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Differential Activity-Dependent Development of Corticospinal Control of Movement and Final Limb Position During Visually Guided Locomotion

K. M. Friel¹, T. Drew³, and J. H. Martin^{1,2}

¹ Center for Neurobiology and Behavior, Columbia University, New York

² New York State Psychiatric Institute, New York, New York

³ Department of Physiology, Université de Montréal, Montreal, Quebec, Canada

Abstract

Although we understand that activity- and use-dependent processes are important in determining corticospinal axon terminal development in the spinal cord, little is known about the role of these processes in development of skilled control of limb movements. In the present study we determined the effects of unilateral motor cortex activity blockade produced by muscimol infusion during the corticospinal axon terminal refinement period, between postnatal weeks 5–7, on visually guided locomotion. We examined stepping and forepaw placement on the rungs of a horizontal ladder and gait modifications as animals stepped over obstacles during treadmill walking. When cats traversed the horizontal ladder, the limb contralateral to inactivation was placed significantly farther forward on the rungs than the ipsilateral limb, indicating defective endpoint control. Similarly, when animals stepped over obstacles on a treadmill, the contralateral limb was placed farther in front of the obstacle, but only when it was the first (i.e., leading) limb to step over the obstacle, not when it was the second (i.e., trailing) limb. This is also indicative of an endpoint control deficit. In contrast, neither during ladder walking, nor when stepping over obstacles on the treadmill, was there any consistent evidence for a major impairment in limb trajectory. These results point to distinct and possibly independent corticospinal mechanisms for movement trajectory control and endpoint control. Although corticospinal activity during early postnatal development is needed to refine circuits for accurate endpoint control, this activity-dependent refinement is not needed for movement trajectory control.

INTRODUCTION

The corticospinal (CS) system is the principal system for controlling skilled voluntary movements. This system develops during early postnatal life, reaching maturity several weeks to months after birth in animals and after many years in humans (for review, see Martin 2005). During early postnatal development, there is an important interplay between CS tract axon terminations in the spinal cord, neural activity in primary motor cortex (M1), and motor experience. In the cat, CS tract axons terminate diffusely as they grow into the spinal gray matter, with extensive dorsoventral and bilateral axon branching (Alisky et al. 1992; Li and Martin 2001; Theriault and Tatton 1989). Refinement of the topography of CS tract axon terminals into the mature form requires neural activity in M1 and limb motor experience during a brief critical period, without which the axons fail to develop dense and topographically

specific terminations in the spinal cord (Friel and Martin 2005; Martin and Lee 1999; Martin et al. 2004).

Even though we understand that activity- and use-dependent processes are important in determining the regional distribution and morphology of CS axon terminals (Friel and Martin 2005; Li and Martin 2001, 2002; Martin et al. 2004), little is known about the role of these processes in development of skilled movement control. Effective posture, interjoint coordination during movement, and visual guidance of movement are expressed within moments after birth in many animals (Muir 2000). By contrast, many species, including cats, monkeys, and humans, develop these motor skills later in development. This raises the possibility that not only postnatal activity in particular motor systems, but also use-dependent mechanisms are important in establishing functional motor circuits. We began to address the role of these factors in motor development by blocking neural activity or by preventing limb use during the critical period for CS axon termination refinement and examining performance changes later in development. Overreaching was produced in kittens after we blocked M1 activity and grasping impairments occurred after either activity blockade or preventing limb use during the same period (Martin et al. 2000, 2004). These impairments could reflect defects in activity-dependent development of the CS system and its targets in the brain stem and spinal cord that are critical for maturation of circuits for controlling specific movement features.

In the present study we determined the effects of unilateral M1 activity blockade during the critical period for CS axon terminal refinement on visually guided locomotion. We examined two locomotor tasks, one when an animal adjusts limb position to step on a ladder rung and another, when the animal steps over obstacles during treadmill locomotion (Armstrong and Marple-Horvat 1996; Drew 1988). These locomotor tasks, similar to prehension, depend on the CS system for control in mature animals (Armstrong and Marple-Horvat 1996; Beloozerova and Sirota 1993; Biernaskie et al. 2004; Drew 1991; Emerick and Kartje 2004; Metz and Whishaw 2002). Visually guided locomotion is well suited for developmental studies because stable performance can be achieved with less training than reaching. We show that there was consistent overstepping in both tasks as animals placed the limb contralateral to inactivation on the substrate. Surprisingly, our analysis revealed no apparent impairments in limb trajectory control preceding paw placement. These results point to distinct and possibly independent corticospinal mechanisms for movement trajectory control and endpoint control. Although corticospinal activity is needed to refine circuits “functionally” for accurate endpoint control, this activity-dependent refinement is not needed for movement trajectory control. Preliminary results were previously published in abstract form (Friel et al. 2004).

METHODS

Animals were obtained from an AAALAC-accredited supplier. Kittens were delivered in litters of 4 or 5 along with a lactating mother at postnatal day 28. One untreated animal, which served as a control, was delivered after weaning. Columbia University and the New York State Psychiatric Institute IACUC committees approved all experimental procedures. Procedures (treadmill walking) performed at the Université de Montréal were approved by the local animal care committee. Table 1 summarizes the number of animals used to study locomotor performance and the treatments they received.

Sensory-motor cortex activity blockade

Animals were administered atropine [0.04 mg/kg, intramuscularly (im)] before surgery to reduce tracheal secretions. A mixture of acepromazine (0.03 mg/kg, im) and ketamine hydrochloride (32 mg/kg, im) was given to induce anesthesia. The trachea was intubated in each cat after anesthesia was induced and the cats were maintained in an areflexive condition on 1–2% isoflurane during surgery. Animals were given a broad-spectrum antibiotic

(cephazolin) at the time of surgery and an analgesic (buprenorphin) afterward. Animals resumed nursing on recovery from anesthesia and were given supplemental milk (KMR feline milk replacement) as needed.

A small craniotomy (~5 mm diameter) was made over the forelimb area of primary motor cortex (M1), in the lateral sigmoid gyrus, just lateral to the tip of the cruciate sulcus. This region projects to the cervical spinal cord (Martin 1996) and microstimulation evokes contralateral forelimb joint movement (Chakrabarty and Martin 2000). To block neuronal activity in forelimb M1, we infused the γ -aminobutyric acid type A receptor (GABA_A) agonist muscimol (10 mM in sterile saline; Sigma). This concentration was previously shown to effectively and reversibly block activity in primary visual and motor cortex in kittens (Martin et al. 1999; Reiter and Stryker 1988). A 28-gauge hypodermic needle cannula (Alzet), beveled at the tip, was connected with vinyl tubing (Scientific Commodities, size 4) to an osmotic minipump (Alzet, model 2002) filled with the muscimol solution. In one animal, the pump was filled with sterile saline only. We previously showed using the metabolic marker cytochrome oxidase that this infusion maximally inhibits a 2.5- to 3-mm radius of cortex at the infusion site and has a smaller effect for an additional 4- to 5-mm radius (Martin et al. 1999) (i.e., total spread of inactivation, 6.5–8 mm). The cannula was inserted 2 mm below the pial surface and was fixed to the skull with screws and dental acrylic cement. The pump delivered muscimol or saline at the rate of 0.5 μ l/h during postnatal weeks 5–7. At the end of the infusion period, the cannula and pump were removed.

