Impairments in Prehension Produced by Early Postnatal Sensory Motor Cortex Activity Blockade

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Martin, John H., Laura Donarummo, and Antony Hacking. Impairments in prehension produced by early postnatal sensory motor cortex activity blockade. J. Neurophysiol. 83: 895–906, 2000. This study examined the effects of blocking neural activity in sensory motor cortex during early postnatal development on prehension. We infused muscimol, either unilaterally or bilaterally, into the sensory motor cortex of cats to block activity continuously between postnatal weeks 3–7. After stopping infusion, we trained animals to reach and grasp a cube of meat and tested behavior thereafter. Animals that had not received muscimol infusion (unilateral saline infusion; age-matched) reached for the meat accurately with small end-point errors. They grasped the meat using coordinated digit flexion followed by forearm supination on 82.7% of trials. Performance using either limb did not differ significantly. In animals receiving unilateral muscimol infusion, reaching and grasping using the limb ipsilateral to the infusion were similar to controls. The limb contralateral to infusion showed significant increases in systematic and variable reaching end-point errors, often requiring subsequent corrective movements to contact the meat. Grasping occurred on only 14.8% of trials, replaced on most trials by raking without distal movements. Compensatory adjustments in reach length and angle, to maintain end-point accuracy as movements were started from a more lateral position, were less effective using the contralateral limb than ipsilateral limb. With bilateral inactivation, the form of reaching and grasping impairments was identical to that produced by unilateral inactivation, but the magnitude of the reaching impairments was less. We discuss these results in terms of the differential effects of unilateral and bilateral inactivation on corticospinal tract development. We also investigated the degree to which these prehension impairments after unilateral blockade reflect control by each hemisphere. In animals that had received unilateral blockade between postnatal weeks (PWs) 3 and 7, we silenced on-going activity (after PW 11) during task performance using continuous muscimol infusion. We inactivated the right (previously active) and then the left (previously silenced) sensory motor cortex. Inactivation of the ipsilateral (right) sensory motor cortex produced a further increase in systematic error and less frequent normal grasping. Reactivation of the contralateral (left) cortex produced larger increases in reaching and grasping impairments than those produced by ipsilateral inactivation. This suggests that the impaired limb receives bilateral sensory motor cortex control but that control by the contralateral (initially silenced) cortex predominates. Our data are consistent with the hypothesis that the normal development of skilled motor behavior requires activity in sensory motor cortex during early postnatal life.

INTRODUCTION

Skilled limb use during early postnatal life develops in parallel with the growth and refinement of corticospinal (CS) terminations. In the monkey, digit use develops between the third through sixth months (Armand et al. 1997; Galea and Darian-Smith 1997a,b; Lawrence and Hopkins 1976), a period that is during the phase of rapid growth of CS terminations into the motor nuclei (Armand et al. 1997; Kuypers 1962). In the cat, the contact placing reaction matures (Leonard and Goldberger 1987a) as the laterality of CS axon terminals in the spinal cord is refined, between postnatal weeks (PWs) 3–7 (Alisky et al. 1992; Theriault and Tatton 1989). These similarities in the timing of anatomic and behavioral events suggest an interplay between limb use or other behavioral or biological factors and the development of CS connectional specificity necessary for skilled limb movements.

We recently have shown that formation of the mature CS termination patterns in the cat depends on neural activity in sensory motor cortex during early postnatal life (Martin et al. 1999). In that study, we focused on refinement of CS terminations from a bilateral pattern, which is present at PW 3, to a predominantly contralateral pattern, which is present after PW 7 (Alisky et al. 1992; Theriault and Tatton 1989). We found that unilateral blockade of neural activity in sensory motor cortex, by continuous intracortical muscimol infusion during this period (PWs 3–7), profoundly disrupts refinement of the laterality of CS terminations from both the silenced and active sides. Projections from the active side not only maintain their immature bilateral termination pattern but expand into a wider ipsilateral territory. In contrast, projections from the inactive side fail to maintain the normal density of terminations on the contralateral and ipsilateral sides. When CS competition is eliminated with bilateral sensory motor cortex inactivation, the extent of the contralateral spinal gray matter labeled is substantially greater and ipsilateral terminations are sparse, similar to controls (Martin and Lee 1999).

In the present study, we examined the relationship between development of skilled motor behavior and activity-dependent anatomic development of the CS system. Our principal objective was to determine if animals deprived of an active sensory motor cortex during early postnatal life have persistent impairments controlling skilled limb movements. Although we know sensory motor cortex activity blockade can alter development of CS termination patterns (Martin et al. 1999), we do not know what effect, if any, activity blockade has on skilled movement control. We conducted two sets of experiments in this report. In the first, we subjected kittens to unilateral or bilateral sensory motor cortex activity blockade during the CS refinement period (PW 3–7) and tested performance in a prehension task after the blockade was stopped. We will show that these animals had persistent defects in the spatial planning of
reaching movements and in grasping with the limb contralateral to the early postnatal blockade. Unilateral and bilateral inactivations, despite differences in the patterns of their CS terminations (Martin and Lee 1999), produced the same phenotype of prehension impairments. In the second set of experiments, we determined to what extent the abnormal contralateral movements produced by unilateral inactivation reflect bilateral control by the sensory motor cortex. We were interested particularly in the role of the ipsilateral cortex (active during CS development; PWs 3–7), because it has aberrant ipsilateral CS terminations (Martin and Lee 1999) that could rescue performance. We will show that the ipsilateral sensory motor cortex did play a significant role in controlling the impaired movements, although the role of the silenced cortex was greater. Some of the results were presented in an abstract (Martin et al. 1997), and five of the animals used in this study were also part of our earlier anatomic studies (Martin and Lee 1999; Martin et al. 1999).

