, a 1-h session was conducted each day in which the monkeys were trained to become increasingly proficient in retrieving pellets from a designated well. The monkeys were trained first on their preferred hand on the open board task. Typically, squirrel monkeys adopt one hand (the preferred hand) to perform this task within one or two sessions. The training began with the largest well. Once the monkeys met criterion performance on one well, they were trained on the next successively smaller well the following day. The criterion for monkeys to complete training on a particular well was successful retrieval of 100 consecutive pellets on a given well. Successful retrieval was defined as grasping of the pellet and placing it in the mouth. This procedure was carried out until the monkeys could meet criterion performance on the smallest well (well 5) on two consecutive days. Once the monkeys were proficient on the open board task with their preferred hand, a barrier (translucent Plexiglas block) was placed in front of the target well, which forced the use of either the left or right hand). This will be referred to as the “barrier board” task. Training on the barrier board task was conducted first with the preferred hand, then with the nonpreferred hand on well 1. Monkeys were consecutively trained on the smaller wells alternating the preferred and nonpreferred hands using the 100-pellet criterion until they were proficient with both hands on each of the five wells.

Probe trials (open and barrier board tasks) were conducted weekly during both the preinfarct and the entire 13-wk spontaneous recovery period. Probe trials consisted of a total of 100 trials in 25 or 50 trial blocks. In the first block of 25 trials (open board task), pellets were placed into one of the five wells in pseudorandom order (i.e., randomized blocks requiring 5 trials per well). Then the Klüver board was reversed for an additional 25 trials to counterbalance the well
positions. The final block of 50 trials was conducted using the barrier board task with the board arranged to evaluate the use of either the preferred or nonpreferred hand. First, five trials were conducted on well 1 (the largest well) with the barrier allowing use of the preferred hand. Then an additional five trials were conducted on well 1 with the barrier allowing use of the nonpreferred hand. Similarly, trials were conducted on wells 2–5 in order of decreasing size. Pre- and postin- fant probe trials were documented with a Hi 8 video recorder (30 frame/s) for later analysis.

In previous studies employing more restrictive ischemic lesions in M1, manual dexterity was assessed by tallying finger flexions per pellet retrieval on the Klüver board (Open board task) or time to retrieve pellets (e.g., Frost et al. 2003). However, with the more extensive ischemic lesions employed here, retrievals were typically not successful, and a more general, ordinal motor performance assessment scale was used. Trial-by-trial performance was evaluated by use of the ordinal scale, with performance scores ranging from 0 to 5. Performance scores were assigned as follows: 0, no attempt made; 1, hand crosses the plane of the cage bars; 2, hand touches the board; 3, digits placed inside the well; 4, digits flexed inside the well; 5, pellet successfully retrieved from the well and placed into the monkey’s mouth.

To reduce the influence of motivation on task performance, no value was assigned if the monkey would not perform the task with the nonimpaired (sometimes called “less-impaired”) hand on the same well for which the impaired hand was being tested. Typically the monkeys were highly motivated to remove the pellets with their nonimpaired hand. The very few cases in which the monkey would not attempt to retrieve pellets with the nonimpaired hand, the session was interrupted and reintiated a few hours later. Finally, the trial was discarded if the pellet was dropped; however, this occurrence was rare.

Surgical techniques

Each monkey was preanesthetized with ketamine (25 mg/kg im) and then intubated with a tracheal tube (2.5 mm OD). The monkey was then placed on a gas mixture of 75% nitrous oxide-25% oxygen with 1.5–2% halothane (to effect). Intraoperative fluids (3% dextrose in Ringer solution) were then administered and maintained throughout the procedure. Mannitol (8 ml) was administered intravenously to control for possible edema. The monkey’s head was stabilized in a stereotaxic frame, and under aseptic conditions, the scalp and the temporal muscle were reflected, exposing the skull. A craniectomy was then performed and a portion of the dura removed to expose M1, PMv, PMd, and SMA contralateral to the preferred hand. Movements were evoked by delivering a small electrical current into the brain through a stimulating microelectrode. The microelectrode consisted of a glass micropipette (1.5 mm OD) filled with a 3.5 M NaCl solution. The pipette was tapered so that the electrode measured ~10–25 μm OD at the tip and ~100 μm OD 2 mm from the tip. The tip was then beveled to an angle of 25° to aid in penetration through the pia. The ICMS stimulus was delivered as a train burst of 13.0–2.0 ms cathodal monophasic pulses, delivered at 350 Hz. This pulse train was triggered through a pulse generator (Master-8; A.M.P.I.) and delivered by a stimulus isolator (Model BSI-2, BAK Electronics) at a rate of one train per second. At each penetration site the microelectrode was held perpendicular to the cortex, suspended from a stereotaxic micropositioner (Narishige SM-11) and then lowered to ~1.750 μm below the surface with a hydraulic microdrive (Kopf Model 650) targeting layer V (the location of the corticospinal neurons). Because the squirrel monkey’s cerebral cortex is relatively lissencephalic and the microelectrode penetrations are perpendicular to the surface, movements evoked at different depths are usually identical because the microelectrode passes through a single cortical column. However, by convention, evoked movements are defined only from stimulation of layer V (~1.750 μm) because movement thresholds are lowest at this depth. Stimulation current was increased in increments of 1 μA until a movement was visibly evoked. The threshold current level was defined as the joint movement evoked in ≥50% of the pulse trains using the lowest possible current level.

Movements were described using conventional terminology (Gould et al. 1986). We defined and included in the DFL all sites at which electrical stimulation elicited movements of the digits, wrist or forearm. We grouped these movements based on functionality. Elbow and shoulder movements direct the entire arm and/or forearm through space. Wrist and forearm supination/pronation movements orient the hand within a fixed space. The M1, PMv, PMd, and SMA DFLs were delineated by defining the borders between sites at which stimulation evoked digit or wrist–forearm movements and sites at which stimulation evoked more proximal (elbow, shoulder, and face) movements or by sites at which stimulation evoked no movement at the maximum current level (30 μA for M1, PMv, and PMd and 60 μA for SMA). As defined, the DFLs generally comprise individual contiguous representations within each of the cortical motor areas.

