INTRODUCTION

There are multiple postnatal developmental periods during which there is progressively greater corticospinal (CS) connectional specificity, cortical motor functional capacity, and motor performance capabilities (Eyre et al. 2000; Flament et al. 1992; Li and Martin 2001; Olivier et al. 1997; for review, see Porter and Lemon 1993). In several species, connectional specificity is achieved partly by eliminating spinal terminations because CS axons terminate in the gray matter early in development but in maturity (Alisky et al. 1992; Martin et al. 1999; Theriault and Tatton 1989). In addition to— or in place of—axon terminal elimination, there is also extensive local CS axon terminal growth (Armand et al. 1997; Li and Martin 2001, 2002). Together, the combined elimination and addition of terminals could lead to development of the capability of the CS system to drive spinal motor circuits for controlling skilled movements.

Early postnatal life is thus a dynamic time for development of the CS system and ascendency to its important role in controlling skilled movements. Not surprisingly, this is also a time of transition, from when CS functions are not outwardly expressed, either physiologically or behaviorally, to when the same experimental manipulations point to a clear role for the CS system in activating spinal motor circuits for producing muscle contraction. For example, motor cortex stimulation in both cat and monkey—either electrical or transcranial magnetic stimulation (TMS)—fails to produce motor effects early in development before CS termination refinement (Alisky et al. 1992; Armand et al. 1997; Martin et al. 1999; Theriault and Tatton 1989). But immediately thereafter, stimulation of motor cortex produces muscle contraction (Bruce and Tatton 1980; Chakrabarty and Martin 2000; Flament et al. 1992; Olivier et al. 1997). In the monkey, the expression of relatively independent finger movements—a measure of CS function—also coincides with achieving the mature topographic pattern of terminations (Armand et al. 1997). The effects of CS system stimulation in humans during early postnatal development are similar to those in cat and monkey. Generally there are heightened thresholds for evoking movements using TMS, and motor effects are inconsistent during the first year or two of postnatal development. This is before acquisition of relatively independent finger movements (Eyre et al. 2000; Porter and Lemon 1993). This is followed by a later postnatal drop in TMS threshold (Eyre et al. 2000), more consistent responding to TMS stimulation (Nezu et al. 1997), and a correlated improvement in hand skills (Eyre et al. 2000). In the cat, lesions or inactivation of the motor cortex before CS termination refinement produces minimal motor control disturbances but robust effects immediately thereafter (Armand and Kably 1992; Martin et al. 1999, 2000). What could account for these differences in expression of CS function that occur just before and after the period of topographic refinement of CS terminals? While the presence of compensatory mechanisms or maturation of motivational and cognitive systems may play a role, we have been struck by the possibility that the CS system is incapable of exciting spinal motor circuits to a sufficient degree for controlling limb movements until their axon terminals are refined topographically.

While development of the capacity to drive spinal neurons is certainly important for the CS system’s essential role in skilled movement control, it is also critical for refining the topography of its spinal terminations. We showed that CS activity blockade, by intracortical infusion of the GABA agonist muscimol, impairs development of CS terminations (Martin et al. 1999).
The silenced cortex fails to develop dense spinal terminations, whereas the active side develops both dense contralateral and ipsilateral terminations. The ipsilateral terminations of the active cortex are produced both by maintenance of transient terminals and new growth beyond the normal territory of transient terminations. Given this important role for activity in shaping development of CS terminations, it is all the more paradoxical that cortical motor functions are not apparent at early postnatal ages.

The principal purpose of this study was to determine if the delay in expression of cortical motor system function is due to the inability of the CS synapse to activate its postsynaptic spinal targets. We studied this question in the cat so that the results could be interpreted in relation to the time course of activity-dependent refinement, development of CS terminal morphology, and development of the cortical motor map (Chakrabarty and Martin 2000; Li and Martin 2000–2002; Martin and Lee 1999; Martin et al. 1999). To probe development of the capacity of CS terminations to evoke spinal postsynaptic responses, we bypassed the influence of motor cortical circuit development by stimulating CS axons directly in the medullary pyramid (PT). We recorded surface-evoked potentials from the spinal cord and local field potentials within the gray matter in response to PT stimulation. We chose these extracellular recording approaches for two reasons. First, these are classical population recording techniques that are effective in sampling the actions of ensembles of CS axon terminals and their postsynaptic targets (Baldissera et al. 1981; Maier et al. 1998). Second, because it is difficult to maintain acute neonatal spinal preparations, these extracellular techniques are efficient substitutes for the more physiologically invasive single-cell recordings requiring paralysis and artificial ventilation. We show that CS terminals are capable of driving spinal circuits well before anatomical specificity is achieved and before primary motor cortex stimulation is effective in producing motor responses. Given the importance of activity-dependent refinement of CS axon terminals, this capacity for activating spinal neurons during early postnatal life could play an important role in development of CS circuit connectivity. Some of the findings in this paper were presented in an abstract (Meng et al. 2002).

