Motor But Not Sensory Representation in Motor Cortex Depends on Postsynaptic Activity During Development and in Maturity

Samit Chakrabarty1 and John H. Martin1,2
1Center for Neurobiology and Behavior, Columbia University; and 2New York State Psychiatric Institute, New York, New York

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INTRODUCTION

The primary motor cortex (M1) is a site of interaction between a variety of sensory and integrative systems and spinal motor circuits. It is the principal origin of the corticospinal tract. For the corticospinal tract to achieve an important role in skilled movement, it depends on development of intrinsic M1 connectivity, the myriad of inputs, and spinal motor circuits. The movement representation in M1 develops between postnatal weeks 7–12 in the cat (Chakrabarty and Martin 2000), immediately after the period when corticospinal (CS) terminations develop a mature topography (Li and Martin 2001, 2002). This sequence suggests the significance of relatively stable spinal connections in development of the motor representation. Most of the characteristics of the immature motor map at 11–12 wk are maintained into maturity, including the percentage of sites where stimulation evokes movement and the current thresholds for evoking movements (Chakrabarty and Martin 2000).

The mature motor map is not static; it can be modified by experience throughout life (Keller et al. 1996; Sanes and Donoghue 2000). Although the organization and plasticity of the mature motor map are well understood, little is known of the determinants of development and maintenance of map characteristics. Recently, we showed that motor experience during motor-map development does not affect representation topography but does affect development of several map parameters (Martin et al. 2005). However, these developmental changes are not permanent as the map reverted to a normal organization several months after cessation of training. This shows that maintenance of the map depends on continued limb use.

In addition to a movement representation in M1, there is also a somatosensory representation (Asanuma 1981). In maturity, most M1 neurons have well-defined mechanosensory receptive fields. M1 neurons receive somatosensory inputs at ages before motor map development (Bruce and Tatton 1980; Chakrabarty and Martin 2000), suggesting that the somatic sensory map helps shape motor-map development (Bruce and Tatton 1980).

In this study, we examined the role of neural activity in development and maintenance of the M1 movement and somatosensory representations. Neural activity is important for development of the normal organization of various sensory systems (Shatz 1990) and for corticospinal terminations (Martin 2005). We blocked postsynaptic activity in sensory-motor cortex for 1 mo during the motor-map development period by intracortical infusion of the GABA agonist muscimol. In adult cats, we blocked activity for 1 mo. We reasoned that activity is selectively important in development of the somatic sensory and motor representations if activity blockade during development, but not maturity, altered the representations. By contrast, if activity blockade had the same effects at both ages, then motor cortex activity—while possibly important in map development—was equally necessary for map maintenance.

METHODS

Sensory-motor cortex activity blockade

All experiments were conducted with Institutional Animal Care and Use Committee approval. To block activity in sensory-motor cortex.
Inactivation

<table>
<thead>
<tr>
<th>Age at Mapping, Days</th>
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<tr>
<td></td>
<td>Active</td>
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<td>Adult</td>
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Values are means ± SE.

Intracortical microstimulation and recording

For motor and sensory mapping, anesthesia was induced with ketamine (30 mg/kg im) and xylazine (0.6 mg/kg im), and anesthesia was maintained using ketamine infusion (10 mg $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$ iv; adjusted to maintain an areflexive state) as previously (Chakrabarty and Martin 2000; Martin et al. 2005). Ketamine anesthesia maintains muscle tonus, and therefore is well-suited for motor mapping studies. Animals were placed in a stereotactic frame. Body temperature was maintained at 39° by a heating pad. For animals subjected to infusion, craniotomies were made on each side over the lateral portion of the anterior parietal and frontal lobes to expose the forelimb areas of the sensory-motor cortex. The dura was incised, exposing a radius of $\geq$3–5 mm of cortex around the lateral margin of the cruciate sulcus. In most experiments, we photographed the exposed cortex using a CCD camera attached to a dissecting microscope and referenced each electrode penetration to a specific location on the cortical surface. In experiments in control 7-wk-old animals and adults (see Table 1), a craniotomy was made on only one side and only that side was examined.