Behavioral testing

LADDER-WALKING—We examined animals on the ladder task between 1 and 3 mo after cessation of the inactivation (see Table 1). The ladder was made of Plexiglas and placed on a horizontal surface. It was 88 cm long and 18.4 cm wide, with a rung interval of 5.8 mm. Rungs were square bars 9 mm wide. Stationary platforms were located at either end of the ladder. Cats were placed at one end of the ladder and meat cubes were placed at the other end. Testing was videotaped. During testing, the cat walked across the ladder from the start platform to the food reward. Halfway through testing each day, the positions of the start platform and food reward were switched so that each side of the cat could be captured on film. To prevent cats from memorizing rung position, we placed them at different positions on the platform for each trial while keeping the distance between rungs constant. This resulted in their starting to step on the rungs with either forelimb. Moreover, the first ladder rung to be stepped on differed from trial to trial. For sessions in which data for kinematic analysis were obtained (see following text), we used a permanent marker or white correction fluid, depending on the color of the cat, to mark the shoulder, elbow, wrist, and metacarpophalangeal (MCP) joints, as well as the paw tip.

GAIT MODIFICATION DURING TREADMILL LOCOMOTION—To examine gait modification, inactivated animals were prepared in the lab in New York and transported to the lab in Montreal for testing on a treadmill with a 1.7-m-long surface distance that ran at a speed of 0.35 m s⁻¹. Two obstacles (either 2.5 cm high for cats 1 and 2; or 4.5 cm for cat 3) were attached to the treadmill belt equally spaced. The cats were trained to walk on the treadmill and to step over the obstacles over a period of 2–3 wk and data for analysis were obtained only after the cats had learned to walk steadily on the treadmill for periods of approximately minutes. Cats were filmed at 60 fields/s using a Sony Video camera and a shutter speed of 2 ms.

Behavioral analyses

ANALYSIS OF LADDER STEP MOVEMENTS—Videotapes of testing sessions were imported into a video-editing program (iMovie; for the Apple Macintosh computer). Trials were not scored if the cat halted movement during the step, changed direction of movement,

jumped to the end of the ladder, or stepped off the side of the ladder. Approximately 20% of trials were excluded from scoring. Images from the video files were analyzed at 30 Hz, pausing the frame in which the paw made contact with the rung. We examined forepaw performance only. The front edge of the rung is defined as the edge closest to the cat, as the paw approached the rung. The opposite side is termed the rear edge. We measured the distance that the tip of the cat's forepaw extended beyond the rear edge of the rung of the ladder (termed forward distance). The forward distance was measured on a flat computer screen. Distance measures from the computer screen were converted to centimeters by scaling according to a calibrated distance on each video file.

For kinematic analysis of ladder-step movements, digitized files of testing sessions were imported into the program MaxTRAQ (Innovision Systems) for marking joint centers and the paw tip and MaxMATE (Innovision Systems) was used for computing kinematic measures. Steps were selected for analysis if the cat made a smooth, uninterrupted movement to a ladder rung. Using MaxTRAQ, shoulder, elbow, wrist, MCP joint centers, and the paw tip were marked. These measurements were made on the three video frames before contact with the rung and the contact frame. Because the frame rate is 30 Hz, our measurement points were at -90, -60, and -30 ms before contact and at the time of contact ($t = 0$). For the terminal portion of each step (i.e., between -90 ms before and the time of contact), we generated movement paths for each joint and the paw tip, stick figures of the movement, joint (elbow, wrist, MCP) angles and segment (upper arm, defined by the shoulder and elbow points; forearm, defined by the elbow and wrist points; paw, defined by wrist and paw tip points). Standard parametric and nonparametric statistics were used to test the significance of differences in step measures of the limb contralateral to the cortical infusion (inactivated limb) and the ipsilateral limb (control limb).

STEP HEIGHT AND DISTANCE DURING TREADMILL LOCOMOTION—

To characterize gait modification on the treadmill, we examined the first 25 steps over the obstacle in each period of testing for both the contralateral and ipsilateral (right and left for the untreated control) forepaws. For each step and for each forepaw we made three measurements: 1) the distance between the front edge of the obstacle (i.e., the edge that was closest to the animal before stepping over it) and the tip of the paw (termed distance before obstacle), 2) the distance between the paw tip and the rear edge of the obstacle (termed forward distance), and 3) the height of the paw as it stepped over the obstacle (termed step height). For each step, these measurements were made for both the leading forepaw (i.e., the first to step over the obstacle) and trailing forepaw (the second forelimb to step over the obstacle). Approximately equal numbers of steps were analyzed in which the inactivated and control paws were used as either leading or trailing paws.

TRAJECTORY ANALYSIS DURING TREADMILL LOCOMOTION—The paw trajectory from lift to contact was analyzed in two cats. The steps that we analyzed were selected from the same group of steps for which we measured step height and distance. The vertical and horizontal coordinates of the paw were determined at a 60-Hz sampling rate. The location of the paw at lift-off, when it was directly over the obstacle, and at contact were recorded so that we could align the paw path trajectories with one of these points. Alignment over the obstacle gave the clearest view (see Fig. 8).

To obtain ensemble averages of the paw path trajectories and to compare trajectories statistically, we used the program Matlab (The MathWorks) to interpolate the step data using the Fourier interpolation function (Interpftf). Normalized trajectory data, for the control and inactivated limbs, were averaged using Excel (Microsoft) and plotted using KaleidaGraph (Synergy Software). We used the program Statview for statistical analysis.

Histological analyses

At the conclusion of experiments, cats were deeply anesthetized with sodium pentobarbital (30 mg/kg, intravenous) and, using a peristaltic pump, perfused transcardially with warm saline, followed by a solution of 4% paraformaldehyde. Heparin was injected into the heart (200–500 units) at the onset of perfusion. The total perfusion time was 20–30 min. The brain and spinal cord were removed, postfixed in the same fixative at 4° for 2–3 h, and then transferred to 20% sucrose in 0.1 M phosphate buffer overnight.

Frozen parasagittal sections through the cortex and transverse sections through the cervical enlargement were cut (40 μ m). Cortical sections through the infused and noninfused cortices were stained using the Nissl method and immunostained for antibodies to SMI-32 and in two 7-wk-old cats, parvalbumin. Cortical and second cervical segment (C2) sections were processed for immunohistochemistry with a mouse monoclonal antibody to a nonphosphorylated epitope in neurofilament H (SMI-32; Covance, Berkeley, CA). Although this antibody labels diverse neurons in the CNS, in sensorimotor cortex it appears to be selective for pyramidal neurons (Kaneko et al. 1994; Preuss et al. 1997; Wannier et al. 2005). This antibody also labels axons in the dorsolateral white matter of the spinal cord (Wannier et al. 2005), where the lateral corticospinal tract is located. We used SMI-32 immunostaining to monitor potential effects of muscimol infusion on the population of layer 5 output neurons. We compared SMI-32 staining in the infused and noninfused cortex and in the C2 lateral white column.

For SMI-32 immunohistochemistry, sections were incubated in 3% goat serum with 0.2% Tween at room temperature for 1 h. Next, the sections were incubated in the primary antibody at 4°C overnight. The optimal concentration of primary antibody was 1:2,000. Tissue was washed and then incubated in the secondary antibody (peroxidase-anti-mouse; 1:100). After further washes, the sections were incubated in the DAB solution (10 mg DAB; 20 ml TBS; 2 μ l H₂O₂), washed, defatted, and coverslipped. Cortical tissue from the infused and noninfused sides for each animal was processed within the same vial; therefore they are directly comparable.

Cortical sections were also stained using an antibody to parvalbumin (mouse monoclonal #235; Swant, Belinzona, Switzerland). Sections were incubated in 3% goat serum at room temperature for 1 h. The sections were then incubated in the primary antibody at 4°C overnight, at a concentration of 1:5,000. Tissue was washed and incubated in the secondary antibody (peroxidase-anti-mouse; 1:200) for 2 h, subsequently incubated in the DAB solution (10 mg DAB; 20 ml TBS; 2 μ l H₂O₂), washed, defatted, and coverslipped.