A digital picture of the blood vessel pattern over the cortex was taken using a video frame-capture card (Scion, Frederick, MD) and an image analysis program (National Institutes of Health Image). The file was saved and re-opened in an illustration program (Canvas, Deneba) where a computer-generated grid pattern scaled to 1 mm for M1, PMv, and PMd, or 250 μm for SMA, was superimposed onto the digital image of the blood vessel pattern. This image was used to guide microelectrode penetrations with reference to the surrounding vasculature while accurately measuring the distance between the microelectrode penetrations (inter-penetration distance). Movements associated with stimulation at each of the penetration sites were designated on the digital image. One investigator, who was blind to the location of the electrode, was primarily responsible for defining evoked movements, which were subsequently confirmed by a second investigator.

ICMS-evoked movement analysis

Digit movements included all extensions, flexions, abductions, and adductions of fingers and thumb. Wrist–forearm movements included all wrist extensions, wrist flexions, wrist radial deviations, wrist ulnar deviations, forearm supinations, and forearm pronations. Shoulder, elbow, and oro–facial movements, evoked by stimulation of cortical regions that border the distal hand area, were not included in the data.
analysis. A computer algorithm was used to unambiguously delineate functional boundaries for analysis of representational areas (Nudo et al. 1992).

**Ischemic infarct**

Of the five monkeys, two monkeys (684 and 424D) received the cortical infarct in two stages: First, the arterial branches of the MCA that supply the frontal cortex were occluded as they emerged from the lateral sulcus (distal MCA branch occlusion). We found this procedure to be ineffective due to reperfusion, and substantial recovery of behavioral performance. Thus a second, more permanent ischemic lesion was made 3 or 8 wk after the MCA branch occlusion (in monkeys 684 and 424D, respectively). All five monkeys received the permanent ischemic lesion, described in the following text. Because the results of these two monkeys after the permanent lesion did not differ substantially from the other three monkeys, the results of all five monkeys were considered as a single group. In the cases with two-stage lesions, behavioral and neurophysiological data prior to the permanent lesion were used as preinfarct data in the subsequent analyses.

For the permanent lesion, ICMS was used to derive maps of the cortical representations of the upper extremity (distal and proximal forelimb) within M1, PMv, and PMd as described in the preceding text. The surface vasculature within this area was permanently occluded by microforceps connected to a bipolar electrocoagulator (Codman and Shurtleff). This technique produces a well-defined, focal infarct, confined to the targeted area of cortex, and sparing underlying white matter (Nudo et al. 1996; Stowe et al. 2007). The outline of the infarct was delineated according to the physiological representation areas, but respecting also a general rule regarding the major blood vessel pattern. Thus the infarcts were delimited by caudally: the central sulcus, bordering but not including the distal branch of the Rolandoic artery irrigating the sensory cortex; laterally: the lateral border of the M1 representation, bifurcation of the main vein draining the M1-PMv area, exclusion of face representations and lateral border of PMv; rostrally: inclusion of distal branch of the Prerolandic artery and the rostral border of PMv and PMd forelimb representations; medially: distal fine branches of Rolandoic and pre-Rolandic arteries (“border” between middle and anterior cerebral artery territories), border between proximal and leg representations of M1 more rostrally, and border between proximal distal forelimb and trunk representations of M1 more caudally (Fig. 1). In some cases, anastomoses between the distal branches of the middle cerebral artery and the anterior cerebral artery distal branches could be seen clearly. These anastomoses were electrocoagulated to prevent reperfusion of the ischemic area.

The area of the infarct was visually inspected for reperfusion 10 min after electrocoagulating the targeted vasculature. If reperfusion was observed, the electrocautery procedure was repeated over the reperfused vasculature to create a permanent occlusion of the targeted region. A laser Doppler blood flow imaging system (Moor Instruments, Devon, UK) was used before and 1 h after the infarct to confirm elimination of blood flow within the defined infarct area but not in adjacent areas. The electrocoagulation method produced a reliable, nonvariable lesion avoiding reperfusion by eliminating all blood flow at once and by eliminating anastomoses and venous drainage from the territory of interest (Figs. 1 and 2).

To measure the infarct areas, the laser Doppler image was superimposed on the appropriately scaled preinfarct photograph of the surface vasculature. The border of the infarct was defined as the area where no perfusion was detected. These borders correspond well to the histologically defined borders (Nudo et al. 1996). Using this method, the circumference of the lesion was drawn in a separate drawing layer in Canvas (Version 3.5.5, Deneba Systems, Miami, FL). The scaled polygon representing the lesion border was then used to calculate the area (Fig. 2).

**Histology**

Four months after the permanent ischemic lesion, animals were killed with ketamine (20 mg/kg iv) followed by Euthasol (1 ml; 390 mg pentobarbital sodium/ml ip) and then perfused through the left ventricle with 0.1 mol/l sodium phosphate buffer, pH 7.25 and 4% paraformaldehyde. Coronal sections (50 µm thick) collected across the rostrocaudal aspect of the lesion were stained for Nissl substance following standard protocols. Serial sections were cleared and rehydrated, placed in cresyl violet (15–20 min), then rinsed and dehydrated through graded alcohols. Sections were placed in xylene, then coverslipped with DPX mounting medium. Nissl-stained sections were examined visually to determine the laminar extent of the infarct across the necrotic area.

**Statistical analysis**

Because the motor performance scores are ordinal data, and thus not normally distributed, a nonparametric statistical test (Wilcoxon signed-ranks; Wilcoxon statistic = “T”; lowest P value of 0.031 with n = 5) was employed for analysis of selected pre- and postinfarct comparisons. As multiple comparisons can increase the probability of type I error, these results should be considered with caution. However, because the comparisons were limited to the most critical time points (3 and 13 wks postinfarct), we have limited this possibility to the extent possible.

SMA DFL area measurements of the maps at postinfarct week 3 and 13 were compared with preinfarct measurements using repeated-measures ANOVA with alpha = 0.05. Physiological mapping results were correlated to lesion size and behavioral outcome using correlation Z test analyses.

**Results**

**Verification of cortical infarcts**

Ischemic infarcts targeted the ICMS-defined upper extremity representations in M1, PMd, and PMv and intervening representations within this territory (Figs. 1 and 2). This included the entire distal and proximal forelimb representations in M1, PMd, and PMv and a portion of the face representation along the M1/PMv border. Infarcts were matched as closely as possible based on these neurophysiological criteria. The infarct areas averaged 86.8 ± 12.7 (SD) mm², ranging from 70.5 to 99.9 mm² based on measurements from the scaled digital picture of the blood vessel pattern over the cortex. The variation was due to the normal anatomical differences in the blood vessel patterns and spatial configuration of the forelimb representation.