METHODS
Animal groups
Four sets of animals were used in this study; each corresponded to a different treatment group (Table 1; see also Table 2). First, to examine the effects of continuous inactivation during the CS refinement period on prehension, we infused muscimol between PWs 3–7 (Table 1: early-unilateral; n = 5; early-bilateral; n = 2). Second, we infused muscimol after the CS refinement period (i.e., ≥PW 8) to examine the effect of a prolonged period of inactivation and the effect of cortical inactivation on ipsilateral limb control in normals (Table 1: late). Third, to examine the laterality of CS control in animals with impairments after early unilateral inactivation, we used late inactivations (PWs 11–17) to block on-going neural activity during task performance (Table 1: early and late). The fourth group consisted of controls (Table 1: age matched, unilateral saline infusion). A total of 14 animals was used in this study.

General surgical procedures
For all surgical procedures (cannula and pump implantation and removal and tracer injection; see following text), animals received atracurium (0.04 mg/kg im) and were tranquilized with diazepam (0.1 mg/kg im). Anesthesia, to maintain an areflexive condition, was induced using a mixture of ketamine hydrochloride (30 mg/kg im) and acepromazine (0.03 mg/kg im) and maintained with ketamine alone (1.2–3.0 mg/kg im as needed). Animals were administered a broad spectrum antibiotic at the time of surgery and during the first postsurgical week. Older animals (≥PW 8) received supplemental barbiturate anesthesia (Nembutal, 33 mg/kg iv as needed). Experiments reported here were conducted with the approval of the New York State Psychiatric Institute Animal Care and Use Committee.

Sensory motor cortex activity blockade
For unilateral inactivation, we blocked activity in the forelimb area of the left sensory motor cortex by continuously infusing the γ-aminobutyric acid agonist muscimol (Sigma; 10 mM, in saline). (See Martin et al. 1999 for a detailed description of the methodology.) We used a 28-gauge hypodermic needle cannula, connected by vinyl tubing to an osmotic pump (Alza Corp. either model 2 ML4 or 2002). The infusion cannula was implanted at a depth of 2.5–3 mm below the pial surface, into the center of the forelimb representation of motor cortex (Bruce and Tatton 1980; Groos et al. 1978), in the lateral sigmoid gyrus just lateral to the cruciate sulcus.

In 3-wk-old animals, the bone over the lateral sensory motor cortex is translucent, permitting visualization of vasculature in the cruciate sulcus and the relatively avascular prospective cannula implantation site in the lateral sigmoid gyrus. The bone was opaque in older animals (7 wk), which required thinning of the bone (several millimeters posterior to the caudal end of the developing frontal sinus) to see the cortical implantation landmarks. For reactivation of the left cortex (see Table 1; early and late group), the cannula was implanted within 1 mm of the original infusion site (identified by bony landmark). Because the late cannulae were left in situ at the time the animals were killed, we were able to verify their placement (see following text). For bilateral infusions, the two cannulae were implanted at symmetrical anteroposterior and mediolateral locations based on skull surface landmarks.

Muscimol (or vehicle) was infused at the nominal rates of 2.2 μl/h (n = 2, muscimol; n = 1, saline) or 0.56 μl/h (n = 10, muscimol). Results of muscimol infusion at the two rates were identical. At the higher rate, muscimol spreads between 5 and 10 mm from the infusion site (Hata and Stryker 1994). We have shown that these infusions

### Table 1. Summary of treatment groups and controls

<table>
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<tr>
<th>Group</th>
<th>Cat</th>
<th>Early Infusion Period</th>
<th>Late Infusion Period</th>
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<th>Training Duration*</th>
<th>Age at Start of Testing</th>
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All ages are indicated in postnatal weeks (PWs). * PWs rounded to the nearest week. † S, trained to a single target; M, trained to multiple targets. ‡ Late infusion periods of right cortex followed by late infusion period for left cortex. § Training history described in Table 2.
reduce cytochrome oxidase histochemical staining for up to 7 mm from the infusion site (Martin et al. 1999).

Early postnatal infusions were begun between postnatal days 21 and 24 and were stopped between postnatal day 51 and 55 (nominally PWs 3–7). After unilateral infusion, the cannula implant and pump were removed (see General Surgical Procedures), and animals subsequently were trained. During late infusions, we implanted the cannula after the period of CS refinement (see Table 1). To minimize the number of major surgical procedures in animals subjected to early and late infusions (see Table 2), the cannula, cannula fixation, and tubing connecting the osmotic pump and cannula for the early infusion were removed at the time of implanting the right (first late) cannula. We halted the early infusion by removing the osmotic pump and ligating the tubing. This is a minor surgical procedure, performed under ketamine anesthesia (30 mg/kg im) and supplemented with local anesthesia because the pump and tubing were located superficially under the dorsal neck skin.

During the infusions, we routinely examined several untrained behaviors: contact placing reaction, the response of the fore- and hindlimbs to perturbations that either displaced the limb laterally or brought the dorsal surface of the paw in contact with the floor (“knuckling”), and locomotion on a smooth level surface. These tests were done to confirm infusion of muscimol (see RESULTS).