Cortex and spinal sections were photographed using a digital camera (Microfire; Optronics) attached to an Olympus BH-60 stereomicroscope. In comparisons of sections from control and treated sides, all camera exposure and microscope lighting settings remained the same. Digital images were processed using Photoshop (Adobe). Any changes in brightness and contrast to the images to improve image quality were identical for the control and treated sides.

RESULTS

In this study, the forelimb area of M1 was inactivated ($n = 5$ cats) unilaterally between postnatal week (PW) 5 and PW 7. Animals were allowed to recover for 1 wk after the muscimol infusion and from the surgery to remove the cannula and infusion pump. Then, during postnatal months 2–3, cats were tested on one or two visuomotor tasks: traversing a horizontal ladder and stepping over obstacles on a treadmill. In all of these animals (termed inactivated animals), the contralateral forelimb is the affected (termed inactivated) limb and the forelimb ipsilateral to the inactivation served as the control limb. Two additional control animals (age-matched; saline

infusion) were examined in the ladder task; the saline-infused animal was allowed to recover for 1 wk at the end of the infusion period before training. Table 1 summarizes animals, treatment, infusion times, behavioral testing times, and on which tasks each animal was tested.

SMI and parvalbumin immunostaining in M1 and the lateral columns of the spinal cord

The infused and noninfused M1 cortex was stained for SMI-32 to reveal the general distribution of pyramidal neurons to determine whether muscimol infusion produced a lesion. Figure 1 shows data from one representative animal, where *A* and *B* show sections through the region of the infusion site. These sections, which are through the lateral pericruciate cortex, pass through cortical layers that are superficial to the pyramidal cell layer; thus only dendritic/neuropil labeling of SMI-32 was present. Labeling in the infused (*A*) and noninfused (*B*) sides is similar. Figure 1*C* is a section through the infused cortex of the same animal several hundred micrometers medial to the infusion, showing all cortical layers. Labeled cell bodies are present in layer 5 (Fig. 1, *C1* and *C2*); labeled dendrites are present predominantly in layer 5 and in layers 2 and 3. There was little staining in the subcortical white matter. This pattern was identical for the noninfused cortex (Fig. 1, *D1* and *D2*). SMI-32 staining in the territory occupied by the lateral CS tract in the lateral column of the C2 white matter was symmetrical (Fig. 1, *C4* and *D4*). The other cats also showed symmetrical SMI-32 staining in infused and noninfused cortex and in the lateral white columns at C2.

The duration of behavioral training and testing resulted in euthanizing animals many weeks after cessation of the infusion. To more directly determine the effects of the muscimol infusion, we inactivated M1 between wk 5 and wk 7 in two animals and killed them at 7 wk, while the muscimol infusion had occurred. Figure 2 shows histological sections directly through the infusion site (*A*) and the contralateral noninfused cortex (*B*) from one of the animals; *A1* and *A2* are Nissl-stained sections. The cavity in *A1* corresponds to the space occupied by a portion of the cannula. Note that the cannula was in situ during fixation. The dashed lines correspond approximately to the position of the cannula farther into the depths of the tissue. Apart from glial cell proliferation (small nuclei on Nissl staining), the tissue on the two sides was similar. Importantly, there is no evidence of a lesion apart from the tissue directly damaged by the presence of the cannula. In terms of the extent of the inactivation produced by muscimol infusion, an earlier study using cytochrome oxidase, a metabolic marker, showed that the infusion maximally inhibits a 2.5- to 3-mm radius of cortex at the infusion site and has a smaller effect for an additional 4- to 5-mm radius (Martin et al. 1999) (i.e., total spread of inactivation, 6.5–8 mm). At 5–7 wk of age, this region would encompass the lateral pericruciate portion of M1 and extend rostrally into area 6 and caudally into somatic sensory cortex. For M1, this area develops into the region where intracortical microstimulation produces shoulder, elbow, wrist, and digit responses (Chakrabarty and Martin 2000).

In the two animals from the present study, we also stained the tissue with an antibody to the calcium-binding protein parvalbumin, whose tissue levels were previously shown in other brain regions to be activity dependent (Patz et al. 2004). Parvalbumin staining in the infused cortex showed laminae and labeled cell bodies, similar to the noninfused cortex (Fig. 2, *A2* and *B2*), also indicating that the infusion did not produce a lesion. At higher magnification, it is apparent that staining was weaker in the infused than in the contralateral noninfused cortex (Fig. 2, *A3* and *B3*); labeled cell density and neuropil staining were less. In this animal, for example, we found that neuropil staining in lamina 1 was nearly absent for 3.75 mm from the center of the infusion site. This corresponds approximately to the region of maximal cytochrome oxidase reduction shown earlier (Martin et al. 1999). The *inset* in Fig. 2*A1* shows approximately this region of maximal reduction in parvalbumin immunostaining. There was a further, but reduced, decrease in parvalbumin staining over a larger distance, which corresponds approximately to the wider region of reduced cytochrome oxidase staining shown earlier (Martin et al. 1999).

Our present and prior findings show that muscimol infusion reduced activity-dependent markers within M1 areas where the representation of all forelimb joints develop and did not produce a lesion because there was minimal structural damage to cortical neurons, especially layer 5 output (i.e., corticospinal) neurons, at the infusion site (e.g., Fig. 1, *C1* and *C2*). The absence of a structural lesion is also consistent with our prior results based on layer 5 cell counts and measures of the cross-sectional area of the medullary pyramids (Friel and Martin 2005; Martin et al. 1999, 2000).

Generalized limb control impairments during and after inactivation

Modest forelimb motor control impairments were produced during M1 inactivation (PW 5–7). Descriptions of the motor effects during the period of muscimol infusion were previously described in detail (Martin et al. 2000). In brief, contact placing was impaired (reduced probability to elicit; habituates more easily) on the forelimb contralateral to the infused cortex during the infusion period. Within-cage behavior appeared normal, as did locomotion on a flat surface. There was no paw drag. Contact placing improved within several days after cessation of infusion, although it often remained asymmetric compared with the noninfused side. After cessation of inactivation, animals showed no impairment, either when walking on a flat surface or while walking unobstructed on the treadmill. After saline infusion, the animal did not show any limb motor impairments.

Performance on the horizontal ladder task

GENERALIZED FORELIMB PERFORMANCE CHANGES—When control animals (saline infusion, age-matched) traversed the ladder, the contralateral and ipsilateral forepaws landed squarely on each rung. By contrast, placement of the forelimbs contralateral and ipsilateral to the cortex that received muscimol infusion in the inactivated animals was markedly different. The ipsilateral (control) forelimb was placed squarely and securely on the rung, as in the control animals. By contrast, the contralateral (inactivated) forelimb was typically placed well over the rear edge of the rung and often slipped off. We scored paw placement for individual steps from videotaped data in five animals that received muscimol infusion. On average, the forelimb contralateral to inactivation slipped off the rung on $28.2 \pm 7.9\%$ of trials. By contrast, the forelimb ipsilateral to the inactivation never slipped off the rung. This difference is significant (Mann–Whitney, $Z = 3.811$, $P < 0.0001$). The contralateral and ipsilateral forepaws of both the untreated animal ($n = 3$ sessions) and the one in which saline was infused into M1 from PW 5 to PW 7 ($n = 3$ sessions) did not slip off the ladder rung on any trials.