Histological examination of coronal sections of the lesion stained with cresyl violet confirmed that the injury comprised all cortical layers. Toward the center of the lesion some of the white matter was lost presumably due to the degeneration of axons of neurons within the infarct zone. Histological sections verified that the lesion was confined to the intended targeted areas (Fig. 3).

**General postinfarct behavioral observations**

During preinfarct training, each monkey met criterion performance on the smallest well (well 5) within ~4 wk. The day following the permanent ischemic infarct, and after return to their home cages, each of the five monkeys showed a clear monoplegia of the forelimb contralateral to the cortical infarct. The affected arm was flaccid. The shoulder adopted a medial
rotation and declined position with the elbow turned away from the midline; the hand adopted a neutral position with the fingers extended. The unaffected arm showed no obvious impairments when compared with the affected arm. Weakness of the leg on the affected side was evident only during the first postinfarct day.

After the first 2 or 3 day postinfarct, the monkeys were able to feed themselves normally with standard monkey chow from the food bins. They used the unaffected arm to scratch and groom but not the affected one. No spatial neglect was observed as evidenced by retained ability to retrieve food rewards from the contralateral space. They presented no observable sensory deficits as evidenced by tactile stimulation and reflex withdrawal with both hands with no delay.

During the first 2 wk postinfarct, the monkeys were somewhat inactive compared with their preinfarct behavior. The monkeys were able to climb and jump from different locations within the cage but clearly exhibited postural abnormalities indicated by occasional slips of the contralateral forelimb off the perch and sliding down of the forelimb on vertical cage front rails (presumably denoting weakness). They were able to use their affected hand for climbing and posturing but not for fine coordination.

Beginning approximately in postinfarct week 3 and during the subsequent 9 wk (postinfarct weeks 4–13), general behavior was variable from monkey to monkey. Some improved rapidly and maintained a high level of home cage activity, whereas others were less active. Some sat on the perch and emitted normal vocalizations. Others kept their heads faced down, rested on the perch or on the floor in the back of the cage, and did not emit many vocalizations. In general, however, when considering the five monkeys as a group, by postinfarct week 13, there was an increase in the general activity and use of the previously dominant hand (i.e., the hand that was preferred prior to the infarct) during testing sessions as compared with postinfarct week 3 and especially compared with the first days immediately after the infarct. During the 13-wk period, there was an increase in compensatory use of the impaired hand during normal cage activities.

**FIG. 1.** Cortical infarct. A: lateral view of blue pigment casted squirrel monkey brain. An additional animal was used to study the brain arterial vascular pattern of the squirrel monkey by injection of a plastic mixture through the ascending aorta. Highlighted in black are the main sulci. The pre-Rolandic and Rolandic branches of the middle cerebral artery that emerge from the depths of the lateral sulcus are highlighted in red. All craniectomies (outlined in white) in the 5 monkeys included the central sulcus on the caudal aspect, and the arcuate sulcus on the rostral aspect, bordered the lateral sulcus on the lateral aspect, and the sagittal sinus on the medial aspect. B and C: intracortical microstimulation (ICMS) mapping techniques were used to determine the extent of upper extremity motor representations in frontal cortex. In each case, the target of ischemic lesions was defined by a contiguous area bounded by the physiologically defined primary motor (M1) and ventral and dorsal premotor cortices (PMv and PMd) upper extremity representations, including digits (red), wrist/forearm (green) and proximal (blue) representations, and excluding M1 leg (purple) and trunk (light blue) representations and S1. Most of the face (yellow) representation was excluded, except for an area on the border between M1 and PMv. The outline of the lesion was delineated according to the physiological representation areas, but respecting also a general rule regarding the major blood vessel pattern shown in C (see text). Distal branches of the Rolandic (arrow) and pre-Rolandic (arrowheads) arteries are labeled. D and E: laser-Doppler blood flow images of the area of interest before (D) and 1 h after (E) the ischemic lesion. The scale depicts relative perfusion.
Effect of infarct on hand preference

Prior to the permanent ischemic infarct, all monkeys were tested during two consecutive days for preinfarct Klu¨ver board performance. They performed 50 trials per session on the open board task to assess spontaneous forelimb use and hand preference. The monkeys demonstrated a clear asymmetry in hand use as they preferred one hand over the other on ~75% of the 50 trials. After the infarct, when presented with the open board task, monkeys showed a change in hand preference, retrieving the pellets almost exclusively with their nonimpaired arm (i.e., the previously nonpreferred hand), and using the affected arm an average of 3% of the 50 trials. This change in hand preference persisted throughout the 13-wk period of observation (Fig. 4). One-way ANOVA for number of pellets retrieved with the dominant hand revealed a significant difference ($F_{17.09} = 17.09, P < 0.0001$) for the main effect of week. Preinfarct hand preference was significantly different from each of the postinfarct weeks in post hoc analyses (Dunnett’s test; $P < 0.0001$).

There did not appear to be any trend toward increased use of the impaired limb on this task between weeks 3 and 13 in spite of the observations of increased limb use noted in the general behavioral section in the preceding text.

Effect of lesion on motor performance scores

In previous studies employing more restrictive lesions in M1, manual dexterity was assessed by tallying finger flexions per pellet retrieval on the Klu¨ver board (open board task) or time to retrieve pellets (e.g., Frost et al. 2003). However, with the more extensive lesions employed here, retrievals were typically not successful, and a more general, ordinal motor performance assessment scale was used. As described in METHODS, scores ranged from 0 (does nothing with impaired hand) to 5 (successfully retrieves pellet). Mean open board task performance scores for the five monkeys during the prelesion assessment was $4.8 \pm 0.2$ SE. Mean scores for all monkeys throughout the 13-wk postlesion recovery period on the open board task with the dominant (impaired) hand was $0.6 \pm 0.1$ SE. The
average mean scores of the 13-wk across all monkeys ranged from 0.2 to 1.0. Monkeys attempted retrievals mostly with the nonimpaired hand. They only attempted to retrieve pellets with the impaired hand on average 1.8 ± 0.2 SE times of the 50 trials (3.6%), ranging from 0 to a maximum of 9 attempts (18%) for each session.