Electrode penetrations, orthogonal to the pial surface and $\sim$1 mm apart, were made into homotopic regions of the silenced and contralateral active M1 in each animal subjected to muscimol infusion. To minimize animal state-dependent effects, especially due to anesthesia level, we alternately examined sites in rostral and then caudal regions after a series of penetrations and, if both sides were examined, one and then the other side. In all animals, penetrations were made between the surface and 2 mm to examine the surface cortex. In all animals, the region sampled was the same. We made penetrations within approximatively a 3-mm radius of the tip of the cruciate sulcus. This area corresponds to the forelimb representations in kittens (Chakrabarty and Martin 2000) and adults (Armstrong and Drew 1985; Keller 1993; Pappas and Strick 1981) of area 4-gamma (Hassler and Muhs-Clement 1964). This area projects densely to the cervical enlargement in kittens and adult cats (Li and Martin 2000; Martin 1996).

We used low-impedance tungsten microelectrodes (Microprobe; 0.5 MΩ impedance) for microstimulation and recording multiunit activity. Motor effects produced by microstimulation occurred at lowest stimulus currents at depths where we recorded multiunit activity with the largest amplitude spikes (typically 1.2–1.5 mm below the pial surface). Multiunit activity for recording peripheral inputs was present throughout most of the depth of the cortex but was most clear from $\sim$300 μm below the pial surface to the depth of the large cell layer. The characteristics of the multiunit recordings were similar for the infused and noninfused or control sides.

We recorded activity and determined the presence, modality (cutaneous or deep), and location of peripheral mechanosensory receptive fields. A penetration site was considered to receive somatosensory input if multiunit activity that was driven by peripheral input could be recorded at least at one depth within the penetration. Cutaneous fields were driven by gentle tapping of the skin or brushing of the hairs. Deep receptive fields were activated by joint rotation or pressure applied to muscle. Marking lesions were made to help identify the location of the region sampled. The sampled cortical area was removed and postfixed in 10% formal-saline. Sections were cut and Nissl-stained.

Stimuli (45-ms duration train, 330 Hz, 0.2-ms biphasic; every 3 s) were delivered using a constant current stimulator (AM Systems). We kept the limb in a posture in which the shoulder was slightly extended, the elbow was approximately half-way between flexion and extension, and the wrist was plantarflexed. We determined the threshold, defined as the lowest current that consistently produced a motor effect. We raised the current to suprathreshold values (in increments of 1–10 μA depending on the absolute threshold value), then reduced the current to below threshold, noting the lowest current at which the effect was present. Next, we increased the current from a subthreshold value to $\geq$1.5 times threshold and noted when effects reappeared.

We used a maximal current of 100 μA. For each penetration, we determined the type of motor effect produced by a threshold and 1.5 times threshold stimulus on the basis of the evoked phasic kinematic change. For each hemisphere in every cat, we computed the mean percentage of effective sites (i.e., number of sites where forelimb effects $\leq$100 μA were evoked in relation to all sites examined), mean

**TABLE 1. Summary of experiments and microstimulation current thresholds**

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current threshold, and mean percent of multijoint sites, and mean number of effects produced at these sites by \( \leq 1.5 \) times threshold stimulus.

Standard statistical analyses were conducted using the program Statview for the Apple Macintosh computer. An unpaired \( t \) statistic was computed for comparing microstimulation current threshold values and receptive field modality for the treated and untreated sides. A \( \chi^2 \) test was used for comparing the proportional distributions of receptive fields and microstimulation effects (proximal vs. distal) for the treated and untreated sides.

**RESULTS**

**Primary motor cortex representations in kittens prior to inactivation**

Prior to day 49 (week 7) microstimulation in M1 does not evoke muscle contraction or limb movement but recordings indicate that M1 neurons receive somatosensory information (Bruce and Tatton 1980; Chakrabarty and Martin 2000). This was confirmed in this study (see Table 1; untreated control animals). We determined the percentage of M1 sites receiving somatosensory inputs at this age, receptive field location, and modality (cutaneous or deep). Peripheral receptive fields were recorded in three untreated control cats (43–45 days old; see Table 1) at 91 ± 3% (mean ± SE) of penetrations. All forelimb segments were represented, but the majority of sites received input from the proximal forelimb and adjoining axial sites (50.2 ± 0.8% proximal; 10.6 ± 0.02% paw; 10.7 ± 0.5% forearm and arm). The remaining sites (28.5 ± 1.3%) received input from most of or the entire forelimb. Multiunit activity at most sites (for each animal: 73, 80, 58%) was driven by deep stimulation. Typically, the receptive field and modality of the multiunit activity within a penetration was the same at all depths. These results indicate that most of the developing forelimb representation of M1 receives somatosensory information from the entire forelimb and adjoining body axis before the motor map develops.