ANALYSIS OF MOVEMENT BEFORE RUNG CONTACT—To assess impairment in control during the terminal phase of the movement, we determined whether there were asymmetries between the contralateral (inactivated) and ipsilateral (control) limbs in the following measures: digit path variability, movement speed reduction as the paw approached the rung, path orientation, and forelimb joint and segment angles. We digitized individual video frames in two of the inactivated cats and the two controls. For each cat, we digitized the X–Y locations of the shoulder, elbow, wrist, MCP, and digit tip for 15 steps (selected randomly among steps in which the paw did not slip off the ladder rung) during the last three video frames before paw contact with the ladder rung and the frame at contact. From these coordinates, we created a database of joint locations, joint and paw speeds based on the anterior distance traveled during each frame, joint angles, and limb segment angles. We generated stick-figure representations of the right and left forelimbs as each paw approached the ladder rung from mean X and Y joint locations for each step. Figure 3, *A1* and *B1* shows averaged stick figures for the three frames preceding rung contact (thin lines) and the frame in which contact occurred (thick line). For M1 inactivation (Fig. 3*A1*) the contralateral limb oversteps compared with the ipsilateral limb. This is reflected in a greater distance between the rear edge of the ladder rung

(black square) and the paw tip in the stick figure. This is termed forward distance, discussed in detail in the next section. Individual paw tip paths are shown below the stick figure. The ensemble extends farther into the gray band, which highlights the space beyond the rear edge of the rung. For the control cat, this distance was the same for the right and left limbs.

We compared vertical trajectory variability for the limbs contralateral and ipsilateral to the inactivation using the Y coordinate of the paw for the three frames before contact (i.e., the terminal phase of the movement, as the paw approached and landed on the rung). Variability of this measure was not different for the ipsilateral and contralateral limbs (ANOVA; M1: $F = 0.54$, $P = 0.66$; M2: $F = 1.22$, $P = 0.31$). We computed the average speed for the inactivated and control limbs during the terminal phase of the step based on the distance traveled during each 33-ms frame. We found that there were no significant differences between the inactivated and control limbs for the inactivated animals (ANOVA; muscimol cat 1: $F = 0.141$, $P < 0.869$; muscimol cat 2: $F = 0.412$, $P < 0.631$), suggesting that inactivation does not impair the ability of the animal to slow the limb as the paw approached the ladder rung.

We also determined whether there were significant differences in the elbow, wrist, and MCP joint angles and the proximal arm, forearm, and paw segment angles for the inactivated and control limbs during the terminal phase of the step (i.e., three steps preceding contact). The only consistent difference we found was increased extension of the elbow contralateral to muscimol infusion, relative to the ipsilateral elbow (repeated-measures ANOVA, across time compared with the control elbow; muscimol cat 1: 19° mean increase in extension, $F = 6.87$, $P = 0.015$; muscimol cat 2: 16.6° mean increase in extension, $F = 58.53$, $P < 0.0001$). This extended posture is consistent with a limb that is placed farther forward. Our kinematic analysis of the terminal phase of the step did not reveal any significant trajectory asymmetries between the inactivated and control limbs, apart from the increased forward distance, suggesting that the inactivation did not impair control of the terminal phase of the movement, preceding contact.

PAW PLACEMENT ERROR—In contrast to the absence of effects on the terminal trajectory during ladder walking, there was a clear and consistent contralateral limb endpoint error. As indicated earlier, on 28% of steps the paw slipped off the rear edge of the rung (i.e., the edge that was farther from the animal as the paw approached the rung). We reasoned that these slips occurred on particularly errant steps and that a more consistent control impairment, present on most steps, was a forward paw placement error.

To quantify overstepping across animals, we measured the distance from the rear edge of the ladder rung to the paw tip in the video frame at which the forelimb first contacted the rung (see Fig. 4*B*, *inset*). Limbs contralateral and ipsilateral to the inactivated cortex were both measured. Figure 4 shows histograms of forward distance for a representative inactivated cat (muscimol cat 1) and one that received no treatment (age-matched control). In the control cat (Fig. 4*B*), the distances after the rung were symmetrical for both forepaws; they were not significantly different [Kolmogorov–Smirnov (K-S); $c = 1.17$, $P = 0.87$]. Because of the size of the paw relative to the size of the ladder rung, even when the cat paw landed squarely on the rung, the paw tip extended beyond the forward edge of the rung (see Fig. 4, *inset*).

In the inactivated cat, the distribution of forward paw distance for the contralateral limb was significantly broader and shifted forward than for the ipsilateral limb (K-S; $c = 14.35$, $P = 0.0015$). The distribution of distances after the rung for the control side was not significantly different from the distributions for the untreated cat (K-S; $c = 3.22$, $P = 0.40$). The distributions for the impaired paws for all inactivated animals were similar, each peaking between 3 and 4.5 cm. Distances for steps in which the paw slipped off the ladder rung are shown in light gray. Occasionally the control paw was as advanced as for the inactivated side. However, these

forward directed steps never led to a slip. For example, a 3-cm forward distance often resulted in a slip on the inactivated side (Fig. 4A, *top*), whereas the same distance on the control side did not (Fig. 4A, *bottom*). This suggests an impairment in addition to an endpoint defect, such as postural instability.

Figure 5 shows mean forward distances for the five cats receiving unilateral inactivation and the two control animals (untreated age-matched and saline infusion). For four of the five muscimol-treated cats, this forward displacement was significant, whereas for one, it failed to reach significance ($t = 1.8$, $P < 0.082$). For all cats, the average ratio of the forward distance for the contralateral and ipsilateral paws was 2.1. Additionally, the small differences in distances after the rung for the paw contralateral to inactivation were not different between cats (ANOVA; $F = 1.34$, $P > 0.26$). The forward distance was not significantly different for the two limbs in the control cats (unpaired t -test for each animal; control 1: $t = 0.18$, $P > 0.86$; control 2: $t = 0.46$, $P > 0.63$). Across all test and control animals, the small differences in the forward distance for the paw ipsilateral to inactivation, ipsilateral to saline infusion, and the right/left in the untreated animal were not significant (ANOVA; $F = 0.96$, $P > 0.44$). This indicates that the overstepping measure (forward distance) for the ipsilateral limb in the inactivated animals is not different from either of the two limbs in the controls. These findings indicate that prior inactivation of M1 results in a systematic endpoint bias during visually guided stepping. This impairment is similar to overreaching after early postnatal M1 inactivation (Martin et al. 2000).

In three cats we increased the ladder rung distance from 6 to 12 cm on random runs to verify that the animals were not using a stereotypic stepping strategy but were visually placing their limbs on the rungs during each step. We also were interested in determining whether the magnitude of the overstep was relatively constant for two different intended step distances. Figure 6 compares data for the 6- and 12-cm rung distances for the limbs contralateral (dark gray) and ipsilateral (light gray) to inactivation. In each cat the forward distance for the contralateral limb was slightly more for the 12-cm rungs than the 6-cm rungs, but not significantly greater. For the ipsilateral (control) limb, step distance to the more distant rung also was not significantly greater than that for the closer rung. These results indicate that the animals are using visual guidance in placing their paw on the rungs rather than a stereotypic/automatic strategy.

Overstepping on the ladder task is a persistent impairment. In two animals we determined whether there were significant changes in the forward distance during the examination period. We examined five sessions for each animal, between 13 and 21 wk. We found that the overstepping was stable; there were no trends toward decreases or increases in the measured forward distance for the contralateral limbs (muscimol cat 1: $f = 0.775$, $P = 0.5935$; muscimol cat 2: $f = 1.546$, $P = 0.094$). Similarly, the distance after for the ipsilateral (control) limbs did not change (muscimol cat 1: $f = 0.638$, $P = 0.479$; muscimol cat 2: $f = 0.837$, $P = 0.233$).