Animals were killed with an overdose of barbiturate and perfused transcardially with saline and aldehyde fixatives. The frontal and anterior parietal lobes were removed bilaterally and blocked, and tissue was sectioned (40 μm) and Nissl-stained. Cannula implantation sites were verified histologically. In each animal, we verified that the gliosis associated with the cannula (Martin et al. 1999) was in the lateral sigmoid gyrus, within ~1–2 mm of the identified lateral margin of the cruciate sulcus. For reactivation of the left cortex (early and late group; see Table 1), where gliosis from the late cannula could obscure identifying the early cannula implantation site, there was no evidence of cannula placement outside of the target field. In all animals, the center of the infusion site was within area 4, based on surface landmarks and cytoarchitecture (Avendaño and Verdu 1992). Although the size of the sensory motor cortex was larger in the older animals (late and combined early and late groups), gliosis associated with cannula placement was within the same part of area 4.

As we have previously reported (Martin et al. 1999), muscimol infusion produced cortical structural changes. The mediolateral length of the cruciate sulcus was reduced (~30%), and there was an increase in the width of the lateral sigmoid gyrus. The overall width of the frontal lobe at the level of the cruciate sulcus was somewhat reduced. The infused cortex was between 75 and 85% as thick as the noninfused side, and there was gliosis within several mm of the infusion site (Fig. 1, right). There was a more prominent sublamina in layer 2 on the infused side (Fig. 1, B and D) and an apparent increase in neuron density (D and F). The muscimol-infused cortex was more vascular compared with the noninfused side. There also was up to a 25% reduction in the cross-sectional area of the medullary pyramid on the side ipsilateral to muscimol infusion. Similar changes in cortical structure were noted in animals subjected to bilateral inactivations, including the prominent layer 2 sublamina. However, the magnitude of cortical thinning was less than that observed with unilateral inactivation.

**Prehension**

We trained animals to reach through a horizontal aperture (12 cm wide, 3 cm high) in a cage to grasp a small cube of meat (~0.5 cm on each side) placed on a horizontal surface (5.5 cm above cage floor). Animals stood between two vertical supports (separated by 12 cm) and faced the target. Sessions were videotaped with a camera (Panasonic S-VHS; 1/250th sec shutter; 60 Hz temporal resolution) mounted directly above the workspace that allowed full view of the workspace and limbs.

Animals were trained during consecutive daily sessions. During the first session, the meat initially was placed close to the cage aperture so the animal could bite it. Gradually, the meat was placed farther from the aperture to encourage the animal to reach. Most directed attempts at reaching for the meat were rewarded. By the end of the first or second session, animals reached with either limb, albeit inconsistently. At this time, the meat was placed at random locations on the platform. We have found that this results in faster and more robust task acquisition, possibly by minimizing reinforcement of adventitious stereotypic behaviors. At the beginning of the next daily session, the animals were forced to use either the right or left limb, in alternate blocks, by bandaging the nonused paw. During this phase of training, the meat was placed at the center of the platform. Animals were considered trained when they reached immediately after baiting the platform and did so consistently for ~25–50 trials for each limb. For most animals, after 1 wk of training (see Table 1), we videotaped the sessions and assessed performance. We typically recorded 3–4 consecutive daily sessions to collect at least 75–100 reaches per limb. Four animals (see Table 1) were trained for an additional several days to become accustomed to performing 200 reaches in a single session to examine reaching to multiple targets (see Fig. 4). In pilot studies conducted in control animals, we found that end point accuracy was no different for random or block presentation of multiple targets (mean target-end-point error for block presentation: 1.73 cm; random presentation: 1.64 cm; t = 1.37; P = 0.17).

**FIG. 1.** Nissl-stained sections from an animal that received unilateral muscimol infusion between postnatal weeks (PWs) 3 and 7. Left: noninfused side; right: infused side of cortex within 1 mm of the infusion site. A and B: low-power views through the posterior sigmoid gyrus, near the lateral end of the cruciate sulcus. → (from top to bottom), layer 1–2 junction, superficial layer 5, and layer 6-white matter border. C and D: higher magnification views at the junction of layers 1 and 2. E and F: higher magnification views of layer 5. Calibrations: A and B, 1,000 μm; C–F, 200 μm.
During the first day or two of training, animals subjected to unilateral muscimol infusions typically favored the ipsilateral (left) limb. Nevertheless, the contralateral limb was used repeatedly. After several days of training, the number of reaches performed by the right and left limbs was approximately the same. Control animals did not favor the use of one or the other limb. Moreover, measures of spatial accuracy (see following text) for the right and left limbs in controls did not differ (age-matched: $t = -0.347; P = 0.729$; $K22: t = -0.343; P = 0.734$). We analyzed 1,905 reaches for the left (ipsilateral) limb and 1,702 for the right limb for animals subjected to the early infusions and controls. For the early and late group, we analyzed 812 reaches for the left limb and 927 reaches for the right limb.