To encourage monkeys to use the impaired hand, and thus to more adequately test motor abilities, a clear Plexiglas block was used as a barrier (barrier board task). The monkeys could see through the barrier but could only reach with one hand according to the barrier’s position with respect to the well and hand being tested. This allowed assessment of motor performance with each hand independently. In general, throughout the 13-wk time period, and for all wells, monkeys were able to reach and attempt pellet retrievals with the impaired arm but unsuccessfully. They extended their impaired arm, reached through the bars of the cage front and directed their hand toward the board, touched the board, but were not able to retrieve the pellet. This appeared to be due to an inability to place the fingers inside the well. Throughout this period, however, their performance progressed from not moving their arm at all in postinfarct week 1, to only reaching through the cage bars in postinfarct week 2 to being able to actually reach far enough to touch the board in postinfarct week 3 (Fig. 5). No differential use of specific fingers was observed, and no forearm pronation and/or wrist flexion/extension was used in contrast to preinfarct behavior and in contrast to the nonimpaired limb.

Motor performance scores for all monkeys averaged across all five wells declined from a mean of 4.8 ± 0.3 SE preinfarct to 0.0 ± 0.0 SE on postinfarct week 1. The motor performance score increased to 1.8 ± 0.5 SE on postinfarct week 3 and was 1.9 ± 0.8 on postinfarct week 13. Because motor performance scores are based on an ordinal scale and thus not normally distributed, a nonparametric statistical test (Wilcoxon signed-ranks; Wilcoxon statistic = T; lowest P value of 0.031 with n = 5) was employed for analysis of selected pre- and postinfarct comparisons. This test revealed a significant difference between preinfarct performance and performance on postinfarct week 1 (T = 7.5; P = 0.031) confirming the effectiveness of the infarct. There was an improvement in motor performance scores from postinfarct week 1 to postinfarct week 3 (T = 7.5; P = 0.031), although scores at postinfarct week 3 remained significantly different from preinfarct (T = −7.5, P = 0.031). No difference was found between postinfarct weeks 3 and 13 (T = 1.5, P = 0.375), suggesting that further recovery on this task did not occur (Fig. 5A). Klüver board performance scores for individual wells showed a trend in the degree of recovery from well 1 to 5 reflecting the increasing

![Image](http://jn.physiology.org/)

**FIG. 4.** Change in hand preference. Prior to the infarct, all monkeys (n = 5) were tested during 2 consecutive days for baseline Klüver Board performance to assess spontaneous forelimb use and hand preference. The monkeys demonstrated a clear asymmetry in hand use as they preferred one hand over the other on ~75% of the 50 trials. After the infarct, when presented with the open board task, monkeys showed a change in hand preference, retrieving the pellets almost exclusively with their nonimpaired arm (i.e., the previously nondominant hand). This change in hand preference persisted throughout the 13-wk period of observation.

**FIG. 5.** Klüver board performance. A: throughout the 13-wk postinfarct period, when the barrier was employed on the Klüver board to encourage the use of the impaired hand (filled squares), the monkeys were able to reach and attempt pellet retrievals but mostly unsuccessfully. Open squares, performance with the nonimpaired hand; asterisk, improvement in motor performance scores from postlesion week 1 to postlesion week 3. Scores at postlesion week 3 were significantly different from prelesion. B: motor performance scores for individual wells showed a trend in the degree of recovery from wells 1 to 5 reflecting the increasing level of difficulty in retrieving pellets from the smaller wells. For example, compare performance on well 1 (largest well; blue lines) to well 5 (smallest well; orange lines). For clarity, error bars are not shown. Note that the temporary decline in performance on week 4 is most likely a residual effect of the mapping procedure conducted after the probe trials on week 3.
level of difficulty in placing digits into the wells while attempting to retrieve food pellets from the smaller wells. The average scores across postinfarct weeks 1–13 for each well were: well 1 = 1.9 ± 0.3 SE; well 2 = 1.3 ± 0.2 SE; well 3 = 1.2 ± 0.2 SE; well 4 = 1.1 ± 0.2 SE; well 5 = 1.0 ± 0.1 SE (Fig. 5B).

Effect of lesion on ICMS maps of motor representations in SMA

Postinfarct neurophysiological maps of the SMA DFL (Figs. 1 and 6) were compared with maps at the end of postinfarct weeks 3 and 13 (Fig. 7). The total SMA DFL area included all digit and wrist/forearm representations, which comprised 61% (0.51 mm²) and 39% (0.32 mm²), respectively, in preinfarct maps and then in different proportions at postinfarct weeks 3 and 13. Compared with the preinfarct map, the week 3 postinfarct map demonstrated a decrease in total SMA DFL area in four of the five animals. The percentage decrease ranged from 22 to 89%. In the fifth monkey (697), the total DFL area increased 37%. At postinfarct week 13, an increase in DFL area (relative to preinfarct area) was seen in four of five animals. The percentage increase ranged from 29 to 73%. In the fifth monkey (424D), the total DFL area decreased by 19% compared with the preinfarct map. A one-way repeated-measures ANOVA used to determine changes in area over time (preinfarct, 3 wk, 13 wk) approached statistical significance \(F(4,10) = 4.234, P = 0.056\).

Further analysis of specific movement representations within DFL revealed no statistically significant changes in digit representations across the three maps due to substantial variation across animals. Three of the five animals lost all digit representations at postinfarct week 3, whereas digit area for monkey 424D was largely unchanged, and digit area in monkey 697 increased substantially. Two of the three monkeys that had lost all digit sites on postinfarct week 3 did not recover digit representations on postinfarct week 13. In the third monkey, the digit area was reduced compared with the preinfarct map. The digit area of monkey 424D also decreased in postinfarct week 13. However, the digit area for monkey 697 further increased at postinfarct week 13. Overall, the mean digit representation was 0.51 ± 0.041 SE mm² preinfarct, 0.47 ± 0.33 SE mm² on postinfarct week 3, and 0.43 ± 0.35 SE mm² on postinfarct week 13 (Fig. 8; 1-way repeated-measures ANOVA, \(F = 0.042, P = 0.96\)).