**Effect of inactivation on development of the motor and somatosensory maps**

To determine the role of neural activity in motor and somatosensory map development, we infused muscimol into the lateral pericruciate cortex (Fig. 1; gray dots) between 49 and 77 days (7–11 wk; see Table 1). We waited either 3–4 or 14 days between cessation of the infusion and motor and sensory mapping for activity to return. The short delay was to capture the maps as close to the inactivated period as possible, whereas the longer delay was to ensure that the effects were not due to residual inactivation. As we show in the following text, there were no systematic differences in the number of effective motor sites for these two delay periods nor in the number of sites in which receptive fields were recorded.

Representative motor and somatosensory maps 2 wk after cessation of activity blockade in a day 91 animal (week 13) are shown in Fig. 1, A and B. Each square corresponds to a penetration site; color codes current threshold. Red squares are the sites where the maximal stimulus current was used. The letters indicate movement type: digits (D), wrist (W), elbow (E), or shoulder (S). Multiple letters at a single site correspond to multijoint effects (W,E, wrist and elbow; W,S, wrist and shoulder; M, wrist, elbow, and shoulder). Stimulation of few sites within the silenced cortex (left) evoked a motor response. By contrast, stimulation of most sites on the active side was effective in evoking responses. The bars in Fig. 2A plot the mean ± SE percentage of effective sites for evoking motor responses in the silenced (dark gray) and contralateral active
The responses evoked from the silenced cortex were predominantly proximal (elbow and shoulder). In the three cats in which stimulation of the silenced cortex evoked movement (no effects were evoked in 1 cat), 94.1% were proximal (either at the elbow or shoulder), whereas stimulation of the active side in the same three animals evoked proximal (elbow and shoulder) responses at 71.2% of the sites \( (P < 0.05; \chi^2 = 3.88) \).

Although inactivation had a profound effect on the motor map, there was little or no change in the somatosensory representation after the blockade was removed. At all sites we were able to record single- and multiunit activity driven by mechanical stimulation from the contralateral side. This activity was recorded from superficial to deep layers. Figure 1B re-plots the motor threshold maps for the silenced and active sides (from A) without listing the motor particular effects but with the location of the receptive field for each penetration coded as letters. Fields were located on the digits (D), paw (P), forearm (FA), upper arm (A), in the region of the shoulder (S), and on other proximal body sites (most commonly the scapular and pectoral regions; marked by ×). Sites where no receptive field was recorded are unlabeled. For the case shown, there were three sites from which no receptive fields were detected on the silenced cortex (left). All sites, in the active cortex (right), received somatosensory input.

There were no differences in the incidence of mechanical inputs to M1 sites sampled in the four animals in which activity was blocked. The bars in Fig. 2B plot the percent of penetrations from which receptive fields were recorded. This figure shows that activity blockade did not affect the incidence of M1 sites which receive somatosensory input.

Effects of inactivation on mature motor and sensory maps

The absence of motor effects after inactivation could reflect the importance of neural activity in the expression of the motor effects in response to microstimulation. This would indicate a role for activity in motor map maintenance not necessarily in development. To determine this, we examined the effects of month-long inactivation on stimulation-evoked responses in two adult cats. We also examined the effects of this inactivation on the location and modality of receptive fields. Figure 3 shows the motor (A) and somatosensory (B) maps from one of the adult cats subjected to inactivation. As with the older kittens (83–91 days), comparison of the previously silenced and active sides revealed a profound reduction in the number of sites from which stimulation evoked a motor response. Data from the two adult cats are plotted as the gray dots in Fig. 2. The number of motor sites was markedly reduced in one cat (from 84 to 36%; same cat as in Fig. 3) and entirely eliminated in the other (from 77% to 0). Mean current threshold for the active sides was 36.6 ± 0.3 \( \mu \text{A} \) (see Table 1) and for the silenced side in the animal that showed some effects was...
A The threshold for the active side was very similar to that of untreated control adult animals (Table 1). Comparison of the motor map data in kittens and adult cats show that—while activity could be necessary for motor map development—it is also essential for the maintenance of the motor map.

