Effect of Forelimb Use on Postnatal Development of the Forelimb Motor Representation in Primary Motor Cortex of the Cat

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Submitted 8 October 2004; accepted in final form 23 November 2004

INTRODUCTION

The primary motor cortex (M1) contains an orderly representation of the muscles and joints of the body (Porter and Lemon 1993). Several studies have shown that in the cat this representation develops during late postnatal life (Bruce and Tatton 1980a,b; Chakrabarty and Martin 2000). The map appears relatively suddenly after postnatal week (PW) 7, which is when the pattern of developing corticospinal (CS) axon terminations in the spinal gray matter is similar to that of the mature animal (Alisky et al. 1992; Li and Martin 2002; Theriault and Tatton 1989). By this age most ipsilateral and ventral terminations have been eliminated, resulting in a predominantly contralateral projection to the deeper parts of the dorsal horn and to the intermediate zone.

We found, using intracortical microstimulation, that normal motor map development is accompanied by 3 changes in the characteristics of stimulation-evoked motor responses (Chakrabarty and Martin 2000). First, the thresholds for evoking movements show a systematic age dependency from PW 8 to 13. Median thresholds in anesthetized animals drop from 80 to 36 μA during this period. The decrease in threshold is accompanied by an increase in the number of sites from which stimulation evokes a response. This suggests that as animals grow older connections between local zones in M1 and spinal motor circuits become more effective in transducing cortical stimulation into movement. Second, the topography of the forelimb motor map develops from proximal to distal. Between PW 8 and 13, muscles that act at the shoulder and elbow became represented before muscles that act on the wrist. Digit muscles were the last to become represented. It has been reported that human infants develop control of the proximal upper limb before the distal limb (Berthier et al. 1999). This parallel suggested to us that an individual’s pattern of motor behaviors during postnatal life might be important in establishing the motor map, and specifically, in setting up the proximal-to-distal representation development. This seems all the more plausible because skilled motor behavior can reshape the motor representation in M1 of mature monkeys and rats (Kleim et al. 1998; Nudo et al. 1996). Third, as animals grow older stimulation of M1 at or close to threshold evokes multijoint responses at more sites (Chakrabarty and Martin 2000). This is similar to what has been reported in monkeys after motor skill training (Nudo et al. 1996) and could reflect an emerging representation of muscle synergies.

In this study we used intracortical microstimulation in anesthetized cats to examine how promoting or preventing limb movement influence development of these 3 characteristics of the motor map. We promoted skilled motor experience by engaging the animal in a prehension task that required endpoint accuracy and distal control (Ghez et al. 1996; Martin et al. 1995). We prevented experience either by injection of botulinum toxin A into forelimb muscles or by restraining movement of one forelimb. We timed these manipulations with the period of motor map development, which is between PW 8 and 13–14.

We were also interested in determining whether experience-dependent changes in motor map development were permanent. We examined the motor map between 1 wk and 1 yr after cessation of prehension training.

We found that motor experience had several effects on motor map organization, although these effects were not permanent. Our findings show that the motor map is capable of adapting to novel motor demands as it is forming during development but that it can revert back to the control form if...
those motor demands are not maintained in the animal’s behavioral repertoire.

**METHODS**

All cats were obtained from an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited supplier. All animals were delivered to the animal care facility after weaning and cats were housed individually. We used intracortical microstimulation (ICMS) to probe the organization of the M1 motor representation in anesthetized animals. All experiments were conducted with the approval of the New York State Psychiatric Institute and Columbia University IACUC.

**Anesthesia and general surgical procedures**

Anesthesia was induced with ketamine hydrochloride [30 mg/kg intramuscularly (im)] and xylazine (0.6 mg/kg im) and anesthesia was maintained using intravenous (iv) ketamine infusion (10 mg kg h⁻¹; adjusted as needed to maintain an areflexive state); iv ketamine anesthesia maintains muscle tonus. This was similar to the method we used previously (Chakrabarty and Martin 2000). Animals were placed in a conventional stereotaxic frame. Body temperature was maintained at 39° by a heating pad. A craniotomy was made over the lateral portion of the anterior parietal and frontal lobes of each hemisphere to expose the forelimb areas of the sensory–motor cortex. The dura over the lateral pericruciate cortex was incised and reflected, exposing a portion of the anterior parietal and frontal lobes of each hemisphere to examine the surface cortex within the rostral, lateral, and caudal limits of the frontal lobe. The rostral portion 3–5 mm of cortex around the lateral margin of the cruciate sulcus. 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.