**Behavioral analysis**

Videotaped reaches were viewed frame-by-frame on a video monitor. The starting laterality of each reach and the end point of the first attempt of the movement (i.e., before any corrective movement) were scored to the nearest x-y grid coordinate where the tip of the third digit first contacted the work surface. We chose digit 3 tip (leading edge of the paw) because it can be scored accurately and it is a measure that does not presume a particular aiming strategy, as would scoring the metacarpophalangeal joint or an arbitrary paw location. It does, however, introduce an overestimate of end-point error that is constant for a given animal but varies for different animals. To estimate this error, we measured the length of the digit (when paw reached the target location) in controls and animals receiving unilateral muscimol infusions. The mean lengths did not differ across subjects (e.g., unilateral inactivation, left digit: 1.12 cm; controls, left digit: 1.14 cm). For controls (both limbs) and test animals (left limb only, which was not impaired), mean end-point error was correlated with measured digit length ($R^2 = 0.83$). [This relationship did not hold for the right (impaired) limb in the test animals ($R^2 = 0.06$) presumably because of factors contributing to the prehension impairments.]

For analysis of the spatial accuracy of reaching, we determined the target-end-point distance for a given target location for every trial. The mean target-end-point distance was used as a measure of systematic error. Means for each session typically were computed from 15–20 trials, unless otherwise noted. We took advantage of trial-to-trial variability in the starting position of the movement to examine compensatory adjustments in trajectory length and angle for maintaining end-point accuracy. We used the area of the computed ellipse (i.e., as average gray scale pixel values) in the spinal gray matter using a CCD camera, Apple Macintosh computer, and the program NIH Image (for a detailed description, see Martin et al. 1999). We uniformly sampled homotopic bilateral locations (40 μm by 40 μm) over a 3-mm rostrocaudal portion of the cord and computed the average labeling density.

**Results**

**Behavioral changes during muscimol infusion**

Beginning 1–2 days after implantation, early unilateral muscimol infusion produced contralateral defects in forelimb contact placing (absent, delayed onset, or rapid habituation). The affected limbs typically maintained (or delayed adjustment of) the atypical posture produced by the perturbation rather than immediately returning to the initial position, as for unimpaired limbs. These defects did not appear to limit the range of spontaneous limb movements, impair locomotion, or affect interactions with littermates or the mother. Bilateral infusions produced bilaterally symmetrical deficits and were otherwise similar to impairments produced by unilateral inactivation. Muscimol infusion after PW 8 more severely impaired placing and limb responses to perturbations than in younger animals but did not impair locomotion. Saline infusion had no observable behavioral effects. These findings suggest that muscimol infusion was effective in disrupting on-going cortical contributions to limb control.

**End-point errors during reaching**

Figure 2 shows the distribution of end points for reaches to a single target placed at the center of the workspace. Each square corresponds to the location of end points (±1, depending on the gray scale shading). The animal that received unilateral saline infusion (A) consistently placed the metacarpophalangeal joint (i.e., palm just distal to the foot pad) directly over the beef during a single, smoothly executed movement. This resulted in minimal systematic error. Mean end points for the two limbs were not significantly different (ipsilateral limb: 1.95 cm; contralateral limb: 1.92 cm; $P = 0.84$). As indicated in methods, the small systematic error seen for the controls and for the unimpaired limb in animals that received unilateral muscimol infusion largely reflected scoring the tip of digit 3 as the movement end point. For the data presented in Fig. 2A, digit 3 length was 1.2 cm.

All animals that received unilateral muscimol infusion showed robust impairments in directing reaches with the contralateral limb with increases in both systematic and variable end-point errors. A representative example is shown in Fig. 2B. The trials with the largest errors forced the animal to make corrections to contact the meat. Although the corrective adjustments were directed toward the target, their extent and direction were variable. The paw ipsilateral to the infusion (Fig. 2B1), like the control, was placed consistently directly over the beef during a single movement. Mean end points for the two limbs were significantly different (ipsilateral limb: 2.42 cm; contralateral limb: 1.92 cm; $P = 0.734$). We analyzed 1,905 reaches for the left (ipsilateral) limb and 1,702 for the right limb for animals subjected to the early infusions and controls. For the early and late group, we analyzed 812 reaches for the left limb and 927 reaches for the right limb.

**Anatomic experiments**

The two animals that were subjected to early and late muscimol infusions also received wheat germ agglutinin-horseradish peroxidase (WGA-HRP) injections into the right cortex. The methods we used for tracer injection, perfusion, and histochemistry are identical to our prior study (Martin et al. 1999). We quantified the laterality of terminations at C5 by computing the ratio of ipsilateral to contralateral labeling (i.e., HRP reaction product) in laminae 6 and 7. Using polarized dark-field microscopy, we measured the density of reaction product (i.e., as average gray scale pixel values) in the spinal gray matter using a CCD camera, Apple Macintosh computer, and the program NIH Image (for a detailed description, see Martin et al. 1999). We uniformly sampled homotopic bilateral locations (40 μm by 40 μm) over a 3-mm rostrocaudal portion of the cord and computed the average labeling density.
cm; contralateral limb: 3.67 cm; \( P = 0.0001 \). Four of the five animals with early unilateral infusions overshot the target, primarily in the forward direction (i.e., anteroposterior axis), whereas the other animal reached lateral to and overshot the target. Systematic errors for the two animals subjected to early bilateral infusions were symmetrical for both limbs. End points for one animal were strictly overshot and for the other, lateral and overshot.