Methods

All cats used in this study (n = 19) were obtained from a supplier accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC, International). Kittens are defined as cats <16 wk old, which is the age of sexual maturity. Kittens less than postnatal week (PW) 6 at the time of arrival were delivered with a lactating female (biological or fostered mother). Kittens were housed in a group cage that exceeded the standards established by the USDA, with up to four animals of similar age. All adults were ≥2 yr old. Adults were housed individually. All experiments were conducted with the approval of the New York State Psychiatric Institute and Columbia University institutional animal care and use committees.

Surgical procedures

Anesthesia was induced with ketamine (30 mg/kg im) and xylazine (0.6–0.8 mg/kg im) and maintained using intravenous ketamine infusion (10–30 mg·kg⁻¹·h⁻¹). Wound margins were infiltrated with lidocaine (2%). This is the same anesthesia protocol used in our earlier study on development of the cortical motor map (Chakrabarty and Martin 2000). Using this anesthesia protocol, animals remained alert and mobile throughout surgery and testing. Animals were placed in a conventional stereotaxic frame. Body temperature was maintained at 39° by a heating pad. We performed a cervical laminectomy, exposing the dorsal surface of the cord from C₂ to C₈. After exposing the spinal cord, it was covered with warm mineral oil. The C₈ spinous process was clamped to stabilize the vertebral column. The combination of C₈ spinal fixation and placement of the head in the stereotaxic frame produced adequate stabilization of the cervical cord for recording surface and depth field potentials.

Pyramidal tract stimulation

A craniotomy was made over the posterior portion of the occipital bone to access the pyramid. In adults, an electrode was placed at standard stereotaxic anteroposterior (AP) and mediolateral (ML) coordinates (AP 13.5 ML 0.75 mm). In kittens, we adjusted the AP location so that the electrode was adjacent to the caudal edge of the occipital bone, at ML 0.5 mm. The electrode was inserted parallel to the vertical plane. This resulted in consistent PT stimulating electrode placement within the caudal medulla (see Fig. 1A).

We inserted a pair of concentric electrodes (separated by 2 mm, oriented in the sagittal plane; Microprobe) into the medulla to stimulate CS axons in the pyramid. We found that we were able to activate CS axons more effectively (i.e., produce larger spinal potentials) when we used a pair of electrodes (caudal electrode as anode) than a single electrode with the anode at the center and the cathode as the surround electrode. We lowered the electrodes to a depth at which contact occurred with the ventral surface of the occipital bone, just beneath the pyramid. The point of contact was noted under a dissecting scope as a small jarring motion of the electrodes. We then raised the electrodes between 0.5 and 1.5 mm, to a depth that evoked the largest surface potential at C₈ at the lowest stimulus amplitude. We used a constant current stimulator (AM System, Model No. 2100; biphasic stimulus 20–μs duration); current varied in relation to the animal’s age (see RESULTS). In all experiments, we determined the position of the electrodes histologically. In six experiments, we unilaterally sectioned the dorsolateral column of the spinal cord in the horizontal plane at C₈ while recording at C₈ to verify the conduction path for PT activation.

Spinal recordings and data acquisition

Surface potentials were recorded above the dorsal root entry zone in the center of the target spinal segment (C₆, C₇, and/or C₈) using a silver ball electrode. Depth recordings were made using a glass micropipette (tip diameter between 10 and 20 μm, filled with 4 M NaCl; 0.1–0.4 MΩ). The micropipette was mounted to a Kopf electrode carrier. The pipette was connected to a unity gain preamplifier, which in turn was connected to an AC-coupled amplifier (AM Systems, Model No. 1800). Filters cut signals <100 Hz and >10 kHz. In each experiment in which depth recordings were made, penetrations were made at 10 sites, equally spaced within the C₆ segment. We recorded at 500-μm-depth intervals from just beneath the pial surface to the a point within the ventral gray or white matter. The output of the amplifier was digitized on-line (Axograph, Digidata; Axon Instruments) and responses to individual stimuli or a set of 10 stimuli (as an ensemble average) were saved on disk for subsequent analysis (see RESULTS for particular measurements.) The records all show positive up. Data were collected at a sampling rate of 20 kHz, in 20-ms trials.