**ICMS protocol**

Microstimulation was applied through tungsten microelectrodes (Microprobe; 0.5-mΩ impedance). Stimuli (45-ms duration train, 330-Hz, 0.2-ms balanced pulses) were delivered once every 3 s using a commercial constant current stimulator (AM Systems). These are the same parameters we used in a prior publication (Chakrabarty and Martin 2000). Electrode penetrations were made orthogonal to the cortical surface. To minimize animal state-dependent effects, especially those arising from the level of anesthesia, we alternately stimulated one then the other side after every 5–10 penetrations. For a given animal, the same number of penetrations (30–40) was made in the motor cortex on each side.

Penetrations were made between the surface and 2–2.5 mm to examine the surface cortex within the rostral, lateral, and caudal pericruciate cortex. We made penetrations within approximately three mm of the tip of the cruciate sulcus. This corresponds to area 4-gamma of the primary motor cortex (Hassler and Muhs-Clement 1964). The area we sampled corresponds to the forelimb representations in kittens (Chakrabarty and Martin 2000) and more mature cats (Armstrong and Drew 1985a; Groos et al. 1978; Nieoullon and Rispal-Padel 1976). Our choice to sample this region was guided by 3 factors. First, this area projects densely to the cervical enlargement in kittens and adult cats (Li and Martin 2000; Martin 1996). Second, several studies have sampled this area systematically in kittens and mature cats, indicating that it contains the forelimb representations (Armstrong and Drew 1985a; Chakrabarty and Martin 2000; Keller 1993; Pappas and Strick 1981b). Other studies sampled more medial areas of the cat motor cortex and areas within the sulcus, where they found a preponderance of hind-limb effects, with comparatively fewer (proximal) forelimb effects (Ghosh 1997; Nieoullon and Rispal-Padel 1976). Third, the forelimb area of cat motor cortex is located at the dorsal, lateral, and rostral limits of the frontal lobe. The rostral portion of the forelimb zone abuts the posterior bony wall of the frontal sinus. The lateral portion of the forelimb zone of motor cortex is the surface cortex of the coronal gyrus. The somatic sensory cortex is located more laterally, predominantly on the lateral wall of the frontal-parietal lobes. Although our sampling strategy may have resulted in missing some forelimb sites, we minimized the risk of sampling out of the motor cortex forelimb limb zone.

We were careful to sample homotopic regions of the motor cortex in each animal. This was ensured on the basis of surface landmarks and by only stimulating the most superficial cell layer. Motor effects produced by ICMS occurred at lowest stimulus currents at depths where we recorded multiunit activity with the largest amplitude spikes. The cell layer depth was between 1.2 and 1.5 mm from the pial surface. This was measured directly from the micromanipulator (Kopf) vernier scale. This cell layer almost certainly corresponded to layer 5 because we lost multiunit activity after advancing the electrode an additional several hundred micrometers. Nevertheless, we typically advanced the microelectrode an additional 1 mm to verify that we had sampled the lowest threshold point.

In determining the threshold and topography (i.e., type of movements) of motor effects evoked by ICMS, we kept the limb in a posture in which the shoulder was slightly extended, the elbow was approximately halfway between flexion and extension, and the wrist was plantarflexed. The fixed limb position was necessary to prevent afferent (i.e., mechanoreceptor) facilitation from reducing current thresholds. Sometimes it was necessary to stabilize a proximal joint during stimulation to verify that distal joint movement was not a consequence of an inertial interjoint interaction. When this was done, or whenever the limb was moved, we waited several seconds before stimulating to minimize the effect of mechanical limb stimulation on current threshold. We characterized changes at the shoulder (extension, flexion, abduction, adduction), elbow (flexion, extension), wrist (flexion, extension, supination, pronation), and at digit joints (flexion/ digit closure, extension).

When ICMS produced a motor effect, we determined the current threshold, defined as the lowest current that consistently produced a motor effect. At first, we quickly raised the current to suprathreshold values, 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 ≥1.5 times threshold and noted when the effect reappeared. If necessary, we repeated this procedure until we were confident of the threshold value and particular motor effects. Each threshold value represents testing over at least one descending and one ascending stimulation run.

We used a maximal current of 80 μA, which is higher than what is commonly used in mature animals, to minimize the likelihood of missing effective sites. A site at which a forelimb motor effect was evoked at or <80 μA is termed an effective site. We occasionally exceeded this value but found that using higher currents (e.g., ≤125 μA for one or 2 trains) rarely produced an effect when one was not present at 80 μA. Moreover, 80 μA produces a DC spread of <350 μm (Asanuma and Sakata 1967), which was an acceptable distance considering the sizes of joint representation zones in cat M1 (Keller 1993). Moreover, in most animals there was a mix of high and low threshold sites (see Fig. 3); the presence of an ineffective site tested at 80 μA did not preclude identifying adjacent effective sites at much lower thresholds. 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 each cat, we computed the mean percentage of effective sites (i.e., number of sites where forelimb effects ≤80 μA were evoked in relation to all sites examined), mean current threshold, and mean percentage of multijoint sites and mean number of effects produced at these sites by ≤1.5 times threshold stimulus. We determined the significance of treatment-induced effects using the program Statview. We compared the distribution of threshold currents and joint effects for the hemisphere contralateral and ipsilateral to the treatment (prehension or lack of use).
Promoting and preventing limb use