Figure 3 contrasts the effects of inactivation on spatial accuracy (A) and end-point dispersion (B) for controls, unilateral inactivation, and bilateral inactivation. For the animals subjected to unilateral muscimol infusion, there were significant increases in both measures for the contralateral limb (mean end points, left: 1.46 cm; right: 3.10 cm; \( P < 0.0001 \); end-point dispersion, left: 9.1 cm\(^2\); right: 25.4 cm\(^2\); \( P = 0.0021 \)). There was no apparent difference between the magnitude of the target-end-point distance for the ipsilateral limb in these animals and controls (control mean target-end-point distance, left: 1.5 cm; right: 1.6 cm). End-point dispersion, however, was greater than controls (control mean end-point dispersion, left: 4.7; right: 6.8). It is important to note that the ipsilateral limb of animals subjected to unilateral muscimol infusion was not impaired in its ability to perform the task (i.e., to aim to, grasp, and retrieve the meat) despite any differences in accuracy measures from controls.

Figure 3 also presents data from two animals subjected to bilateral muscimol infusions. These animals had similar reaching defects as those subjected to unilateral infusion, but the magnitudes of the end-point error and dispersion were intermediate between controls and the impaired limb of animals receiving unilateral muscimol infusion (mean end points, left: 2.54 cm; right: 2.15 cm; end-point dispersion, left: 17.7 cm\(^2\); right: 19.7 cm\(^2\)). These results show that muscimol infusions, whether unilateral or bilateral, significantly impaired reaching for the limb contralateral to activity blockade.

We next determined if the movements had the same systematic error when reaching to different locations in the workspace (Fig. 4). The saline-infused animal (Fig. 4A) reached accurately to all targets with either limb. (The small systematic error is related to the digit 3 end-point criterion.) The animal that received early muscimol infusion had small systematic errors when reaching with the ipsilateral limb (B1), as the control, but reaches with the contralateral limb (B2) were consistently overshot and more dispersed to each target. Note that the reaches to the near target actually ended closer to the more distant target, 3 cm anterior. The second animal trained to reach to multiple target locations overshot all targets, whereas end points for the third animal were overshot and lateral to all targets.

We verified that these reaching impairments were not due to differences in the position of the body during the reach. In both controls and unilateral inactivated animals, axial displacement was not different for reaches using either limb (noninfused control: \( P = 0.254 \); unilateral muscimol infusion: \( P = 0.238 \)).

Figure 3. Effects of inactivation on spatial accuracy (A: target-end-point distance) and end-point dispersion (B: the area of the 95% ellipse). Bars plot mean values ± SE for all animals in each condition group (see Table 1; controls: \( n = 2 \); unilateral muscimol infusions: \( n = 5 \); bilateral muscimol infusion: \( n = 2 \)); dots plot values for each animal (mean of 2 sessions). Lines connect data points for the same animal.
adjusted reaches, starting at different lateralities and ending on the central target. The relations among start laterality and reach (i.e., vector) length and angle were fitted to a linear slope with high $R^2$ values (laterality-reach angle: $R^2 = 0.964$; laterality-reach length: $R^2 = 0.976$).

Results from all animals are presented in the histograms in Fig. 6. The slopes of the relationships for trajectory length and angle were reduced significantly, as were the $R^2$ values, for the contralateral limbs in animals that received early unilateral inactivations. The reductions in the slopes and $R^2$ values for animals subjected to bilateral muscimol infusions were similar to those for the unilateral infused animals, but there was no difference between two limbs. We also observed a small decrease in $R^2$ for controls using the right limb. Other than this difference, we noted no other significant differences between the two limbs in controls. We verified that the reductions in the slopes and $R^2$ values in animals subjected to unilateral or bilateral infusions were not due to differences in the range of starting laterality, the range of reach lengths (which was greater for the impaired limb in animals that overshot the target), or saturation in reach length. These data suggest that the animals were less effective in accounting for the starting laterality when planning the length and angle of reaching with the impaired limb.

Grasping

We distinguished two categories of grasps. 1) In normal grasping, the digits were slightly extended before contact with the meat, followed by digit flexion, and then forearm supination. 2) In raking grasping, the meat was dragged from the work surface, typically without preceding digit extension and without digit flexion and forearm supination after contacting the meat. Occasionally, during raking grasps the digits were fanned as the paw approached the meat. Normal grasping occurred on 82.7% of the trials in controls and 80.4% with the ipsilateral limb in animals with early unilateral activity blockade (Fig. 7). By contrast, the contralateral limb grasped the meat normally in only 14.8% of reaches. Animals with early bilateral activity blockade also rarely used the normal grasping movement (20 and 19.5%).

Laterality of control

We examined to what extent the impaired reaching and grasping movements, produced by early unilateral activity blockade, reflected bilateral sensory motor cortex control. The impaired movements could have been controlled by the aberrant ipsilateral CS terminations of the cortex that was active between PWs 3 and 7, by the sparse contralateral CS terminations of the previously silent cortex (Martin et al. 1999) or bilaterally. We used late muscimol infusions to block on-going activity (Table 2).