By contrast to the profound changes in the motor map, sites from which receptive fields were recorded were reduced only slightly (89.2 and 84.9% compared with 100% on the silenced side). While this could reflect a small effect of inactivation, the percentage is similar to the percent of penetrations with receptive fields in two untreated control adult cats (90.2 and 90%). M1 sites in which proximal receptive fields were recorded outnumbered those in which distal receptive fields were recorded for both the silenced side and controls (silenced side, average of 2 cats: 69.9% proximal, 14.2% distal; control cats, average of 2 cats: 59.7% proximal, 31.2% distal). While the active side in one animal was like that of the controls (55.9% proximal, 17.6% distal), there was a distal bias in the second animal (9.5% proximal, 52.4% distal). In one treated adult, like the treated kittens, there was a preponderance of deep receptive fields on both the silenced (60% deep) and active (59% deep) sides. In the other treated adult, a minority of sites received deep inputs (13% deep on silenced side and 14% on the active side). The two untreated adult cats also had a preponderance of sites receiving deep mechanoreceptive inputs (75 and 80%).

We pooled the receptive field data for all of the muscimol-infused cats (4 kittens and 2 adult cats) and found that there was no difference in the proportion of deep fields recorded from the silenced and active sides ($t = 0.979; P = 0.3604$).

**DISCUSSION**

The movement representation in M1, both during development and in maturity, depends on motor cortex neural activity. Activity blockade in older kittens and adult cats profoundly decreased the number of sites from which motor effects are evoked by stimulation and elevated stimulus current thresholds. The effect of activity blockade in kittens could be due both to an inability to develop new connections for constructing the motor map and an inability to maintain preestablished connections for supporting the motor map. The effect in maturity clearly argues a role for M1 neural activity in map stability or maintenance, and in consequence, the need for M1 activity for maintenance of CS system functions. This could have important implications for promoting function after stroke or spinal cord injury.

The motor map was examined using microstimulation. Elimination of the map after inactivation was not due to electrical inexcitability. At both 3 and 14 days after cessation of inactivation, peripheral somatic sensory stimulation was effective in evoking single- and multiunit activity in the previously inactivated cortex. And the characteristics of this activity was indistinguishable from the contralateral active cortex. It is unlikely that the M1 neurons can be synaptically activated by afferent input but not electrically activated by microstimulation. The return of contact placing three days after cessation of muscimol infusion is consistent with our earlier work of the reversibility of the infusion at various ages (Friel and Martin 2005; Martin et al. 1999; Martin et al. 2000). In light of the defective M1 motor map at the time of testing, this finding implies that the placing reaction, although dependent on the level of cortical excitability, can function independent of an organized motor map. This is consistent with earlier studies in normal and spinalized kitten (Amassian 1977; Amassian and Ross 1978; Forssberg et al. 1974). These assessments of cortical neuronal excitability are indirect and may not distinguish subtle changes in the excitability of the networks engaged by microstimulation. Therefore we cannot rule out that M1 neuronal excitability has not fully returned to normal at the time of testing. Nevertheless, it is implausible that a small reduction in excitability would lead to nearly complete eradication of the motor map while sparing the sensory effects.

M1 neurons receive somatosensory information before the motor map develops (Bruce and Tatton 1980; Chakrabarty and Martin 2000) and the organization of this input did not undergo
a major reorganization between days 49 and 84 (weeks 7–12). Maintenance (or further development) of the somatosensory map did not depend on neural activity during this period because the pattern of somatosensory inputs to M1 (both topography and modality) was the same before and after inactivation. It is possible, however, that more subtle features of the representation such as receptive field properties were affected by the activity blockade.

Maintenance of the somatic sensory map in M1 after activity blockade is surprising, especially because muscimol infusion blocks activity by postsynaptic hyperpolarization (Martin and Ghez 1999). During infusion, thalamocortical and corticocortical afferents are presumably active, yet this afferent activity does not result in postsynaptic activation. This suggests that synapses transmitting the basic mechanoreceptive signaling that we recorded as peripheral receptive fields are not hebbian. On the other hand, corticocortical and thalamocortical afferents to M1 show activity-dependent potentiation of synaptic excitatory potentials (Iriki et al. 1991; Rioult-Pedotti et al. 1998; Sanes and Donoghue 2000), indicating that they are capable of activity-dependent modulation. Whether our finding reflects a class of stable inputs originating from a particular location or a basal state of synaptic transmission deserves further investigation.