To promote limb use, kittens were trained to perform a prehension task while standing in their cage. Training began 2 or 3 days before PW 8. During this time they were trained to reach from a quadrupedal standing posture, through an aperture in the cage to grasp a cube of beef (about 0.5 × 0.5 cm) from a horizontal plate just outside the cage. At this time they could use either forelimb. Next, they were trained to reach through the aperture to grasp the beef from a narrow cylindrical food well (3.2 cm inside diameter; 5 cm deep) using their preferred limb only. The food well imposed an accuracy constraint that the animals had to satisfy during the initial training and later testing periods. This is identical to the task used in earlier experiments (Martin et al. 2004). After several days of shaping, the animals were engaged in performance testing for the remaining month. During this period their movements became more consistent, with shorter periods between movements, and appeared to become faster. Initially, training sessions were about 45–60 min. At the end of the testing period the sessions were about 30–40 min. This is consistent with an optimization of performance over the month-long testing period.

We used 2 strategies for preventing limb use. In 2 animals, forelimb use was prevented between PW 8 and 13 by intramuscular injection of botulinum toxin A (BTX; Allergan). BTX is a clostridial neurotoxin that acts presynaptically to block neurotransmitter release by proteolytic activity directed on the SNARE protein SNAP-25 (Schiavo et al. 2000). After injection into muscle, the toxin is taken up by the motoneuron axon terminal, where it acts to block acetylcholine release. In earlier experiments (Martin et al. 2004) we determined that 15 units injected directly into the belly of several forelimb muscles was sufficient to prevent limb use for at least 1 wk in kittens. This amount is similar to what is injected into human intrinsic hand and small forearm muscles for treating focal dystonias, but the duration is much less (Pullman et al. 1996). In our earlier study we determined that injections into the elbow flexors and extensors, together with the wrist plantarflexor compartment, were sufficient for preventing limb use. The shoulder and wrist extensor muscle compartments were not injected. Having several muscle groups intact offered the opportunity to determine whether the animals contracted these intact muscles during testing. As we describe below, this appears not to have been the case because animals neglected use of the limb.

BTX was diluted to a concentration of 100 units/ml saline just before injection. The skin over the ventral forearm and arm was shaved to permit palpating the muscles to be injected. Separate injections (15 units BTX/150 μL sterile saline per muscle) were made directly into the large plantar flexor belly on the ventral forearm to target the palmaris longis and other wrist and digit plantarflexor muscles, into the elbow flexor compartment to target the biceps and brachialis, and 2 separate injections into the elbow extensor compartment (from its lateral and medial aspects) to target the 3 heads of the triceps muscle. Animals were returned to their home cage after the injections. Injections were made weekly between PW 8 and 12 to block movements through PW 13.

We determined the efficacy of the BTX injections using the following behavioral tests to evoke limb movements: contact and proprioceptive placing reactions, withdrawal response to moderate (but not noxious) paw pressure, and assessment of elbow strength. Within 2 days after the initial injection contact and proprioceptive placing reactions were essentially absent. Kittens normally attempt to withdraw the paw when it is held or lightly squeezed; this reaction was not present 2 days after BTX injection. To assess strength at the elbow, the animal was supported under the chest and the hind limbs were raised off the testing surface. While standing only on one or the other forelimb, pressure was applied to the shoulder of the supporting limb. The control limb would support considerable downward force before the elbow yielded and flexed. In contrast, the injected limb not only would yield at the onset of force testing but commonly did not have sufficient strength to support the weight of the animal’s forequarter against the force of gravity alone. We reinjected the same muscle compartments at weekly intervals to prevent the muscles from regaining strength because, in the kitten, the time course of BTX actions in limb muscles is less than that in the adult cat (Misiaszek and Pearson 2002). After the fourth injection, animals regained elbow strength and limb responsivity within about 1–2 wk.

The third cat in which limb use was prevented was fitted with a jacket that restrained the right forelimb. The jacket had one sleeve in which the right limb was placed. The sleeve was then strapped to the animal’s chest. The left limb had free mobility. The jacket prevented use of the right limb during walking, feeding, and postural support, but did permit small movements within the confines of the jacket. After the first day, the animal became accustomed to wearing the jacket. The jacket was removed immediately before anesthetizing the animal for the terminal ICMS experiment. The animal was allowed to bear weight on the limb and to walk briefly. This verified that the limb was not grossly impaired. The principal purpose of using the jacket was to demonstrate an affect of limb disuse that did not depend on impairing neuromuscular transmission. In addition, we wanted to determine whether the ICMS effects obtained several weeks after cessation of BTX injections were muted because of the postinjection recovery period.