Gait modifications during visually guided treadmill locomotion

Our findings indicate that after M1 inactivation between PW 5 and PW 7, animals express a consistent impairment in control of the endpoint of visually guided locomotion on the ladder, for which there is no compensation. In three of the five animals studied in the ladder task, we additionally examined their capacity to step over obstacles while walking on a treadmill. We examined visually guided gait modification to step over obstacles for three reasons. First, control of the height of the modified step over the obstacle depends not only on vision (i.e., obstacle size and distance), but also on corticospinal system control (Drew et al. 1996). Therefore as in the ladder task, the absence of M1 neural activity during development would be expected to lead to a control defect. Second, location of the tip of the limb during the movement may be under the same explicit control as during the ladder task, to prevent bumping

of the obstacle. Trajectory measures during this phase may be more sensitive indicators of impairment. Third, location of the endpoint of the step, after clearing the obstacle, is not as critical as in the ladder task; i.e., the animal will not stumble if it oversteps.

We measured three characteristics of gait modification to step over the obstacle: 1) the distance from the front edge of the obstacle (i.e., the edge closest to the animal before stepping over it) to the tip of the paw on the video field before when the paw was lifted from the treadmill belt (termed distance before obstacle); 2) the maximal height of the paw tip when it was above the obstacle (termed modified step height); and 3) the distance from the rear edge of the obstacle (i.e., the edge farthest from the animal before stepping over it) to the tip of the paw on the video field when the paw made contact with the treadmill belt after stepping over it (termed forward distance). The forward distance is similar to the measured forward distance between the rear edge of the ladder rung and the paw tip in the ladder task. These measures were obtained for both the contralateral (inactivated) and ipsilateral (control) limbs, during trials when each limb was the leading or trailing limb during the step.

For all three animals, the contralateral (inactivated) limb was the leading limb as frequently as the ipsilateral (control) limb. Based on an analysis of 224 steps ($n = 53$ for muscimol cat 1; $n = 94$ for muscimol cat 2; $n = 77$ for muscimol cat 3), we found that both forelimbs stepped over the obstacle with equal success and without bumping. We measured the height of the modified step when the limb was leading (Fig. 7A1) and trailing (Fig. 7B1). The differences between the inactivated and control sides were not significant during either the leading or the trailing conditions (leading condition: ANOVA, $F = 0.057$; $P = 0.8123$; trailing condition: ANOVA, $F = 1.333$; $P = 0.2502$). Thus early postnatal M1 activity blockade does not affect control of stepping over obstacles.

We next compared the paw distances before the obstacle and the forward distance beyond the rear edge of the obstacle for the inactivated and control limbs. The distance before the obstacle, before lifting the paw from the treadmill belt, for the contralateral and ipsilateral limbs was not significantly different for the leading condition (ANOVA, $F = 0.726$; $P = 0.3957$). For the trailing condition, the contralateral limb was placed significantly farther before the obstacle (ANOVA, $F = 5.41$; $P = 0.0215$). Post hoc testing revealed that this effect was not consistent across animals; differences in two of the cats were not significant (unpaired t -test; muscimol cat 1: 2.53 cm, $P = 0.14$; muscimol cat 2: 0.94 cm, $P = 0.292$) and in one of the cats was significant (muscimol cat 3: 1.5 cm; $P = 0.05$). By contrast, the increased forward distance beyond the rear edge of the obstacle for the inactivated compared with control limbs is consistently significant, but only when the limb leads (ANOVA; $F = 25.024$; $P < 0.0001$; Fig. 7A2); not when it trails (ANOVA; $F = 2.38$; $P = 0.125$; Fig. 7B2). Comparison between the inactivated and control forelimbs under the leading condition was significant for each cat (P values indicated on the figure). Our findings during treadmill locomotion under the leading condition are similar to those during ladder walking: the limb oversteps the foot placement target. However, in the treadmill task the overstepping did not impair function because it does not provoke tripping.

Our finding that animals can step over an obstacle without bumping—together with the lack of consistent control defects, apart from the overstep—suggests that early postnatal M1 inactivation does not produce a trajectory impairment during stepping over obstacles. To examine this question further, we analyzed the trajectory between lift and contact in two cats. Figure 8, A and C shows paw paths from the control (*left column*) and inactivated (*middle column*) forelimbs from the two animals; inactivated and control paths are overlaid in the *right column* (red, inactivated; black, control). Individual steps are aligned when the paw is located directly over the obstacle. Trajectories for the control and inactivated sides had similar shape and variability. For the limbs on both the control and inactivated sides, the paw lifts smoothly

from the treadmill and clears the obstacle with a safety margin. The return of the paw to the treadmill belt beyond the obstacle is over a shorter distance than the lift before the obstacle. These findings also show that the animals were capable of producing trajectories over obstacles without bumping with either forelimb.

To more directly compare the responses of the control and inactivated limbs, the trajectories were interpolated to 100 points between lift and contact (see METHODS) and averaged ($\pm$ SE, dotted lines) for the two animals (Fig. 8, *B* and *D*). Comparison of the averaged control and inactivated trajectories for the two cats (*right column*) showed minimal differences. Using a repeated-measures ANOVA, we found that the values of the normalized trajectories on the control and inactivated sides were not different (cat shown in *B*: $F = 0.212$; $P = 0.6483$; cat shown in *D*: $F = 1.068$; $P = 0.3097$).

DISCUSSION

This study demonstrates that neural activity in M1 during the period that CS axon terminals undergo topographic refinement in the spinal cord (PW 5–7) is needed for development of visually guided locomotion. Blocking activity during this period resulted in persistent and robust defects in control of the endpoint of the movements. This indicates that M1 activity is needed to refine CS circuits for accurate control of final position. By contrast, during treadmill locomotion animals were able to step over obstacles without bumping, strongly suggesting that trajectory control was not substantially impaired. The paw on the inactivated side lifted smoothly from the treadmill belt and cleared the obstacle at a height similar to that for the control side. This also shows that animals were capable of adaptive modification of the trajectory in this behavioral context. Analysis of paw paths confirmed that the control and inactivated trajectories showed no major changes in control. Moreover, normalized trajectories for the inactivated and control sides were not significantly different. Also consistent with a lack of a major effect of activity blockade on the trajectory, the terminal phase of the step while ladder walking was without apparent impairment. Although the overstepping could be explained by multiple minor control impairments that sum to produce an errant endpoint, it also could be that the trajectory is normal, but intended for a more distant placement. Nevertheless, these findings show that effective and adaptive visuomotor control of the movement trajectory during locomotion, in contrast to final position, can be expressed without activity-dependent refinement during an early CS system critical period. By manipulating CS system development, and the impact that this has on overall motor system development, we were able to separate aspects of the control of a movement's trajectory from control of the endpoint of the movement.