Data analysis

We used the program Axograph for the Apple Macintosh computer to measure the onset and amplitude of individual responses. The results of the analyses were used to create two databases. One con-
in situ and placed in 10% formal-saline or the brain and cervical spinal cord were removed. The animal was killed with an overdose of Histology

paired significance of age-related and site-related differences using an un-

concurrent surface record close to the penetration. We determined the depth recordings. Most depth recordings were accompanied by a
tained all data from the surface recordings and the other from the depth recordings. Most depth recordings were accompanied by a concurrent surface record close to the penetration. We determined the significance of age-related and site-related differences using an unpaired t-test, ANOVAs, and linear regressions using the program Statview. Specific analyses are described in RESULTS.

Histology

At the end of each experiment a lesion was made through the caudal PT stimulating electrode. The animal was killed with an overdose of anesthesia and either perfused transcardially with saline, followed by 10% formal-saline or the brain and cervical spinal cord were removed in situ and placed in fixative. After a suitable postfixation time, transverse sections were cut through the caudal medulla and either transverse or longitudinal sections were cut through the region of the spinal cord examined. Tissue was Nissl-stained and examined for the PT electrode lesion site and track, spinal recording sites, and spinal lesion sites. Depths in the spinal cord were measured in relation to landmarks (i.e., gray matter borders) or dye spots after tissue was mounted onto slides but before staining.

RESULTS

Pyramidal tract stimulation evoked a pair of short latency surface potentials at all ages

Experiments were conducted on 19 cats between postnatal week (PW) 4 and adult (PW4, n = 2; PW5, n = 2; PW6, n = 1; PW7, n = 1; PW8, n = 1; PW9, n = 2; PW10, n = 1; PW11, n = 1; PW13, n = 2; adult, n = 6) to determine age-dependent changes in spinal cord potentials produced by PT stimulation. Surface potentials were recorded in all cats; a laminar series throughout the gray matter was recorded in two PW4 animals, two adults, and in one of each of the cats at the following ages: 6, 8, 10, and 13 wk. Key developmental parameters are summarized in Table 1. We combined animals into three groups: weeks 4–5, which correspond to ages before CS topographic refinement; weeks 6–13, which correspond to kittens at ages after topographic refinement; and adults.

Stimulation over approximately a 1-mm dorsoventral distance (slightly less in the kitten) in the ventral medulla evoked short latency spinal potentials. Stimulation 1 mm ventral or dorsal to the pyramidal stimulation site either evoked no response or substantially smaller responses, often with a different configuration. Figure 1A is a Nissl-stained section through the ventral medulla in a PW8 animal showing the lesion produced at the tip of one of the stimulating electrodes within the PT. PT stimulation at all ages evoked two distinct potentials recorded from the surface of the cervical cord, at consistent latency (Fig. 1, B and C; PW5). While the onset of the first potential was typically obscured by the stimulus artifact, especially in older animals where the latency was shortest, the peak was discernable. Figure 1B shows ensemble averages of surface potentials (n = 10), aligned with the stimulus artifact and recorded from the fourth, fifth, and sixth cervical segments. The time to the peak of the first potential (arrows) and the onset of the second potential (arrows and vertical tic marks) increased from C4 (thick trace) to C6 (thin trace). This is consistent with an increasing conduction distance from C4 to C6. When the traces were aligned with the peak of the initial response (Fig. 1C), the

TABLE 1. Key developmental parameters

<table>
<thead>
<tr>
<th>Weeks 4–5</th>
<th>Weeks 6–13</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cats</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>PT threshold, mA</td>
<td>395 ± 10</td>
<td>230 ± 20</td>
</tr>
<tr>
<td>Conduction velocity, m/s</td>
<td>36 ± 4.83</td>
<td>59 ± 3.82</td>
</tr>
<tr>
<td>Conduction distance, mm</td>
<td>42 ± 2</td>
<td>54 ± 5</td>
</tr>
<tr>
<td>Time to peak volley, ms</td>
<td>1.007 ± 0.12</td>
<td>0.784 ± 0.03</td>
</tr>
<tr>
<td>volley fall time, ms</td>
<td>0.528 ± 0.04</td>
<td>0.368 ± 0.03</td>
</tr>
<tr>
<td>Conduction distance, ms</td>
<td>1.844 ± 0.15</td>
<td>1.338 ± 0.09</td>
</tr>
<tr>
<td>Conduction distance, ms</td>
<td>0.704 ± 0.07</td>
<td>0.815 ± 0.04</td>
</tr>
<tr>
<td>Segmental latency, ms</td>
<td>0.837 ± 0.07</td>
<td>0.554 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE.
onset times of the late response did not increase systematically with more distal segments (single arrow; vertical tic marks). This is consistent with a relatively constant conduction distance from the CS tract branch points in the lateral white matter at the different segments to the segmental gray matter terminations. We occasionally recorded small potentials near the end of the volley. These could be mediated by slowly conducting corticospinal axons, with a latency about twice that of the initial potential.