Changes in wrist/forearm representations were more consistent across animals, resulting in a statistically significant difference over the three time points. Wrist/forearm representations decreased in all animals on postinfarct week 3 ranging from a 41% decrease to a total loss of wrist/forearm representations. On postinfarct week 13, wrist/forearm representations increased in four of the five animals, ranging from a 57 to 310% increase compared with preinfarct maps. The wrist/forearm representation in the fifth monkey (697) decreased 21% compared with the preinfarct map, although this was an increase from postinfarct week 3. Mean values for wrist/forearm representations were 0.32 ± 0.5 SE mm² preinfarct, 0.09 ± 0.39 mm² on postinfarct week 3, and 1.01 ± 0.3 SE mm² on postinfarct week 13 (ANOVA, \(F = 9.27, P < 0.01\); Fig. 8). While there appeared to be a trend toward a smaller SMA DFL on postinfarct week 3, this result was not statistically significant (Fisher’s post hoc test for preinfarct vs. postinfarct week 3: \(P = 0.33\)). However, significant differences were found between pre- and postinfarct week 13 (Fisher’s post hoc test; \(P = 0.01\)) and between postinfarct week 3 postinfarct week 13 (Fisher’s post hoc test; \(P = 0.003\); Fig. 8). Thresholds for evoking DFL movements in SMA were not significantly different among the pre- and postinfarct maps (34 ± 3 μA preinfarct, 35 ± 4 μA postinfarct week 3, and 38 ± 3 μA on postinfarct week 13; \(F = 0.71, P = 0.52\)).

Relationship between lesion size and physiological changes in SMA

At postinfarct week 3, there were no correlations between physiological changes in the SMA DFL areas and lesion size \(R^2 = 0.105, P = 0.63\). However, at postinfarct week 13, the change in the total SMA DFL area was positively correlated with infarct size \(R^2 = 0.973, P = 0.0004\; (\text{Fig. 9})\). The larger the lesion, the greater the change in SMA total DFL area compared with preinfarct maps. The changes in the individual movement categories (digit and wrist/forearm) were not correlated to lesion size \(R^2 = 0.328, P = 0.36\) for change in digit area and \(R^2 = 0.03, P = 0.81\) for change in wrist/forearm area.

Relationship between motor performance and physiological changes in SMA

We then examined the relationship between the size of the SMA DFL area and motor performance scores at postinfarct weeks 3 and 13. At postinfarct week 3 these two variables were not correlated \(R^2 = 0.191, P = 0.51\). At postinfarct week 13, motor performance scores were linearly related to the SMA DFL area included digit and wrist/forearm representations, outlined in red, are surrounded by proximal representations. Small dots represent microelectrode penetration sites, placed ~250 μm apart. C: 2-dimensional color-coded reconstruction of an SMA DFL map.
total DFL area \( (R^2 = 0.925, P = 0.005; \) Fig. 10). The larger the SMA total DFL area, the higher the barrier board task performance score achieved with the impaired hand. Individual digit and wrist/forearm areas were not correlated to motor performance \( (R^2 = 0.241, P = 0.45 \) for digit area and \( R^2 = 0.036, P = 0.79 \) for wrist/forearm area). The change in behavior from postinfarct week 3 to postinfarct week 13 was not related to the change in area during this same time period \( (R^2 = 0.247, P = 0.44)\).

**DISCUSSION**

**SMA reorganizes after injury to other frontal motor areas**

This study presents several novel results: first, after M1, PMd, and PMv motor representations were destroyed, distal forelimb movements could still be evoked by stimulation of SMA at relatively low current levels. This demonstrates that descending output from M1, PMd, and PMv is not necessary for SMA representations to be maintained. Second, SMA representations were altered after large cortical injuries. Third, changes in SMA representations after motor cortex lesions were time dependent. In particular, wrist-forearm representations underwent a substantial enlargement relatively late, between postinfarct weeks 3 and 13, attaining a size substantially larger than the preinfarct area. Fourth, SMA area in the chronic state (13 wk postinfarct) appeared to be related both to the absolute size of the lesion and to the behavioral performance of the animal. These correlations do not necessarily imply causation as we discuss later. However, they point to a linkage between lesion size, performance, and physiological measures in SMA. Together, these data suggest that changes in SMA are directly triggered by the damage to other motor areas. However, the temporal mismatch between behavioral recovery (through week 3) and physiological plasticity (between weeks 3 and 13) raises questions regarding the role of the spared SMA in the recovery process.

Recent neuroimaging data seem to point to the involvement of spared ipsilesional motor areas (in the same hemisphere as the lesion) and suggest that recovery of function of the paretic hand occurs as a consequence of a dynamic, bihemispheric reorganization after stroke onset. However, given that lesion location and size determine the outcome and degree of cortical plasticity after stroke, reorganization of the motor cortex may...
follow different mechanisms depending on whether primary motor, premotor, or supplementary motor areas in the ipsilateral hemisphere are spared and depending on whether subcortical structures are included in the lesion.

Previous studies following partial or total M1 cortical lesions have demonstrated significant recovery during the first several weeks postinjury. It has been suggested that at least part of the recovery may be due to plasticity of spared brain regions in the peri-infarct region, in the somatosensory cortex (Dancause 2006; Pons et al. 1988) or in more remote motor cortical areas such as PMv (Frost et al. 2003). In the present study, the changes found in the DFL movement representation in SMA following ischemic infarct in the forelimb representation of M1, PMv, and PMd indicate that neurophysiologic reorganization of more remote cortical motor areas occurs also in response to an extensive cortical infarct including other frontal motor areas. Thus it would appear that reorganization of secondary cortical areas is a general feature of injury-induced plasticity.

Several features make SMA an optimal remote area for functionally relevant reorganization following an ischemic lesion. SMA receives its blood supply from the anterior cerebral artery and is thus commonly spared from MCA occlusion, the most common vascular location in clinical stroke. The

![FIG. 9](https://example.com/fig9.png)

**FIG. 9.** Correlation between change in SMA total DFL representation and lesion size at postlesion week 13. The changes in DFL representation areas from preinfarct to postinfarct week 13 positively correlated with lesion size ($R^2 = 0.973, P = 0.0004$).

![FIG. 10](https://example.com/fig10.png)

**FIG. 10.** Correlation between SMA total DFL representation and Kluver board performance score at postlesion week 13. Behavioral performance was positively correlated to the SMA total DFL representation on postlesion week 13 ($R^2 = 0.925, P = 0.005$).