After the initial (early) inactivation, and as in the other animals subjected to early postnatal muscimol infusion, reaching and grasping were impaired. The mean target-end-point distances for the left versus right limbs during the first baseline period for K63 was 2.2 versus 4.2 cm, and for K74, 1.6 versus 3.0 cm. Baseline data, for comparison with effects of the late inactivations, were collected before the first late inactivation, during the 1-wk period without infusion between the two late

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

**Fig. 4.** Mean end points for reaches to multiple targets for animals that received unilateral saline infusion (A) and unilateral muscimol infusion (B). Open symbols show target locations; closed symbols show mean end points for corresponding targets. Bars plot error along the principal (long) and minor (short) axes of the ellipse that encloses 95% of the end points (e.g., Fig. 2) and are oriented according to ellipse orientation. For each data point, there are between 15 and 20 trials. For the animal shown in B, the small fluctuations in the magnitudes of the error (e.g., target-end-point distance) were significant for the contralateral but not the ipsilateral limb (ipsilateral: $F = 1.72$, $P = 0.239$, NS; contralateral: $F = 5.45$, $P = 0.024$). For neither limb did the angle of the error vary significantly (angle formed by target-end-point vector) (ipsilateral: $F = 1.9$, $P = 0.207$, NS; contralateral: $F = 1.2$, $P = 0.37$, NS). Near target was located 3 cm from the aperture; the far targets were located 6 cm from the aperture, each separated mediolaterally by 2 cm.

Adjustments in trajectory length and angle for differences in starting laterality

From trial to trial, animals varied the mediolateral paw position at the start of the reach by moving between the vertical supports on either side of the cage aperture. As the laterality of the animal’s starting position increased (relative to the target), reach length must be increased and reach angle reduced to maintain end point accuracy. For animals that received unilateral inactivation, the magnitudes of these compensatory adjustments were greater for reaches with the unimpaired than impaired limb. Figure 5A shows a vector plot of reaches (i.e., start to end positions) for a representative animal. The vectors plot reach length and direction from the starting point. Although the range of starting lateralities was similar for both sides, reaches made with the limb ipsilateral to inactivation more clearly converged onto the target than the impaired contralateral limb. We quantified this difference by plotting the relationship between the starting laterality and reach length (Fig. 5B) and reach angle (Fig. 5C) for individual trials. For both trajectory measures, the slopes of the relationships were reduced and the variability increased for the contralateral limb. It should be noted that the geometric relationships between laterality and reach length and reach angle were not strictly linear. However, over the 6 cm range of lateralities maximally available in the task, the relationship closely approximated a linear function. We showed this by computing the lengths and angles of sim-
inactivations, and after the second late inactivation (Table 2; Baseline 1–3). Data from the three baseline periods were pooled because there were no systematic differences across the three periods ($K63: F = 0.967; P = 0.383, NS; K74: F = 2.08; P = 0.11, NS$). We found that the day after halting infusion of the right cortex (before baseline period 2; Table 2), reaching using the left limb was not impaired. However, we waited an additional 2–3 days before collecting performance data to ensure that there were no residual effects of muscimol.

Figure 8 plots the percent increase in systematic error (black and white bars) and the decrease in the percent of trials in which normal grasping was performed (gray and stippled bars) for two cats during the late inactivations. In both animals, inactivation of the ipsilateral cortex, the one that had been active between PWs 3 and 7 (Fig. 8; left columns), produced small significant increases in systematic end-point error and large decreases in grasping, relative to the already impaired performance. (There were no significant changes in variable end point error.) Reinactivation of the previously silenced (contralateral to impaired limb) cortex resulted in larger reaching impairments than inactivation of the other cortex, but similar grasping impairments (Fig. 8, right bars). This suggests that the impaired limb receives bilateral control, but contralateral control predominates.

Both of the animals subjected to the combined early and late inactivations had robust bilateral CS terminations from the sensory motor cortex silenced between PWs 3 and 7 (i.e., ipsilateral). Measurements of the density of terminal WGA-HRP labeling indicated that the index of laterality for these animals was 0.76, and 0.86. (An index of 1.0 corresponds to symmetrical bilateral labeling; a value approaching 0 indicates predominantly contralateral labeling.) By comparison, the mean index of laterality for control animals from our published study (Martin et al. 1999) is 0.04 (combined data for 2 age-matched controls and 3 animals subjected to unilateral saline infusion). These anatomic findings show that the side of the spinal gray matter controlling the impaired limb received aberrant ipsilateral CS terminations from the sensory motor cortex that remained active during early postnatal development.

**Effects of late muscimol infusion on prehension**

Three animals received unilateral muscimol infusion after the CS refinement period (i.e., beginning PW 8 or later). In two cats, we verified the absence of observable ipsilateral effects with unilateral inactivation. One animal ($K34$) was trained before inactivation. Mean target-end-point distance was unchanged during inactivation of the ipsilateral cortex compared with data collected before infusion (before infusion: 1.51; $P = 0.79$) and grasping actua...
ally improved by a small amount (from 65 to 74%). The second animal (K33) was trained only during the late infusion to verify that animals can learn the prehension task when the function of one sensory motor cortex is impaired (in this case, by inactivation). The magnitude of ipsilateral reaching errors and the percent of normal grasps were not different from the unimpaired ipsilateral limb in the unilateral inactivation group [K33 mean target-end-point distance = 1.44 ± 0.29; grasping = 81%; unilateral infusion (n = 5 cats) mean = 1.43 ± 0.31; grasping = 80.4%; means ± SE]. The absence of defects using the limb ipsilateral to muscimol infusion shows that the contralateral sensory motor cortex was sufficient for adequate task performance in normal animals. However, bilateral control was present in animals subjected to the early unilateral inactivation (Fig. 8).