Figure 2A plots the time to peak of the first response, the measure used to monitor its latency, for all animals in which recordings were made at C4, C5, and C6. In all cases, the latency of the first response increased with increasing conduction distance \( (F = 67.24; P < 0.0001) \). The interval between the peak of the first and onset of the second potential is plotted in Fig. 2B for recordings at the three cervical levels. We used this interval as a measure of the intrasegmental conduction time, although it is likely also to include pre- and postsynaptic processes. Overall, there were no systematic changes in these values from C4 to C6 \( (F = 0.196; P = 0.6632) \), presumably because conduction distances (and times) remained relatively stable across segments. However, we did note a large decrease in latency in one PW5 animal and a small increase in the adult. These differences could reflect variations in the strength of activation resulting in different summation times, differences in conduction distance, and in conduction velocity of CS axon branches (Shinoda et al. 1976).

These large potentials were eliminated with lesion of the dorsolateral spinal white matter, the principal location of descending CS projection in the cat. After conducting the experiment in six cats, we acutely lesioned the dorsolateral column at C5 while continuing to record at C6. Ensemble averages of the C6 surface potentials recorded immediately before and after making the lesion are shown in Fig. 3, A and C. A Nissl-stained longitudinal section through the lesion is shown in B. The volley was substantially reduced and its configuration changed. The postsynaptic field response was eliminated.

Our findings are consistent with the first potential corresponding to the conducted CS tract volley and the second potential corresponding to the initial postsynaptic response in the gray matter. The two-potential pattern that we recorded is identical to what Elger and colleagues (Elger et al. 1977) recorded in the rat in response to motor cortex stimulation. In that study, the second potential occurred at the onset of monosynaptic excitation of spinal neurons. In the following discussion, we refer to the initial surface response (and corresponding depth records; see following text) as the volley and the later response, as the postsynaptic field potential.

**Postsynaptic field potentials were evoked by PT stimulation in both immature and mature cats**

Ensemble averages of surface potentials recorded at C6 at different postnatal ages are shown in Fig. 4. Potentials recorded at PW4 had substantially longer latencies than in older animals. We show data from two animals because the delay times

---

**FIG. 2.** Relationship between segmental recording level and time to peak of initial potential (A) and interval between volley peak and onset of second potential (B) at various postnatal ages.

FIG. 3. Effect of spinal cord lesion on segmental corticospinal (CS) potential in an 8-wk-old kitten. A: ensemble average \( (n = 10 \) stimuli) of spinal surface recording in response to PT stimulation prior to spinal lesion. B: montage of Nissl-stained longitudinal section through the cervical spinal cord rostral to the recording site. Inset: a schematic diagram of a longitudinal section through the spinal cord; gray bands correspond to gray matter and white bands, white matter. The rectangle corresponds to the micrograph montage. Arrow points to the spinal lesion. C: ensemble average \( (n = 10 \) stimuli) of spinal recording just after making the lesion. The time scale is aligned with stimulus onset; the stimulus artifact was eliminated from the illustration. Calibrations: A and C, 40 \( \mu \)V; B, 2 mm.
lateral white matter was \( \sim 400 \text{\,\mu m} \) at 4 wk and \( 1,700 \text{\,\mu m} \) in the adult. The amplitude of extracellular potentials decrease with distance. The tracings in Fig. 4 also show that there was late activity.