Our recent findings (Martin et al. 2005) show that when an animal does not use one forelimb, similar—but less severe—effects are produced on motor map characteristics as with activity blockade. These include a reduction in the number of sites where stimulation evokes a motor response and an elevation in current thresholds. Somatosensory deafferentation in the rat during both development and in maturity (Keller et al. 1996) or during development alone (Huntley 1997) has been shown to affect the organization of the M1 motor map. These behavioral and sensory manipulations are all likely to profoundly alter M1 neural activity, which in turn could lead to motor representational changes. Activity blockade using a hyperpolarizing drug like muscimol selectively blocks M1 neuron activity (i.e., postsynaptic not presynaptic). This indicates that the synaptic targets of silenced M1 neurons—within the cortex, the spinal cord and elsewhere—are candidates for mediating the effects on the motor map.

The loss of the motor representation could especially be due to functional changes in both the M1 and the spinal cord. If cortical synapses are fewer or weakened because of inactivity, microstimulation would be less effective in activating M1 neurons. This is because high-frequency stimulation, as we use to evoke motor responses, produces a motor effect in large part by activating presynaptic elements, presumably local pretterminal axons (Jankowska et al. 1975). Thus ICMS activates M1 output neurons transsynaptically. In support of a role for the cortex, protein synthesis blockade in M1, which abolishes the motor map, reduces cortical synapse number and horizontal connection strength (Kleim et al. 2003). Several hours of activity blockade using a sodium channel blocker, however, did not have a long-term consequence on motor map stability (Kleim et al. 2003). This duration of activity blockade may be insufficient to alter protein synthesis and synaptic efficacy.

The other likely site where activity blockade could have a profound effect is in the spinal cord. Activity blockade during the period of motor-map development reduces the number of local terminal axon branches and the density of presynaptic boutons within the spinal cord (Friel and Martin 2005). Similarly, our preliminary studies show that M1 activity blockade in mature cats also reduces axon terminal branching and presynaptic bouton density (Salimi et al. 2004). This strongly suggests that activation of M1 neurons (after recovery from blockade) by any of their inputs would produce weakened corticospinal activation of spinal neurons and would be less able to affect limb control. While it is obvious that the patterns and strengths of CS synapses in the cord must be critical for the expression of the M1 motor map, this has not been directly investigated. Our finding of changes in the M1 map after activity blockade that are correlated with reductions in the density of CS presynaptic sites after activity blockade (Friel and Martin 2005) provide, for the first time, evidence of the importance of spinal motor circuits in the expression of features of the M1 map.

Development of the motor map in cats occurs late during postnatal life, possibly contingent on development of both the somatosensory inputs to M1 and corticospinal terminations in the spinal cord. Surprisingly, the map develops after the brain stem motor pathways have projected to the cord (Kudo et al. 1993), after development of the terminations of primary afferents—including the 1A afferents (Gibson and Clowry 1999)—and after spinal reflexes are established (Levine et al. 1980). M1 neural activity shapes development of the corticospinal terminal (Martin 2005), which is the principal effector site for the corticospinal system’s actions and therefore must, in turn, shape motor representation development. Cortical activity is critical for visual cortex circuitry development (Reiter and Stryker 1988). However, overlaying this influence of activity is a fundamental role of activity in maintenance of the motor map, a role that is likely to reflect the importance of the corticospinal system in the acquisition and maintenance of motor skills throughout life. In this way, the CS system is not different from other neural systems, such as those for memory consolidation (Bailey et al. 2004; Lynch 2004), where activity-dependent mechanisms are also critical for function in maturity. Importantly, the finding that corticospinal activity is needed to maintain the M1 motor map suggests that map defects could limit recovery of motor skills after stroke or spinal cord injury. Failure to use an impaired limb after injury would not only decrease mechanosensory input to M1 on the damaged side but also would be likely to reduce the level of incoming subcortical feed-forward control signals. Together this could lead to reduced M1 activity and, in turn, impairments in the motor representation. Indeed forced use rehabilitation strategies after stroke (Gordon et al. 2005; Wolf et al. 2002), where a patient is encouraged to use the impaired limb for daily tasks despite difficulty and reduced efficacy, may be successful because increased movement production raises the overall level of M1 neural activity in the damaged CS system.

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Present address of S. Chakrabarty: Spinal Cord Research Centre, University of Manitoba, 730 William Ave., Winnipeg, Manitoba R3E 3J7, Canada.

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