RESULTS

Experiments were conducted on 9 cats (8 kittens, between PW 14 and 30; 1 adult). Five animals were trained to perform a prehension task using one forelimb; 3 were prevented from using one forelimb (2 with BTX; 1 with a jacket restraint). The adult cat served as an untreated control to verify symmetry of ICMS effects in the right and left motor cortex.

Effects of promoting forelimb functions on motor map organization

The prehension task required endpoint accuracy, and once the paw was in the tube, the animal produced a coordinated digit flexion and forearm supination movement to grasp the beef (Martin et al. 2004). While the beef was grasped, the paw was withdrawn from the tube and the food was brought to the animal’s mouth. Animals were tested daily for 1 mo (31 ± 2 days) and performed, on average, 64 ± 7.8 reaches during each training session.

In each of the 5 animals subjected to prehension training and testing, we mapped the same number of sites in the cortex contralateral to the limb performing the prehension task (termed treated cortex) and the cortex contralateral to the nonperforming limb (termed the untreated cortex). Figure 1 shows the stimulation sites (dots) for all treated animals separately (A) and the combined (B). The left column presents data for the motor cortex contralateral to the treatment. Penetrations were within an approximately 3-mm radius of the lateral tip of the cruciate sulcus (gray horizontal line). The region mapped corresponds to the portion of the forelimb representation on the surface (Chakrabarty and Martin 2000). We have shown that this area projects densely to the cervical enlargement in kittens and adults (Li and Martin 2000, 2001, 2002). This is also the exclusive portion of the distal forelimb representation in cats, examined anatomically and electrophysiologically (Pappas and Strick 1981a,b). Other studies in the mature cat (Armstrong and Drew 1985a,b; Martin and Ghez 1993) confirm this region to be the principal forelimb zone. Although proximal forelimb joints are represented in the cruciate sulcus, albeit less fre-
sequently than in the lateral surface cortex, more medial areas of cat motor cortex and the cortex within most of the cruciate sulcus represent hind limb joints predominantly (Ghosh 1997; Groos et al. 1978; Nieoullon and Rispal-Padel 1976). Comparison of sites on the 2 sides for each animal and comparisons across animals show that there were no systematic biases in the locations of stimulation sites.

For all animals subjected to prehension testing, the percentage of sites in the treated cortex (i.e., contralateral to the limb used during prehension) from which ICMS was effective in producing a motor response was greater than that for the untreated cortex (86.2 ± 4% vs. 75.9 ± 10%). A breakdown of effective sites for each animal (and time after cessation of training) is shown in Fig. 2A. Because of measurement variability between animals—arising from age-dependent effects, limb experiences outside the task, or response to anesthesia—we computed the ratio of the number of effective sites (i.e., forelimb effects evoked <80 μA) for the treated side divided by the value for the untreated side for each animal (Fig. 2). Absolute values are indicated in the figure legends. For effective sites, apart from one of the 1-wk animals, the ratio was not different across all animals (Fig. 2A, light gray bars). For the adult control, the ratio was 0.98. Mean current thresholds for the treated and untreated cortices in all animals were 41 ± 5.3 and 45 ± 6.7 μA, respectively. The threshold ratios for the treated and untreated sides for each animal are shown in Fig. 2B (light gray bars). Although the thresholds for the 2 sides were not different overall, the threshold was significantly lower in the animal examined 1 mo after cessation of training. For the adult control, the threshold ratio (between the right and left motor cortex) was 1.08. Whereas there were slight trends toward increased numbers of effective sites and decreased thresholds for the treated cortex, the effects were neither consistent nor dependent on time after cessation of training. However, these results show that the overall thresholds and percentage of effective sites in the animals studied are similar to our previously published values in anesthetized cats (e.g., PW 13 and adult, percentage of effective sites was 72%; median current at PW 13 was 36 μA and, for adults, 28 μA; Chakrabarty and Martin 2000).

Photographs of the area mapped in the lateral pericruciate cortex are shown in Fig. 3 for 2 representative experiments. The left column shows the treated cortex. Figure 3A shows the cortical map for an animal at 1 wk after cessation of prehension testing. The dots in A1 and B1 show the motor effects produced at each site that was stimulated (≤80 μA). Colored dots show effective sites; the outer diameter of each dot is inversely related to threshold current. Multiple concentric dots indicate that multiple motor effects were evoked at currents ≤1.5 × threshold, but we only report and analyze sites at which the maximal current was <50 μA. It should be noted that the