Separate development of movement trajectory and endpoint control

The endpoint control defect that occurs without M1 activity during early postnatal development is reminiscent of the visuomotor impairments reported by Hein and colleagues (1970) after limiting visual feedback during early development. In those studies, kittens and infant monkeys were prevented from seeing movement of their limbs by restricting visual experiences or dark-rearing. Their findings pointed to impairments in development of visual processing for guiding movement. Apropos to our study, activity may be necessary for refinement of intracortical circuitry underlying visuomotor and other sensorimotor transformations needed for achieving an accurate final limb position. Recently, Lajoie and Drew (2007) showed that area 5 lesion in the adult cat produces impairments in visually guided locomotion, including bumping the obstacle by both the leading and the trailing limbs. These impairments are likely attributable to motor planning defects given the proposed role of the parietal lobe in visuomotor transformations (Jeannerod et al. 1995). Postnatal M1 activity blockade could impair development of corticocortical connections between these two areas (Ghosh 1997; Waters et

al. 1982; Yumiya and Ghez 1983), by affecting the projection from M1 to area 5 or the topography of the incoming projections. This, in turn, could disrupt the capacity of M1 to implement this visuomotor signaling. However, the defects we observed differed from those after area 5 lesion (e.g., changes in paw placement in front of the advancing obstacle), which could arise from the downstream consequences of M1 activity blockade. (This is discussed further in the last section of the DISCUSSION.) We have shown that the blockade redistributes CS axon terminals in the cervical gray matter, thereby changing the population of spinal neurons that are activated by the CS tract (Friel and Martin 2005). This change in termination distribution might disrupt the integration of motor cortical signals with peripheral afferent input and other aspects of descending control. This anatomical change could contribute not only to the expression of the endpoint control impairment, but also to the animal's inability to make the suitable corrections. Similar activity blockade also alters cortical terminations in the brain stem (Martin et al. 1999) and may well affect intrinsic M1 circuits. These changes in CS connectivity also could contribute to the persistent deficits.

The differential effects of an activity dependency on endpoint and trajectory control in the cat are surprising. Given potential redundant control by other motor systems, such as the rubrospinal system, that endpoint control is permanently affected suggests that this is an important controlled variable dependent largely on motor cortical/corticospinal processing. Given the role of M1 in trajectory control in maturity (Drew 1991; Shadmehr and Wise 2005) and the changes in spinal terminals after postnatal inactivation (Friel and Martin 2005; Li and Martin 2001), it is surprising that trajectory control is spared. Trajectory control may be mediated by a component of the CS system whose connections are established either at a different time during development or through nonactivity-dependent genetic or transcriptional codes. In this regard, many animals are capable of producing complex motor behaviors that rely on interjoint and interlimb coordination as well as visual guidance within moments after birth (Muir 2000). These movements are not likely to be dependent on experience or other activity-dependent processes because they occur so soon after birth.

Role of M1 in visually guided locomotion

Visually guided locomotor tasks require M1 and CS tract control (Armstrong and Marple-Horvat 1996; Beloozerova and Sirota 1993; Drew 1991; Drew et al. 1996; Lawrence 1994; T Drew, J-E Andujar, K Lajoie, and S Yakovenko, unpublished observations). We found that when cats stepped over obstacles on a treadmill, the affected limb was placed farther beyond the obstacle than the unaffected limb, highlighting an endpoint control impairment that is remarkably similar to the one present in the ladder task. Single-unit studies in mature cats provide evidence for a role for M1 in ensuring precise placement of the paw (i.e., endpoint control) during locomotion. Some pyramidal tract neurons show increased activity when cats are required to step between two small obstacles than when they are required to step over larger ones (see Fig. 5 in Drew et al. 1996). Experiments of Armstrong and Marple-Horvat (1996) show increased activity in motor cortical neurons just before placement of the paw on the rungs of a horizontal ladder. Experiments by Beloozerova and Sirota (1993) equally suggest a role for motor cortical neurons in precise positioning of the paw.

In our study a difference in forward distance was seen only when the contralateral limb was leading. Drew and colleagues showed, in mature cats, that there are major changes in the activity of many M1 neurons as the cat steps over an obstacle in the lead condition (see Fig. 4 in Drew 1993). However, in the trail condition, activity is enhanced primarily early during the swing phase, before stepping over the obstacle. This is in agreement with the observation that changes in muscle activity during the trail condition occur primarily at the onset of swing, subsequently scarcely changed from control. In the lead condition, however, there are major changes in EMG activation patterns throughout the swing phase. It is thus not too surprising

that blocking activity-dependent developmental processes impairs the overall control of the limb during the lead condition. Studies of human stepping suggest that visual information is used to guide the leading but not the trailing limb during obstacle clearance in locomotion (Patla 1998; Patla et al. 1996), including the role of visual feedback (Mohagheghi et al. 2004). Whether this implies a selective requirement for activity-dependent refinement of CS circuits engaged in particular aspects of visual guidance deserves further study.

Endpoint control deficits during visually guided movement

For a variety of visually guided motor tasks, the same phenotype of defect occurs when M1 is deprived of activity during early postnatal life: cats overstep the rungs on a ladder, overstep obstacles on a treadmill with the leading limb, and overreach targets during reach and grasp (Martin et al. 2000). The hypermetric phenotype was seen in these three visually guided tasks, but neither hypometria nor an increased endpoint variability was seen. We propose two classes of mechanism that could help to explain this hypermetria. First, hypermetria could reflect an impairment in scaling the extent of a movement to a visual stimulus specifying movement endpoint. As discussed earlier, the task-dependent (feedforward) mapping of visuospatial information onto the motor cortex for guiding movement, by premotor and association cortices (Jeannerod et al. 1995), might depend on activity-dependent developmental processes for optimization. For example, a motor synergy consisting of proximal and distal forelimb muscles active as the cat places its limb on the substrate after clearing an obstacle (Krouchev et al. 2006) may be controlled by a signal that is calibrated during development by activity-dependent processes. In addition, integration of limb proprioceptive information in relation to extrinsic (visual) space also could depend on early postnatal activity-dependent processes.

Second, the hypermetric posture could reflect an impairment in expressing the planned motor output. A plausible defect that could produce endpoint hypermetria is an impairment in specifying the appropriate forelimb joint stiffness at the end of the movement (Gandolfo et al. 2000; McIntyre et al. 1996). For example, the normal pattern of inertial and viscous loads generated during the movement could perturb final position. Because these loads act in the direction of the terminal phase of the movement (i.e., forward direction for stepping and roughly along the movement path for reaching) (Sainburg et al. 1999), they would result in a systematic forward displacement of the paw and a hypermetric endpoint, rather than result in increased variable endpoint error.

Recent human psychophysical studies of Ghez and colleagues provide evidence that movement trajectory and endpoint are subject to distinct planning mechanisms (Scheidt et al. 2004). They noted that normal human subjects overshoot the target of a reach when switching from performing tasks constraining either endpoint or the trajectory of movement. Their findings are also in accord with the idea that spatial control of final position and stabilization of the limb at that position are related. They simulated task conditions and showed that the overshoot occurred when the trajectory controller did not take account of the increase in viscosity and limb stiffness that occurs at the end of the movement. Joint stiffness control was specifically proposed for a subpopulation of M1 neurons in the monkey (Humphrey and Reed 1983).

Endpoint control is critical for maintaining a secure stance for the next step in locomotion. In prehension, limb stabilization at the final position is also important for target acquisition and subsequent manipulation. Our observation that forward displaced movements during ladder walking sometimes led to a slip is consistent with the idea that the process controlling final limb position also controls an aspect of stabilizing that position. Normal endpoint control may depend on distinct motor circuits that integrate information about the spatial coordinates of the final position with information about the upcoming motor plan. Our findings show that activity-dependent development of the CS system is needed for this control.