The third animal (K80) received late unilateral infusion to verify that a month-long period of sensory motor cortex inactivation after the period of CS refinement does not lead to prehension impairments. We infused muscimol between PWs 8 and 12, following which the animal was trained. We found that the mean target-end-point distance was not significantly different for the two limbs (left: 1.27 cm; right: 1.24 cm; t =

### TABLE 2. Critical times for experiments in which animals were subjected to both early and late infusions

<table>
<thead>
<tr>
<th>Cat</th>
<th>K63</th>
<th>K74</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial left inactivation</td>
<td>3–7</td>
<td>3–7</td>
</tr>
<tr>
<td>Age start training</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Duration training</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Baseline testing (1)</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Inactivate right and test</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Baseline testing (2)</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Inactivate left and test</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Baseline testing (3)</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

![FIG. 6. Relationships between trajectory length (A) and angle (B) for the 3 groups of animals (controls, n = 2; unilateral muscimol, n = 5; and bilateral muscimol, n = 2). Bars plot means ± SE for both limbs for the different treatment groups; reach length-laterality slope (A1), reach length-laterality slope R² values (A2), reach angle-laterality slope (B1), reach angle-laterality slope R² values (B2). * significant differences. Values for the ipsilateral and contralateral limbs with unilateral muscimol infusion are significantly different (Reach length-laterality slope: P = 0.0001; R²: P = 0.0013; Reach angle-laterality slope: P = 0.0008; R²: P = 0.0001). Values for the 2 limbs in the bilateral muscimol group were not different. Only control values that significantly differed for the 2 limbs was laterality-angle R² relation (P = 0.01). Slopes for trajectory length for the left/ipsilateral limb relation and for the trajectory angle relation for the right/contralateral limb were converted to positive values for plotting.

![FIG. 7. Effect of early postnatal sensory motor cortex inactivations on grasping. Bars plot the mean percent of reaches in which the animals retrieved the food with a normal grasp (see RESULTS for criteria). Data points plot mean percentages for individual animals. For controls, data from the 2 limbs were combined for the ensemble mean because they were not different.](http://jn.physiology.org/)

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in early development. Such a defect would not only impair programming the initial reach to the target but corrections as well.

In our earlier studies (Martin and Lee 1999; Martin et al. 1999), we used the decrease in histochemical staining of the metabolic enzyme cytochrome oxidase to estimate the topography of inactivation produced by muscimol infusion. In the visual cortex, cytochrome oxidase staining levels are proportional to the overall level of neural activity (Wong-Riley 1979). On the basis of our estimates, in the present study the forelimb area of primary motor cortex (area 4γ) would have been maximally affected (≤50% reduction in metabolic staining) (Martin et al. 1999). Staining in adjacent regions of areas 6 and 3 would have been less markedly reduced. Cytochrome oxidase staining in areas 2 and 5 would have been unchanged. Although disruption of the normal function of any of these areas of the sensory motor cortex in the cat might produce prehension defects, two areas stand out as possible substrates for the impairments: rostral motor cortex, where inactivation in mature cats produces prehension defects (Martin and Ghez 1993), and caudal somatic sensory cortex, where lesions in maturity disrupt learning manipulative skills for grasping but not performance of previously learned skills (Sakamoto et al. 1989).

The abnormal contralateral limb control produced by unilateral activity blockade could have been due to altered connections locally in sensory motor cortex or at any of the targets of its diverse efferent projections. The impairments were not likely to be due to gross topographic changes in the patterns of CS terminations, in particular the presence of aberrant ipsilateral terminations from the cortex active during early development. We reached this conclusion because the form of the prehension impairments was the same following unilateral and bilateral muscimol infusion (differing only in magnitude) but our prior studies showed that the CS termination patterns were different for the two conditions. Although unilateral infusions resulted in dense ipsilateral and sparse contralateral terminations (Martin et al. 1999), bilateral infusions preserved the normal density and laterality of terminations (Martin and Lee 1999). Rather the behavioral changes are more likely to be due to changes in the morphology (Lee et al. 1998; Li et al. 1999) or strength of CS connections or to defective control signals from sensory motor cortex.

**Aberrant ipsilateral control and the limitations of ipsilateral projections**

In normal animals, contralateral CS control was sufficient for task performance because ipsilateral sensory motor cortex inactivation did not affect reaching errors or disrupt grasping. After early postnatal unilateral inactivation, the opposite cortex took on a new role in controlling the ipsilateral limb. However, animals that received early unilateral infusions appeared to rely on bilateral CS control because ipsilateral inactivation significantly impaired performance. This result shows that the ipsilateral cortex partially rescued the impaired limb, perhaps by transmitting descending control signals to spinal neurons via the aberrant ipsilateral CS terminations (Martin et al. 1999). We also have reported development of aberrant bilateral corticobulbar projections (although not activity dependent during PWs 3–7) after early postnatal activity blockade (Martin et al.
It is not known if the ipsilateral component of bilateral control specifies particular muscle activation patterns or kinematic changes during prehension. Alternatively, such ipsilateral control could reflect relatively nonspecific facilitation of spinal motor circuits that enables signals from the contralateral cortex to be more effective. One factor limiting the specificity of ipsilateral control is the laterality of somatic sensory information that the sensory motor cortex represents. Control signals based on a contralateral somatic sensory input (Asanuma 1981) would be inappropriate to steer the ipsilateral (impaired) limb. Greater control signal specificity might be achieved with more training. The sensory motor cortex normally receives little ipsilateral input (Asanuma 1981; Brooks et al. 1961), consistent with its minimal role in ipsilateral motor control. However, just as ipsilateral CS control increases with chronic inactivation of the opposite side, ipsilateral somatic sensory inputs might also increase.