FIG. 1. Summary of stimulation sites. A: surface maps of penetration sites in each treated animal. Left column: data for the motor cortex contralateral to the treatment. Each dot corresponds to a single penetration. Notations between the 2 maps indicate the treatment (prehension testing, time after cessation of training: 1 wk, 1 mo, 4 mo, 1 yr; preventing limb movements: jacket restraint, BTX). Horizontal gray bars indicate the location of the lateral portion of the cruciate sulcus. Inset (between the top maps): general location of the sites examined. B: maps are combined for the animals subjected to prehension testing (black dots; n = 5 cats) and preventing limb movements (gray dots; n = 2 cats). Calibration: 2 mm.
maximal currents used for testing multijoint effects at the majority of these sites was lower than 50 μA; the currents used were similar to the overall mean currents used for a given animal (see following text). Note that there were 2 animals at 1 wk after prehension training. Light gray bars indicate values at times after cessation of prehension training. White bar indicates value for adult control. Dark gray bars indicate values after preventing limb use (Restrain, jacket immobilizing right forelimb; BTX, botulinum toxin A). Values of percentage of effective sites for each animal are as follows (treated vs. untreated): wk 1: 82 vs. 41%; 79 vs. 78%; 1 mo, 100% for each cat; 4 mo: 95 vs. 90%; 1 yr: 75 vs. 70%. Adult control: 84 vs. 85%; Restrain: 67 vs. 93%; BTX: 26 vs. 71%. Threshold values (in μA) for each animal are as follows (treated vs. untreated): wk 1: 36.4 vs. 36.5; 57 vs. 68; 1 mo: 29.7 vs. 40.8; 4 mo: 36.9 vs. 39.1; 1 yr: 33.5 vs. 38.2. Adult control: 35.7 vs. 33.1; Restrain: 46.6 vs. 34.7; BTX: 50 vs. 40.6. Asterisks indicate P < 0.01.

FIG. 2. Effects of limb motor experiences on sites from which stimulation was effective in evoking movement (A) and current threshold (B). Bars graph the ratio of the mean number of effective sites (A) or mean current threshold (B) for the motor cortex contralateral to the affected limb divided by the value for the unaffected (no prehension or use prevention) limb. Each bar plots data for one animal. 

At 1 wk (Fig. 3A1) we noticed that the untreated cortex showed a preponderance of single joint effects, in this case mostly wrist and shoulder but few elbow. For the wrist, which clearly dominated the map, sites tended to be clustered into domains where stimulation evoked movements around only that joint. For the treated cortex, sites were intermingled. Importantly, there was a higher percentage of sites where multijoint effects were produced. This is shown more clearly in Fig. 3A2, where the concentric black–white dots show 2-joint effects and black–white–black dots show 3 joint effects. The “X” marks show the ineffective sites. We examined changes in
the percentage of multijoint sites in relation to time after cessation of prehension testing. We tallied the number of sites where stimulation evoked an effect at more than one joint, but restricted this analysis either to currents of 1.5 × threshold or <50 μA, whichever was less (see current value description below). For the 1-wk animals (n = 2), we found that the ratio of the percentage of multijoint sites for the treated and untreated sides was 3.52 (39.1 vs. 11% for the treated and untreated sides, respectively) and 2.84 (43.8 vs. 15%; treated and untreated); for the 4-wk animal, 1.53 (71.4 and 47%; treated and untreated); and for both the 4-mo and 1-yr animal, 0.84 (4 mo: 37.8 vs. 45%; treated and untreated; 1 yr: 37 vs. 44%; treated and untreated). The ratio in control adult was 1.0 (35.3 and 35% for the 2 sides).

We next determined the average number of joint effects evoked from each of the single and multijoint sites for each animal. We reasoned that a higher mean joint number, which ranged from 1 to 3 joints for individual sites, might reflect the greater demand for interjoint coordination during prehension. Figure 4 graphs the ratio of mean joint values for the treated and untreated sides in each animal. There were significantly higher (P < 0.01) mean joint values on the treated sides in the 1-wk and 1-mo animals and a higher, but not significant, number at 4 mo. To compute this statistic we used an unpaired t-test and compared the number of effects at each site for the 2 sides. At one year, the ratio was not different from the control adult (white bar). This effect was not attributed to the use of stronger currents on the treated side, which could spread greater distances within cortex and thereby recruit more diverse cortical sites. For the 1-wk cats, the mean maximal current (i.e., ≥1.5 × threshold for the first response; maximal current <50 μA) used to evoke the multijoint effects at the multijoint sites was 35.3 μA for treated and 31.3 μA for the untreated side (NS; P = 0.22). These values are less than the overall mean thresholds for the treated side (36.4 μA) and for the untreated side (36.5 μA). For the 1-mo cat, the mean maximal currents used to examine multijoint responses was 29.4 μA for the treated side, which was significantly lower than that for the untreated side, which was 36.8 μA (P = 0.0114). Thus more multijoint effects were evoked at lower currents on the treated than on the untreated side. These values were less than the overall mean thresholds for the animal (29.7 μA for the treated side; 40.8 μA for the untreated side). Our findings show that the ratio of mean number of joints decreased as the interval between the last testing session and time of the terminal mapping experiment increased. Although there was an influence of training and extensive daily testing on the motor map, it was not permanent.