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References

- Alisky JM, Swink TD, Tolbert DL. The postnatal spatial and temporal development of corticospinal projections in cats. *Exp Brain Res* 1992;88:265–276. [PubMed: 1374346]
- Armstrong DM, Marple-Horvat DE. Role of the cerebellum and motor cortex in the regulation of visually controlled locomotion. *Can J Physiol Pharmacol* 1996;74:443–455. [PubMed: 8828890]
- Beloozerova IN, Sirota MG. The role of the motor cortex in the control of accuracy of locomotor movements in the cat. *J Physiol* 1993;461:1–25. [PubMed: 8350259]
- Biernaskie J, Chernenko G, Corbett D. Efficacy of rehabilitative experience declines with time after focal ischemic brain injury. *J Neurosci* 2004;24:1245–1254. [PubMed: 14762143]
- Chakrabarty S, Martin JH. Postnatal development of the motor representation in primary motor cortex. *J Neurophysiol* 2000;84:2582–2594. [PubMed: 11068000]
- Drew T. Motor cortical cell discharge during voluntary gait modification. *Brain Res* 1988;457:181–187. [PubMed: 3167563]
- Drew T. Visuomotor coordination in locomotion. *Curr Opin Neurobiol* 1991;1:652–657. [PubMed: 1822312]
- Drew T. Motor cortical activity during voluntary gait modifications in the cat. I. Cells related to the forelimbs. *J Neurophysiol* 1993;70:179–199. [PubMed: 8360715]
- Drew T, Jiang W, Kably B, Lavoie S. Role of the motor cortex in the control of visually triggered gait modifications. *Can J Physiol Pharmacol* 1996;74:426–442. [PubMed: 8828889]
- Emerick AJ, Kartje GL. Behavioral recovery and anatomical plasticity in adult rats after cortical lesion and treatment with monoclonal antibody IN-1. *Behav Brain Res* 2004;152:315–325. [PubMed: 15196799]
- Friel KM, Drew T, Martin JH. Sensorimotor cortex inactivation during a brief postnatal period in cats produces persistent ladder-walking deficits. *Soc Neurosci Abstr* 2004;30:878.872.
- Friel KM, Martin JH. Role of sensory-motor cortex activity in postnatal development of corticospinal axon terminals in the cat. *J Comp Neurol* 2005;485:43–56. [PubMed: 15776437]
- Gandolfo F, Li C, Benda BJ, Schioppa CP, Bizzi E. Cortical correlates of learning in monkeys adapting to a new dynamical environment. *Proc Natl Acad Sci USA* 2000;97:2259–2263. [PubMed: 10681435]
- Ghosh S. Comparison of the cortical connections of areas 4 gamma and 4 delta in the cat cerebral cortex. *J Comp Neurol* 1997;388:371–396. [PubMed: 9368848]
- Hein A, Held R, Gower EC. Development and segmentation of visually controlled movement by selective exposure during rearing. *J Comp Physiol Psychol* 1970;73:181–187. [PubMed: 5493257]
- Humphrey DR, Reed DJ. Separate cortical systems for control of joint movement and joint stiffness: reciprocal activation and coactivation of antagonist muscles. *Adv Neurol* 1983;39:347–372. [PubMed: 6419553]
- Jeannerod M, Arbib MA, Rizzolatti G, Sakata H. Grasping objects: the cortical mechanisms of visuomotor transformation. *Trends Neurosci* 1995;18:314–320. [PubMed: 7571012]
- Kaneko T, Caria MA, Asanuma H. Information processing within the motor cortex. II. Intracortical connections between neurons receiving somatosensory cortical input and motor output neurons of the cortex. *J Comp Neurol* 1994;345:172–184. [PubMed: 7929898]

- Krouchev N, Kalaska JF, Drew T. Sequential activation of muscle synergies during locomotion in the intact cat as revealed by cluster analysis and direct decomposition. *J Neurophysiol* 2006;96:1991–2010. [PubMed: 16823029]
- Lajoie K, Drew T. Lesions of area 5 of the posterior parietal cortex in the cat produce errors in the accuracy of paw placement during visually guided locomotion. *J Neurophysiol* 2007;97:2339–2354. [PubMed: 17215501]
- Lawrence DG. Central neural mechanisms of prehension. *Can J Physiol Pharmacol* 1994;72:580–582. [PubMed: 7954088]
- Li Q, Martin JH. Postnatal development of corticospinal axon terminal morphology in the cat. *J Comp Neurol* 2001;435:127–141. [PubMed: 11391636]
- Li Q, Martin JH. Postnatal development of connectional specificity of corticospinal terminals in the cat. *J Comp Neurol* 2002;447:57–71. [PubMed: 11967895]
- Martin JH. Differential spinal projections from the forelimb areas of the rostral and caudal subregions of primary motor cortex in the cat. *Exp Brain Res* 1996;108:191–205. [PubMed: 8815029]
- Martin JH. The corticospinal system: from development to motor control. *Neuroscientist* 2005;11:161–173. [PubMed: 15746384]
- Martin JH, Choy M, Pullman S, Meng Z. Corticospinal system development depends on motor experience. *J Neurosci* 2004;24:2122–2132. [PubMed: 14999063]
- Martin JH, Donarummo L, Hacking A. Impairments in prehension produced by early postnatal sensory motor cortex activity blockade. *J Neurophysiol* 2000;83:895–906. [PubMed: 10669503]
- Martin JH, Kably B, Hacking A. Activity-dependent development of cortical axon terminations in the spinal cord and brain stem. *Exp Brain Res* 1999;125:184–199. [PubMed: 10204771]
- Martin JH, Lee SJ. Activity-dependent competition between developing corticospinal terminations. *Neuroreport* 1999;10:2277–2282. [PubMed: 10439448]
- McIntyre J, Mussa-Ivaldi FA, Bizzi E. The control of stable postures in the multijoint arm. *Exp Brain Res* 1996;110:248–264. [PubMed: 8836689]
- Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J Neurosci Methods* 2002;115:169–179. [PubMed: 11992668]
- Mohagheghi AA, Moraes R, Patla AE. The effects of distant and on-line visual information on the control of approach phase and step over an obstacle during locomotion. *Exp Brain Res* 2004;155:459–468. [PubMed: 14770275]
- Muir GD. Early ontogeny of locomotor behavior: a comparison between altricial and precocial animals. *Brain Res Bull* 2000;53:719–726. [PubMed: 11165806]
- Patla AE. How is human gait controlled by vision? *Ecol Psychol* 1998;10:287–302.
- Patla AE, Rietdyk S, Martin C, Prentice S. Locomotor patterns of the leading and the trailing limbs as solid and fragile obstacles are stepped over: some insights into the role of vision during locomotion. *J Mot Behav* 1996;28:35–47. [PubMed: 12529222]
- Patz S, Grabert J, Gorba T, Wirth MJ, Wahle P. Parvalbumin expression in visual cortex interneurons depends on neuronal activity and TrkB ligands during an early period of postnatal development. *Cereb Cortex* 2004;14:342–351. [PubMed: 14754872]
- Preuss TM, Stepniewska I, Jain N, Kaas JH. Multiple divisions of macaque precentral motor cortex identified with neurofilament antibody SMI-32. *Brain Res* 1997;767:148–153. [PubMed: 9365028]
- Reiter HO, Stryker MP. Neural plasticity without postsynaptic action potentials: less-active inputs become dominant when kitten visual cortical cells are pharmacologically inhibited. *Proc Natl Acad Sci USA* 1988;85:3623–3627. [PubMed: 3285347]
- Sainburg RL, Ghez C, Kalakanis D. Intersegmental dynamics are controlled by sequential anticipatory, error correction, and postural mechanisms. *J Neurophysiol* 1999;81:1045–1056. [PubMed: 10085332]
- Scheidt RA, Mussa-Ivaldi FA, Ghez C. Posture and movement invoke separate adaptive mechanisms. *Soc Neurosci Abstr* 2004;30:873.14.
- Shadmehr, R.; Wise, SP. *Computational Neurobiology of Reaching and Pointing: A Foundation for Motor Learning*. Cambridge, MA: MIT Press; 2005. p. 544

- Theriault E, Tatton WG. Postnatal redistribution of pericruciate motor cortical projections within the kitten spinal cord. *Brain Res Dev Brain Res* 1989;45:219–237.
- Wannier T, Schmidlin E, Bloch J, Rouiller EM. A unilateral section of the corticospinal tract at cervical level in primate does not lead to measurable cell loss in motor cortex. *J Neurotrauma* 2005;22:703–717. [PubMed: 15941378]
- Waters RS, Favorov O, Mori A, Asanuma H. Pattern of projection and physiological properties of corticocortical connections from the posterior bank of the ansate sulcus to the motor cortex, area 4 gamma, in the cat. *Exp Brain Res* 1982;48:335–344. [PubMed: 7151928]
- Yumiya H, Ghez C. Specialized subregions in cat motor cortex: anatomical demonstration of differential projections to rostral and caudal sectors. *Exp Brain Res* 1983;53:259–276. [PubMed: 6200347]

FIG. 1.