### TABLE 1. Area measurements for total DFL within SMA, absolute and percent change from preinfarct and the distribution of digit and wrist/forearm components

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Monkey</th>
<th>Total DFL</th>
<th>Digits</th>
<th>Wrist/Forearm</th>
<th>Change from Pre-infarct</th>
<th>Digits</th>
<th>Wrist/Forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-infarct</td>
<td>424D</td>
<td>0.88</td>
<td>0.67</td>
<td>0.21</td>
<td>-0.19 (-22)</td>
<td>0.02 (3)</td>
<td>-0.21 (100)</td>
</tr>
<tr>
<td>Post-infarct (PI) wk 3</td>
<td>684</td>
<td>0.69</td>
<td>0.69</td>
<td>0.06</td>
<td>-0.17 (-19)</td>
<td>-0.52 (-78)</td>
<td>0.35 (167)</td>
</tr>
<tr>
<td>PI wk 13</td>
<td>684</td>
<td>0.71</td>
<td>0.15</td>
<td>0.56</td>
<td>-0.85 (-89)</td>
<td>-0.46 (-100)</td>
<td>-0.39 (-80)</td>
</tr>
<tr>
<td>Pre-infarct</td>
<td>681</td>
<td>0.95</td>
<td>0.46</td>
<td>0.49</td>
<td>0.9 (95)</td>
<td>-0.46 (-100)</td>
<td>1.36 (278)</td>
</tr>
<tr>
<td>PI wk 3</td>
<td>681</td>
<td>0.15</td>
<td>0.1</td>
<td>0.05</td>
<td>-0.58 (-77)</td>
<td>-0.46 (-100)</td>
<td>-0.12 (-41)</td>
</tr>
<tr>
<td>PI wk 13</td>
<td>681</td>
<td>1.85</td>
<td>0</td>
<td>1.85</td>
<td>0.73 (97)</td>
<td>-0.46 (-100)</td>
<td>1.19 (410)</td>
</tr>
<tr>
<td>Pre-infarct</td>
<td>695</td>
<td>0.87</td>
<td>0.5</td>
<td>0.37</td>
<td>-0.69 (-79)</td>
<td>-0.5 (-100)</td>
<td>-0.19 (-51)</td>
</tr>
<tr>
<td>PI wk 3</td>
<td>695</td>
<td>0.18</td>
<td>0</td>
<td>0.18</td>
<td>0.25 (29)</td>
<td>-0.33 (-66)</td>
<td>0.58 (157)</td>
</tr>
<tr>
<td>PI wk 13</td>
<td>695</td>
<td>1.12</td>
<td>0.17</td>
<td>0.95</td>
<td>-0.58 (-77)</td>
<td>-0.46 (-100)</td>
<td>1.19 (410)</td>
</tr>
<tr>
<td>Pre-infarct</td>
<td>697</td>
<td>0.7</td>
<td>0.46</td>
<td>0.24</td>
<td>0.96 (137)</td>
<td>1.2 (261)</td>
<td>-0.24 (-100)</td>
</tr>
<tr>
<td>PI wk 3</td>
<td>697</td>
<td>1.66</td>
<td>1.66</td>
<td>0</td>
<td>1.21 (173)</td>
<td>1.35 (293)</td>
<td>-0.05 (-21)</td>
</tr>
<tr>
<td>PI wk 13</td>
<td>697</td>
<td>1.91</td>
<td>1.81</td>
<td>0.19</td>
<td>-0.31 ± 0.33 (30)</td>
<td>-0.04 ± 0.32 (-7)</td>
<td>-0.27 ± 0.04 (-94)</td>
</tr>
<tr>
<td>Pre-infarct</td>
<td>Average</td>
<td>0.83 ± 0.05</td>
<td>0.51 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td>-0.31 ± 0.33 (30)</td>
<td>-0.04 ± 0.32 (-7)</td>
<td>-0.27 ± 0.04 (-94)</td>
</tr>
<tr>
<td>PI wk 3</td>
<td>Average</td>
<td>0.56 ± 0.29</td>
<td>0.47 ± 0.33</td>
<td>0.09 ± 0.04</td>
<td>-0.31 ± 0.33 (30)</td>
<td>-0.04 ± 0.32 (-7)</td>
<td>-0.27 ± 0.04 (-94)</td>
</tr>
<tr>
<td>PI wk 13</td>
<td>Average</td>
<td>1.41 ± 0.23</td>
<td>0.43 ± 0.35</td>
<td>1.01 ± 0.30</td>
<td>0.55 ± 0.24 (71)</td>
<td>-0.19 ± 0.36 (-26)</td>
<td>0.76 ± 0.26 (231)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. Bottom three rows here values expressed as means ± SE.
SMA DFLs of the two hemispheres are strongly interconnected via the corpus callosum (Rouiller et al. 1994) and heavily interconnected ipsilaterally with the M1 DFL (Stepniewska et al. 1996). SMA has been shown to be involved in the control of body and limb posture (Tokuno and Tanji 1993; Wiesendanger 1996) to be related to experience with a particular movement sequence (Lee and Quessy 2003) and in specifying the laterality of the arm to use (Hoshi and Tanji 2004). SMA has also been implicated in regulating synchronization between M1 of both hemispheres (Wiesendanger et al. 1994) and in the initiation and execution of limb movements because it is activated prior to M1 during movement production (Deecke et al. 1999). Thus although plasticity in the ipsilesional SMA might not be sufficient to substitute for function lost following extensive injury to the other major frontal motor representations, it might be specially suited to detect alterations in the normal connection pattern and direct reorganization of descending systems.