The previously silenced (contralateral) cortex maintained a stronger role because reactivation produced further behavioral impairments. This strong contralateral control shows that the early postnatal infusion did not cause a lesion apart from damage at the cannula implantation site. The most likely explanation for exacerbation of impairments during reactivation is a direct effect of infused muscimol on local cortical neurons. In addition to the histological characteristics described earlier (see Fig. 1), the cortex silenced during early development had many of the characteristics of normal cortex. Corticocortical and corticorubral terminations were preserved as well as axonal projections to the medullary pyramids and spinal white matter (Martin et al. 1999). Our earlier findings also showed that muscimol was not toxic to the developing cortex (at the rates and concentration infused): Bilateral infusion, which silences neurons without producing activity asymmetry and competition, led to a relatively normal density and normal topography of CS terminations (Martin and Lee 1999).

Critical period in CS development

Our behavioral and anatomic data suggest that PWs 3–7 is a critical period both for development of CS terminations and for maturation of CS circuits for skilled forelimb control. This is a period of anatomic refinement of CS terminations (Alisky et al. 1992; Theriault and Tatton 1989), as well as a period of susceptibility to the effects of sensory motor cortex activity blockade (Martin et al. 1999). The behavioral deficits in our study were persistent even after 2 mo of training and practice (as in animals subjected to early and late infusions; see Table 2). Moreover, the impairments did not appear to abate even after 7 mo of testing (unpublished observations). These findings show that the changes produced by early postnatal activity blockade pushed the adaptive capabilities of the motor systems beyond its limits. It is likely that these persistent behavioral impairments reflect permanent anatomic changes in CS terminations, similar to what has been reported for structure-function correlations in other systems. For example, in the developing barn owl, reared with horizontal-displacing prisms, animals lose the capacity to adapt head turning to sound sources when the topography of brain stem acoustico-motor connections becomes fixed (Feldman and Knudsen 1997; Knudsen 1998).

It is plausible that there are also earlier and later critical periods in motor behavioral development that reflect motor system structural development. Before PW 3 (the onset of our early muscimol infusions), CS axons are growing into the spinal gray matter (Alisky et al. 1992; Wise et al. 1977) and striated limb muscle is developing its mature electromechanical (Buller et al. 1960) and synaptic (Bagust et al. 1973) properties. Transient functional manipulation of the motor system during this period may have an enduring effect on CS development much like very early visual system activity blockade has on development of retino-geniculate terminations (Shatz and Stryker 1988). The presence of a later critical period (after PW 7, the offset of the early inactivations) is also plausible because the CS system in the cat may not be mature until PW 12 or later. We have preliminary evidence that between PWs 7 and 10 the number of terminal CS axon branches continued to increase (Li et al. 1999) and that the motor representation in primary motor cortex continued to mature (e.g., decreased stimulation current thresholds) (Chakrabarty and Martin 1999). Periods of developmental plasticity in the CS system can be times of heightened vulnerability to traumatic insults (Armand and Kably 1992; Leonard and Goldberger 1987b; for review, see Terashima 1995). Skilled motor behavior might also be vulnerable to environmental deprivations (and enrichments) during these periods.

Implications for development of the functional organization of the corticospinal system

Failure to develop normal contralateral limb motor skills after early postnatal activity blockade is reminiscent of the findings described by Hein, Held, and colleagues for cats (Hein 1974; Hein and Held 1967; Held and Hein 1963) and monkeys (Held and Baur 1967) deprived of visual information about their limb movements. Cats that did not see the kinematic consequences of their self-generated limb movements failed to express normal visually guided behaviors (Hein 1974; Hein and Held 1967). This stresses the importance of receiving visual feedback during postnatal motor skill development. Such visual feedback also could be important in human motor development because newborn infants preferentially move their arms into their view (Meer et al. 1995). Visual feedback would enable the developing motor systems to validate the efficacy of movements (i.e., error magnitude). Limb proprioceptive information is also essential for accurate reaching (Ghez et al. 1995; Gordon et al. 1995), although its role in motor development has not been studied. We propose that the prehension deficits produced by sensory motor cortex activity blockade reflect a failure to learn to integrate somatic sensory, and possibly visual, information with control signals driving the emerging movements. Our chronic inactivations would have affected primary somatic sensory cortex, especially area 3a (Avendaño and Verdu 1992) where limb proprioceptive information is represented (Dykes et al. 1980).

The mature primary motor cortex is a site for integrating target visual information (Crutcher and Alexander 1990; Martin and Ghez 1985) and input from diverse somatic sensory modalities (Martin and Ghez 1985; Pappas and Strick 1981; Tanji and Wise 1981; Vicario et al. 1983) for limb control. The
results of various studies in mature animals suggest a role for primary motor cortex in specifying movement direction (Georgopoulos et al. 1982; Hoffman and Strick 1995; Kakei et al. 1999; Martin and Ghez 1993), gait modification and paw placement during visually-guided locomotion (Drew et al. 1996), and distal muscle/response selection (for review, see Porter and Lemon 1993). Without activity in sensory motor cortex during an early critical period, these functions—which depend on integrating sensory information for motor action—may fail to develop normally. During early postnatal maturation, sensory motor cortex activity may not only contribute to the moment-to-moment control of limb movement but also could serve to shape the anatomic and functional development of CS circuits.

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