SMI-32 immunostaining in motor cortex (M1) and spinal cord from a representative animal that received muscimol infusion. *Left:* SMI-32 staining associated with the inactivated side. *Right:* SMI-32 staining associated with the noninactivated side. *A:* low-magnification view through the infused cortex (primarily through layers above layer 5), and *C1*, within several hundred micrometers from the center of the infusion (through all cortical layers). Staining is largely limited to layers 2, 3, and 5. *C2:* enlarged image of labeled layer 5 pyramidal neurons in the infused cortex. *C3:* low-magnification view of the lateral white column at *C2* and the lateral gray matter contralateral to the side of cortical infusion. Box in *C3* is enlarged in *C4*, showing punctate SMI-32 staining in axons. *Right:* this panel is similar to the *left*, but for the

noninfused cortex (*B*, *D1*) and spinal cord contralateral to the noninfused side (*D2–D4*).
Insets, *A*, *B*, *C1*, and *D1*: planes of section (vertical line) relative to the infusion site (black dot). Calibrations: *A*, *C1*, *B*, *D1*: 1 mm; *C2*, *D2*: 150 μ m; *C3*, *D3*: 1 mm; *C4*, *D4*: 500 μ m.

FIG. 2.

Nissl and parvalbumin staining at the time of infusion. *Left*: sections through the infusion site. *Right*: sections through the noninfused cortex. *A1* and *B1*: Nissl-stained sections. Parallel dotted lines in *A1* indicate the approximate location of the infusion cannula. *Insets*: location of the section (vertical line) relative to the infusion site (dot). Shaded circle corresponds to the distribution of absent layer 1 parvalbumin staining, scaled to the schematic. *A2* and *B2*: sections stained with an antibody to parvalbumin. Sections are located within 200 μm of the section in part 1. Note that lamina 1 staining is virtually absent. *A3* and *B3*: higher-magnification views of laminae 1 and 2 (from parts *A2* and *B2*) showing reductions in neuropil staining. Dotted line corresponds to the edge of the tissue. Calibrations: *A1*, *A2*, *B1*, *B2*: 1 mm; *A3*, *B3*: 100 μm

FIG. 3.

Kinematic features of ladder stepping. *A*: averaged stick figures for 3 frames preceding rung contact (thin lines) and frame of rung contact (thick line) for a muscimol-treated cat (cat 2). Digit paths for 15 trials are shown below the averaged stick figures. Limb contralateral to inactivation oversteps the rung compared with the ipsilateral limb. *B*: averaged stick figures (*above*) and digit paths (*below*) for the right and left forelimbs of control cat (cat 1). Stick figures and digit paths were similar for the 2 sides. Small squares are the ladder rungs drawn to scale. Gray bar corresponds to the distance between ladder rungs (5.8 cm).

FIG. 4.

Histograms of step distances on the ladder task for an inactivated (A, X2) and noninfused control (B, C2). In X2, the forelimb contralateral to the infusion showed a broader distribution and a greater average step distance than the ipsilateral forelimb. Trials in which the limb slipped off the ladder rung are shown in light gray, whereas trials without slips are shown in dark gray. In the control cat, the step distributions were symmetrical for the right and left forelimbs. *Inset*: placement of the forepaw on a ladder rung. Double arrow indicates the step distance measure that we used for the analysis in this figure and the analyses in Figs. 4 and 5.

FIG. 5.

Mean step distances for muscimol-infused cats ($n = 5$) and control cats ($n = 2$; age-matched and saline infusion) on the horizontal ladder task. Limbs contralateral and ipsilateral to muscimol or saline infusion are indicated. For the age-matched control, the right limb is considered contralateral and the left, ipsilateral. Overstep distance was significantly greater for the forepaw contralateral to muscimol infusion for 4 of the 5 inactivated cats (muscimol cat 1: $P < 0.0001$; 2: $P < 0.0001$; 3: $P < 0.083$; 4: $P < 0.0001$; 5: $P < 0.0001$). Forward distance was not significantly different for the contralateral and ipsilateral paws in the control cats.

FIG. 6.

Mean forward distances for muscimol-infused cats ($n = 3$) for surprise trials in which the distance between ladder rungs was changed. Normal rung distance is 6 cm (data replotted from Fig. 4). On random trials the one rung distance was increased to 12 cm. Overstep distance for the 12-cm rung was significantly greater for the contralateral than for the ipsilateral limb (cat 1: $P < 0.0001$; 2: $P < 0.0001$), but was not significantly different from the overstep distance for the 6-cm rung distance.

FIG. 7. Step heights over the obstacle (A1, B1) and forward distances after the cat stepped over the obstacle (A2, B2) are shown for the 3 cats examined in the treadmill task (cat 1 corresponds to M3 in Table 1; cat 2, to M4; cat 3, to M2). Data from the leading (A) and trailing (B) limbs are plotted separately. Schematics (bottom) show cartoons of the leading limb (thick gray line; left) stepping over the obstacle and the trailing limb (thick gray line; right). ANOVAs for step height reveal no significant differences for the contralateral and ipsilateral limbs. ANOVA for the distance after for the leading limb was significant ($P < 0.0001$). P values for post hoc t -test for the individual cats are shown on the graphs. ANOVA for the distance after for the trailing limb did not show a significant difference for the 2 limbs.

FIG. 8.

Trajectory analysis during treadmill locomotion. Data from the active (control) limb are shown in the *left column*, the inactivated limb, in the *middle column*, and the 2 are overlaid in the *right column* (red, inactivated; black, control). *A* and *C*: paw paths from each of the cats used in this analysis. Trajectories are all aligned with the obstacle (zero value on the abscissa) (*A*, $n = 16$ steps; *C*, $n = 20$ steps). *B* and *C*: normalized trajectories (see text). Solid red lines are ensemble averages of the normalized trajectories ($\pm$ SE, dotted red lines). Overlaid normalized trajectories ($\pm$ SE) for the control (gray) and inactivated (red) sides for each cat.

TABLE 1

Summary of animals used in this study

Cat	Treatment	Infusion Period, Postnatal, wk	Ladder, Postnatal, wk	Treadmill Postnatal, wk
M1	Muscimol	5–7		
	Infusion		8–15	
M2	Muscimol	5–7		
	Infusion		8–15	25–26
M3	Muscimol	5–7		
	Infusion		9–13	15–16
M4	Muscimol	5–7		
	Infusion		13–20	20–22
M5	Muscimol	5–7		
	Infusion		8–25	
C1	None		11–16	
C2	Saline infusion	5–7	8–15	

Postnatal weeks are listed during which muscimol or saline was infused into M1, when we tested animals in the ladder task and when we tested animals in the treadmill task. Note that control animal C1 received no infusions.