The extensive ipsilateral projections of SMA (23%) (Dum and Strick 1996) and its role in bimanual coordination should be considered when interpreting the neurophysiological changes observed in the SMA DFL. It is conceivable that map modifications in SMA are related to the unaffected forelimb. Although these ipsilateral projections terminate mainly in laminae VII and VIII in the spinal cord (Dum and Strick 1996), with greater influence on motor neurons pools controlling proximal rather than distal muscles (Colebatch et al. 1979; Kuypers and Brinkman 1970; Nirkko et al. 2001), SMA might be specially accommodated to orchestrate postinjury compensatory strategies employing more proximal joints. The appreciation of the strong SMA homotopic connections have led to the speculation of SMA as having an important role in shifting of attention between activation of both hemispheres (Brinkman 1984) and in reflecting which arm to use during execution of function (Hoshi and Tanji 2004). These functions might become especially relevant following stroke even if this does not necessarily involve recovery of function in the sense of regaining lost motor capabilities. Compensatory strategies include trunk and shoulder-girdle movements, especially in reach-to-grasp strategies (Cirstea and Levin 2000; de Oliveira et al. 2007), further supporting the role of SMA in recovery after stroke. The fact that ipsilateral terminations are found in the proximal and trunk zone of the spinal cord, which receive massive bilateral projections from reticulo- and vestibulospinal systems (Kuypers and Brinkman 1970), suggests that ipsilesional SMA changes might be tightly related to simultaneous changes in the contralesional cortex.

From the results to date, it is not possible to determine if any one motor area is more important in the recovery of motor abilities after stroke. The present study did not monitor changes that might be occurring in cingulate motor areas given that they are less accessible for neurophysiological mapping studies. Nevertheless we hypothesize that the entire cortical and subcortical motor system that is spared by the injury participates to varying degrees depending on the extent and location of the injury and behavioral demands. At least some of the functions of the injured region(s) are redistributed across the remaining cortical and subcortical motor network, yet recovery ultimately depends on the integrity of the entire motor system.

**Relationship of postinfarct map changes to lesion size**

It has been demonstrated previously that the degree of neurophysiological plasticity in spared cortical areas after ischemic infarct is directly proportional to lesion size. In that study, ICMS techniques were used to analyze motor representations in PMv of adult squirrel monkeys before and after an ischemic infarct in M1 DFL. ICMS mapping at 3 mo postinfarct revealed substantial enlargements of the DFL in PMv, suggesting that the disruption of cortical connectivity between M1 and PMv was responsible for PMv reorganization. A follow-up study tested this hypothesis of disruption of cortical connectivity underlying reorganization by inducing restricted lesions in subregions of M1. PMv projects most exclusively to the rostral portion of M1 and only sparsely to the caudal portion of M1. Lesions resulted in a reduction of the PMv DFL at 3 mo postlesion regardless of whether the subtotal lesion was in the rostral or caudal subregion, suggesting that following partial M1 lesions, plasticity in the peri-infarct cortex may be sufficient for recovery to occur. Plasticity (as evidenced by enlarged motor representations) can occur in connected remote regions but only when the injury to the primary region is substantial. Thus in the case of the extensive cortical lesions used in the present experiments, it is conceivable to expect that disruptions in the cortical connectivity between SMA and the other frontal motor areas are driving enlargements of its DFL representations during recovery.

**Relationship of postinfarct map changes to behavioral outcome: the conundrum of temporal mismatch**

While many studies have demonstrated changes in motor maps in remote regions using ICMS in animals or TMS/fMRI in humans, the relationship of postinfarct map changes to behavioral outcome is poorly known. The physiological changes in the SMA DFL found in the present study were linearly correlated with the improved motor function at the end of the 13-wk evaluation period. The larger the SMA total DFL area, the higher the motor performance score achieved. A casual appraisal of this data would suggest that the cortex is responding to new behavioral demands and that the physiological changes underlie improvement in motor function. There are, however, a few caveats to this interpretation.

The correlation between changes in the SMA DFL and motor behavior were present at postinfarct week 13 but not at postinfarct week 3. Analysis of motor performance scores throughout the 13-wk postinfarct spontaneous recovery period revealed a significant improvement from postinfarct week 1 to postinfarct week 3. However, while changes in the SMA DFL were observed at postinfarct week 3, these did not reach statistical significance. In fact, the potential trend that was observed was in the opposite direction; that is, SMA DFL tended to decrease in area. This suggests an initial and transient dissociation between improvement in motor performance and physiological changes. These two parameters seem to evolve at different rates. The early changes in behavior do not translate in, or are not evidenced by, early changes in the maps (Fig. 11).

Temporal disparities between neurophysiological/neuronatomical endpoints and behavioral changes paralleling the present results after lesions have been discussed recently in the...
context of motor learning in intact animals. Kleim et al. demonstrated in adult rats that both motor map reorganization and synaptogenesis occurred in the late phase of motor learning well after motor improvement was evident. In their cogent discussion, several possible reasons for this temporal disparity were raised, which we summarize and expand on here (Kleim et al. 2004). We refer to these potential reasons as wrong location, wrong mechanistic endpoint, and wrong behavior. First (wrong location), it is possible that neurophysiological changes occur during the first three weeks but in a different cortical or subcortical location that was not monitored, such as cingulate motor cortex, striatum or cerebellum. If so, perhaps these other motor structures are more critically engaged in recovery processes during the early stages. Second (wrong mechanistic endpoint), it is possible that functionally significant changes that occur within the first three weeks do not involve map reorganization. It is possible that other changes in network connectivity properties occur much earlier after injury and contribute to behavioral recovery. These might include changes in neurotransmitters or their receptors that have repeatedly been observed, such as GABA<sub>A</sub> receptor downregulation and N-methyl-D-aspartate receptor upregulation (Witte 1998; Witte and Stoll 1997). Alternatively, depression of blood flow and metabolism in remote spared regions might recover relatively early, a concept embodied in the classic definition of diaschisis (Von Monakow 1914).

Third (wrong behavior), it is possible that map changes are related to some other aspect of behavior. Because the monkeys were not able to engage in this particular task requiring skilled and dexterous use of the digits, it is possible that we did not capture other aspects of their behavior that may have been improving between weeks 3 and 13. For example, it is possible that compensatory changes in the kinematics of more proximal body parts may evolve over a longer time period. These behaviors were not examined in the present study. This is especially relevant because physiological changes observed in the SMA DFL primarily involved wrist/forearm movements, more so than digit movements. Also, still more proximal representations (elbow, shoulder, trunk) were not examined systematically in this study. It is well known that in human stroke survivors, proximal musculature control contributes substantially to improvement on functional scales (Cirstea and Levin 2007). Alternatively, it is possible that the late physiological changes are related to some aspect of altered bilateral control of the forelimbs by the SMA. The role of the SMA in bilateral control of the limbs has often been suggested (Wiesendanger et al. 1996).