We next determined whether prehension training augmented the representation of particular forelimb joints. For each animal, we determined the number of shoulder, elbow, wrist, and digit movements evoked by stimulating the treated and untreated sides. Figure 5 plots the ratios of these values for all animals. Ratios for digit, vibrissae, and back movements are not plotted because they were sparsely present. There was a preponderance of elbow effects after training at 1 wk, but this was neither maintained nor did it change in a systematic way after longer delays. A second, smaller spike in the number of elbow effects was present at 1 yr, and this was not particularly different from what was seen in one of the 1-wk animals. Overall, there were no trends in changes in the pattern of joint effects after cessation of prehension training. Therefore we conclude that prehension training did not produce a systematic change in the percentage of joints represented.

**Effects of preventing forelimb use on motor map organization**

Three animals were prevented from using one forelimb between PW 8 and 13. The animal subjected to restraint was...
mapped immediately after removing the jacket. The 2 BTX animals were allowed to recover for 3 wk after the last injection. We found that the effect of intramuscular BTX injections has a 2-wk time course in kittens (Martin et al. 2004), and this was borne out in this study. The additional week recovery was to further ensure that the neuromuscular blocking actions had worn off. Data from one BTX animal are not presented because the animal developed cerebral edema during mapping and died before mapping was complete. For that animal, changes similar to the 2 other cats occurred (reduction in effective sites; increase in stimulation threshold; and a reduction in multijoint sites).

The 2 types of procedures to prevent limb movement were similar in that they prevented the animal from engaging in reaching and batting movements, locomotion, and weight-bearing with the affected limb. The animal adapted to the jacket restraint within one day. Within 2 days after the BTX injections (see METHODS), animals neglected the injected limb. It was not used for support when the intact limb was available for that purpose; the animal adopted a tripod stance. During locomotion, the animal often hopped and the injected limb was dragged. We did not notice spontaneous movements of the injected limb during observation periods despite neuromuscular transmission in the wrist extensors and shoulder muscles.

Figure 1A (bottom 2 rows) shows the locations of stimulation sites in the restrained and BTX animals. The location of these sites are replotted in Fig. 1B (gray dots), along with sites for stimulation after prehension. The average number of effective sites for animals in which the forelimb was prevented from moving was consistently lower for the cortex contralateral to the nonused forelimb (termed treated cortex; 46%) than the other side (termed untreated cortex; 82%). This decrease in the number of effective sites on the treated side was significant for each animal (Mann–Whitney; P < 0.01). The ratios of effective sites and thresholds for the treated and untreated sides for each of these animals are shown in Fig. 2 (dark gray bars). The mean microstimulation threshold for the untreated cortex was 48 μA and, for the untreated cortex, 37 μA. This elevation in threshold for the treated side was significant for each animal (unpaired t-test; P < 0.01). The decrease in effective sites and the threshold elevation were not attributed to state-dependent changes in the preparation because we alternated stimulating the treated and untreated sides after every 5–10 sites.

Cortical maps for the animal whose right arm was restrained in a jacket between PW 8 and 14 are shown in Fig. 3B. The kinds of motor effects produced by stimulation and the distribution of these effects were different for the treated and untreated sides. The treated cortex showed more clustering of a particular effect (e.g., wrist, in the case shown) together with fewer multijoint sites and more unresponsive sites (as described above) than the untreated cortex. Similar changes were observed in the animals treated with BTX.

The percentage of multijoint sites decreased after preventing limb use. For the restrained animal, the ratio of percentage multijoint sites was 0.13 (8.3% on the treated side vs. 64% on the untreated side). The BTX animal had no multijoint sites on the treated side and 30% on the untreated side. Figure 4 (dark gray bars) plots the ratio of the mean number of joints at multijoint sites. The average number of effects evoked at each site by stimulation of the treated cortex in the restrained animal was 1.08 compared with 1.75 for the untreated side and for the animal receiving BTX injections, 1.0 and 1.33 for the treated and untreated sides, respectively. Both animals showed significant reductions in the mean number of multijoint effects (unpaired t-test comparing the mean number of effects produced at each site for the treated and untreated sides; P < 0.01). Similar to the animals subjected to prehension testing, the changes in the number of multijoint effects as a consequence of preventing limb use was not attributed to an increase in the currents used to evoke these responses. The maximal mean current used to evoke multijoint responses in the treated cortex was 49 μA compared with 37.2 μA for the untreated cortex. These values are similar to overall mean thresholds for the treated and untreated cortices, which were 52.7 and 41.2 μA, respectively. Thus fewer multijoint effects were evoked from the treated cortex despite using higher currents. The untreated sides in these animals looked more like the treated side after prehension training, with more multijoint sites and a greater local diversity of effects than expected at this age (Chakrabarty and Martin 2000). This is consistent with a training effect on the untreated side. Despite not using one forelimb for 1 mo, we found no consistent shift in the kinds of joints represented (Fig. 5).