Finally, it should be emphasized that postinjury changes in behavior and neurophysiology are interactive, and the determination of causation may not be feasible. The injury clearly disrupts intracortical connectivity with the remote spared regions, undoubtedly resulting in alterations in output properties. Likewise, it is well known that behavior has a potent modulatory effect on neurophysiological maps in motor cortex, both in intact individuals undergoing skill training, and in cortically injured individuals undergoing recovery (Liepert et al. 1998, 2000; Nudo and Milikien 1996; Plautz et al. 2000). Thus this is a classic “chicken-and-egg” question. Behavior is just as likely to drive map plasticity as the map plasticity is to drive behavior.

Functional relevance of ipsilesional plasticity in SMA

In macaque monkeys, the amount of the SMA devoted to the DFL is nearly equal to that devoted to the more proximal body parts (Lemon et al. 2002). The size of the SMA region that projects most densely to lower cervical segments is comparable to the region that projects most densely to upper cervical segments (He et al. 1993). Although the majority of the corticospinal efferents from the SMA terminate in the intermediate zone of the spinal cord, some terminations are found within lamina IX where motoneurons are located. Further, in macaque monkeys, corticomotoneuronal connections originating from neurons in SMA and M1 converge on single motoneurons (Maier et al. 2002). Thus the anatomical substrate exists for the SMA to directly control arm movements independent of output from M1 (Dum and Strick 1996).

Despite this potential substrate, at least in intact animals, the density of spinal projections from SMA is 13 times less (squirrel monkey) (Maier et al. 1997) and the magnitude of effects on muscle activity 10 times smaller as compared with M1 (macaque monkey) (Boudrias et al. 2006). This markedly weaker output from SMA to motoneurons compared with M1 raises some doubts about the role of SMA corticospinal neurons in the direct control of muscle activity, and hence, its potential role in recovery after M1 lesions. However, the anatomical substrate and functional contributions of SMA to the control of movement in a normal setting might be significantly modified following an extensive cortical infarct. This possibility is supported by a recent study showing long-term reorganization of the corticospinal projection from SMA following aspiration lesions in primary motor and premotor cortex in macaque monkeys. SMA projections appear to terminate in
deeper lamina several months after cortical injury (McNeal et al. 2007).

Use-dependent reorganization of neuronal activity in SMA has been described by Tanji et al. in macaque monkeys (Aizawa et al. 1991; Tanji and Kurata 1982; Tanji et al. 1987). Previously it was shown that neurons in SMA of non-human primates are active before limb movements (Wiesendanger 1996). This premovement activity in SMA has been observed in relation to the execution of relatively recently trained motor tasks (Tanji and Kurata 1982; Tanji et al. 1987). Premovement activity in SMA is no longer observed after a period of overtraining but can be re-elicted three weeks after M1 lesions (Aizawa et al. 1991). Although in this study, changes in the properties of movement-related cellular activity, rather than ICMS effects, were examined, these results correspond well in terms of demonstrating physiological changes in SMA following an M1 infarct.

Relationship to neuroimaging studies of recovery and reorganization in human stroke

Several interrelated variables influence the results and subsequent interpretation of brain reorganization and recovery after stroke. These include lesion location, lesion size, initial stroke severity, degree of recovery, and time after stroke. While it is useful to compare neurophysiological results in animals with fMRI results in human stroke, the dissociation of these confounding variables presents substantial challenges, and thus correlations between human and animal results are still unclear. For example, in an fMRI study of stroke survivors with subcortical lesions (Loubinoux et al. 2003), early recruitment and high activation of the SMA at 11 days after stroke was correlated with a faster or better motor recovery at 4 and 12 mo after stroke. This result would seem to correlate well with the present neurophysiological findings. However, in another study that included chronic stroke survivors with cortical and/or subcortical MCA infarct sparing M1 (Ward et al. 2003b), a negative correlation was found between outcome and the degree of task-related activation in regions such as the SMA. These examples point out the complexity in interpreting and extrapolating results from studies in which different lesion models are used.

One pattern that does appear to be emerging, and that may be common to both neuroimaging and neurophysiological endpoints, relates to temporal progression of activation patterns. Stroke survivors with poorer recovery appear to activate the contralateral hemisphere areas more (e.g., Johansen-Berg et al. 2002; Ward et al. 2003a), especially early after stroke. As recovery proceeds, as well as in stroke survivors with better recovery, a pattern of greater activation is observed in the spared ipsilesional hemisphere motor areas (non-human primate studies: Frost et al. 2003; Liu and Rouiller 1999; human studies: Carey et al. 2002; Seitz et al. 1998; Weiller et al. 1992). The present study provides more clarity for this comparison by demonstrating the temporal progression of cortical motor output patterns. The physiological data in SMA of squirrel monkeys are particularly useful because such midline motor areas are difficult to stimulate in isolation in humans using TMS.

One final point regarding the comparison with laterality results in human fMRI studies is warranted: in the present study, monkeys had extensive cortical lesions and did not demonstrate substantial recovery. Although contralesional plasticity was not measured, one might predict that it would be extensive under these circumstances. It is important for future studies to address neurophysiological changes in contralesional motor areas.

Conclusion

Results from the present study show that extensive cortical lesions involving the forelimb representations of M1, PMv, and PMd induce a permanent deficit in the contralateral upper extremity. Although some improvement in motor performance scores was achieved in the first three weeks, no significant functional recovery was observed during the rest of the 13-wk evaluation period. There was a reduction in the SMA DFL at postinfarct week 3, which, however, did not achieve statistical significance. On postinfarct week 13, there was a significant expansion in the wrist/forearm component. Physiological changes in the SMA DFL on postinfarct week 13 were positively correlated to both lesion size and improved motor performance.

The extension of adaptive processes to remote motor areas after injury to an extensive cortical area including M1, PMv, and PMd has broad implications. While motor deficits following brain injury are often severe and permanent, certain types of physical therapy often can enhance motor ability. Any understanding of the dynamics of cortical map alterability bears significance not only for the development of sensorimotor skills under cortical control but also for the impact of physical therapy on recovery of lost motor skills following injury. Eventually it may be possible to design new therapeutic approaches to treatment of cortical injury that are guided by the rules governing functional and structural plasticity in the cerebral cortex.

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