**DISCUSSION**

We found that promoting limb use by prehension training during the period the M1 motor map develops resulted in an increase in the number of sites from which multiple joint effects were produced by stimulation and the number of joints represented at these sites. We followed this increase for 1 yr after cessation of prehension performance and were surprised to find that the map modifications during development were not permanent. This is similar to reversible changes in M1 map organization after brief neonatal whisker clipping in the rat (Keller et al. 1996). We expected that as developing cortical circuits became committed to controlling a particular motor pattern, expression of this control would have persisted long after the motor experience ceased. This would have been similar to changes in the encoding properties of the visual representation in primary visual cortex after, for example, prolonged exposure to particular patterns of visual stimuli (Katz and Callaway 1992).

Preventing limb use during the period of motor map development resulted in a triad of impairments: decreased number of effective sites, increased thresholds for evoking responses, and decreased representation of joints at multijoint sites. Similar effects were seen using BTX and limb restraint. For BTX, although we waited 3 wk for a normal range of limb motion to return, we cannot rule out that there was a subtle neuromuscular defect at the time of mapping. We propose that there is a use-dependent continuum of cortical zones for controlling movement, beginning in development with zones that are ineffective in activating spinal motor circuits (Chakrabarty and Martin 2000). The presence of ineffective sites could either reflect a current threshold beyond that which is normally tested or the lack of appropriate connections. Limiting limb use during development leads to fewer and less-effective zones (i.e., elevated thresholds) for evoking single-joint effects compared with normal experience, and promoting experience leads to zones that are more effective (i.e., normal threshold) for evoking both single- and multijoint effects. The position of a
motor cortex site along this continuum can shift according to ongoing motor control demands throughout an animal’s life.

**Role of motor experience in determining thresholds for evoking motor effects**

After the initial emergence of the M1 motor map around PW 7–8, there is an age-dependent decrease in threshold and a concomitant increase in the number of effective sites (Chakrabarty and Martin 2000). Development of these characteristics of the map could arise from local changes in cortex, or from changes in the topography and strengths of terminations in the cord and in the subcortical nuclei that give rise to motor paths. Data are available for development only at the spinal level. We have found that as kittens grow older, the number of CS terminals in the contralateral spinal gray matter increases (Li and Martin 2001, 2002). More important, the number of presynaptic sites increases, which would lead to stronger connections (Meng et al. 2004). At about PW 7 the percentage of CS axon terminals that contain neurotransmitter is similar to that of the mature animal (Meng et al. 2004). This suggests that these terminals can be functional, assuming that the postsynaptic machinery is in place. Moreover, the capacity of CS synapses to facilitate synaptic actions also shows an age dependence (Meng et al. 2004). These correlations are consistent with an important developmental change at the level of the CS terminal that enables functional connections between the cortex and muscle. Blocking motor cortex activity during the period of M1 map development (and later) reduces the number of CS axon terminal branches and varicosities (Friel and Martin 2004; Salimi et al. 2004). This would be expected to result in elevated thresholds for evoking motor responses, and concomitant reductions in effective sites. This could account for the reduction in effective sites and threshold elevation we observed after limb disuse. The absence of significant reductions in ineffective sites and threshold after promoting experience may be because the number of effective sites is already high and microstimulation thresholds are close to minimal values in anesthetized animals.

The magnitude of the motor cortical representational changes were quantitatively similar (but qualitatively in the opposite direction) for preventing limb use, which was for 24 h each day, and prehension testing, which was for only 1 h each day. The robust affect of prehension on the motor cortex may be attributable to the young age of the animals and that the developing corticospinal system has a greater potential for plasticity. Also, the particular interjoint coordination demands imposed by the task would contribute (Remple et al. 2001). By contrast, reductions in the representations after preventing limb use reflect only a lack of skilled movement production and not plasticity to mediate a new motor skill. This may be explained by reduced maintenance of constitutive plasticity.

**Development of the distal motor map**

We were surprised that there were no changes in the topography of represented joints in the lateral pericruciate cortex as a consequence of experience. We expected that engaging animals in prehension would have led to a precocious digit map and a preponderance of distal effects, similar to the emergence of increased distal sites after animals are trained to reach and grasp (Kleim et al. 1998; Nudo et al. 1996). It is possible that topographic changes were not observed because there was an insufficient motor “demand” placed on the animals: either too little required skill or too few movements. Kleim and colleagues (Remple et al. 2001) concluded, after comparison of different prehension tasks, that joint representation changes in M1 depended on reaching and grasping skills, not motor strength. This suggests that some critical control parameter is being regulated in relation to map topography. Distal joint control during grasping and proximal–distal control during food retrieval are critical for effective performance of this task and there is a dependency on early (<PW 7) motor experience and CS axon terminal development (Martin et al. 2004). In addition, the prehension task that the cats performed requires coordination between active muscle torques generated by control signals to proximal muscles and mechanical interaction torques generated by inertial effects at distal joints (Cooper et al. 2000; Ghez et al. 1996). This task requirement for more balanced control at the shoulder, elbow, and wrist than just distal control could explain why there were no systematic shifts in the distribution of represented joints after promoting or preventing skilled movements. Although skill appears to be important for regulating M1 motor representation topography, the requirement for a minimum number of trials has not been examined. In our study, animals performed, on average, 65 reaching trials each day. Animals reached satiety after this number of reaches. This is a fewer number of daily trials than used in 2 motor cortex plasticity studies in the rat and monkey (Kleim et al. 1998; Nudo et al. 1996). However, it is important to note that the number of trials the cats performed was sufficient to produce changes in the number of multitjoint sites in the treated cortex. It is also possible that topographic changes occur but are ephemeral. Experiments in the monkey and rat, examining motor cortex representational plasticity during a sequence of training paradigms, mapped after no delay (Nudo et al. 1996) or only 1 or 2 days (Kleim et al. 1998) after cessation of training. They found clear changes in the proportion of proximal and distal joint representations. By contrast, our delays were ≥1 wk. However, if topographic changes were present immediately after training but absent within 1 wk, it would still indicate that development of the topography of the forelimb map in kittens is not determined by early limb motor-use patterns.

If experience is not a major determinant of the proximal-to-distal development of motor map topography, then what could account for this? The early proximal joint representation may reflect early maturation of spinal and brain stem circuits mediating proximal limb and trunk control. Both cats (Levine et al. 1980) and humans (Hadders-Algra et al. 1996) develop posture before precision control of the distal limb. Thus the developing CS system would first encounter a spinal cord better equipped at controlling proximal than distal muscles. Activity at immature CS synapses, which are weaker than those in older animals (Meng et al. 2004; Olivier et al. 1997), would be more effective in activating segmental circuits for proximal than for distal control.

**Development of the circuits underlying the adaptive capabilities of the M1 motor representation may precede emergence of the map itself**

A major characteristic of motor map development, the representation of motor synergies at multitjoint sites, was signifi-
cantly influenced by experience. This is likely to reflect the generalized capacity for representational plasticity of the M1 motor map, active throughout an animal’s life (Kleim et al. 2003), rather than a particular developmental process. This is because skill training in mature monkeys has been shown to produce a similar effect (Nudo et al. 1996). The absence of a permanent effect of early experience in our study could be because the period of motor map development, PW 8–13, is after the period when experience (or M1 activity, which reflects experience) shapes the circuitry underlying its organization. Although this seems paradoxical—in that PW 8–13 is, by definition, the period of motor map development (Chakrabarty and Martin 2000)—there may be an earlier stage when intrinsic cortical and CS connections develop access to spinal motor centers.

Blocking motor cortex activity or preventing limb use before PW 7 permanently impairs limb control (Martin et al. 2000, 2004). One substrate of this impairment is likely to be the profound changes in the topography and morphology of CS terminations that are produced by activity blockade or limb disuse (Friel et al. 2004; Martin et al. 1999, 2004). The activity blockade may also affect development of the inactivated M1 circuits themselves. Development during this early period, which is before the M1 map emerges, establishes the patterns of connections between motor cortex neurons and spinal motor circuits. We propose that the cortical motor system’s capacity for adapting to changing motor demands is embodied in these early-developing circuits, not the particular somatotopic or topographic features. For example, the particular patterns of horizontal connections that develop could determine the intracortical networks that are accessed in response to particular motor-control tasks (Aroniadou and Keller 1993; Matsumura et al. 1996). Similarly, the patterns of CS terminations in the contralateral dorsal horn and intermediate zone would determine the kinds of segmental and propriospinal circuitry that could be activated by descending control signals (Alstermark et al. 1981; Baldissera et al. 1981). Development at this early stage would not establish the particular combination of muscles that are to be controlled. This would occur after PW 7, after the anatomical connectivity is stabilized (Li and Martin 2002) and as the animal’s behavioral repertoire expands with greater motor planning capabilities. The specific topography of motor effects could then be shaped by modulating the strength of CS connections within the broad termination fields of the spinal cord or within local horizontal connections in the cortex.

**Acknowledgments**

We thank M. Choy for help with training and data analysis and G. Asfaw and Dr. M. Osman for veterinary care. We also thank K. Friel and I. Salimi for reading an earlier version of the manuscript.

**Grants**

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-33835; March of Dimes Birth Defects Foundation.

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