The graphs in Fig. 5 summarize the age-related changes in several stimulus and response parameters. A plots the current thresholds for evoking the spinal responses. There was a larger difference between the mean thresholds of animals \( \leq 5 \text{\,wk} \) and adults (251 mA) than between older kittens (6–13 wk) and adults (86 mA). Pair-wise comparison of data from animals in these three groups revealed significant differences between each (\( \leq 5 \text{\,wk} \) and 6–13 wk: \( t = 5.30; P = 0.0003; \leq 5 \text{\,wk} \) and adults: \( t = 39.76; P < 0.0001; 6–13 \text{\,wk} \) and adults: \( t = 3.1; P = 0.008 \)). We found that conduction velocity (Fig. 5B) increased continuously (for the 3 groups in Table 1, \( F = 45.001; P < 0.0001 \)). While in the oldest kitten conduction velocity was not as fast as in adults, by other measures (see following text), the oldest kitten appeared to have a mature CS system. The reduction in stimulation threshold (in mA) for evoking spinal potentials (Fig. 5A) and the increase in CS tract conduction velocity are both likely to be due to increased myelination. These changes are similar to those reported, using TMS, in monkey (Eyre et al. 2000; Flament et al. 1992; Olivier et al. 1997) and human (Eyre et al. 2000; Koh and Eyre 1988).

Although difficult to substantiate in this small sample, volley latency (Fig. 5C) seemed to remain relatively constant after...
week 4. For example, latencies in the two oldest kittens were comparable to those of the adults while conduction velocity was only 60–80%. Moreover, several of the kittens between 5 and 13 wk had volleys of similar latency despite considerable intervening growth (i.e., conduction distance; \( E \)). Between weeks 5 and 13 there was only a 22% reduction in latency despite a 41% increase in conduction path. This suggests relative constancy of central conduction time. On the other hand, the latency to the onset of the postsynaptic surface field potential (Fig. 5D) decreased gradually. Our findings indicate, for all postnatal ages and adults, the presence of a robust postsynaptic field potential and either a stepwise or gradual maturation of many CS electrophysiological characteristics.

**Laminar analysis of local field potentials**

We next examined local field potentials from the surface of the cord to the ventral gray matter in response to PT stimulation in animals of different ages. There were two purposes for these depth recordings. First, we determined the change in the amplitude of the field potentials at the various depths as a measure of the local gray matter territories that could be influenced by CS activation at different postnatal ages. Second, we determined if there was a consistent relationship between the surface and depth recordings, to obtain insight into the generators of the postsynaptic response. For each animal, we made 10 penetrations, equally spaced within C6. Within each penetration, we recorded at 500-μm intervals, from the surface into the ventral horn. In some cases, the deepest recordings were in the anterolateral white matter.

Figure 6 (left) shows a transverse section through C6 in a PW8 kitten. During the experiment, we marked two depths with dye (1,000 and 3,000 μm). Each tracing in the panel on the right is the average of recordings at the 10 different rostrocaudal sites within C6 at the stated depth. For example, the trace marked “surface” is the ensemble average of surface recordings at the 10 sites. The purpose of using averages from sites distributed along the segment was to estimate more accurately the pattern of CS activation within the segment. As we show in the following text, a slightly different pattern of potentials was recorded in a minority of penetrations. These averaged recordings, as in the other kittens and adults, revealed a multiphasic potential. And as discussed in the following text, the dorsoventral distribution varied with age.

The first two phases (positive-negative; Fig. 6; light gray shading) occurred during the surface volley, and the second two phases (positive-negative; Fig. 6; dark gray shading), during the surface-recorded postsynaptic response. Based on classical field potential analysis (Lorente de Nó 1947), the initial positive-negative potential is consistent with recording local outward conductances (i.e., current source) as impulses are distant to the pipette tip, followed by local inward conductances (i.e., current sink) as impulses are conducted toward and recorded at the tip of the pipette. These local inward conductances would predominantly correspond, in sequence, to the impulses conducted along local preterminal axons, their subsequent depolarization, and early postsynaptic depolarization. The subsequent potentials (dark gray shading; positive-negative), based on their longer latency, reflect postsynaptic actions (presumably both excitation and inhibition). In this, and other kittens (≥10 wk), there was a conspicuous late phase negative potential (blackened panel) that occurred during the surface recorded postsynaptic field response. This potential inverts dorsally and ventrally, suggesting that it is a depolarizing potential with a distribution shown by the blackened rectangle. This negative potential had an age-dependent dorsoventral distribution (see following text).

Figure 7 shows depth recordings from two kittens (4 and 6 wk old) and an adult. One major difference between the form of the evoked responses in kittens (including the one shown in Fig. 6) and adults is that the late surface and deep potentials were roughly inverted (i.e., out of phase) in the kittens. This corresponded partly to the prominent negative response in the kittens (Fig. 6; black rectangle). In addition, in adults the duration of the surface volley was shorter so that the late depth potentials roughly paralleled the late surface potential (i.e., in phase). The significance of this latter difference is not clear.

**Age-dependent reduction in the dorsoventral distribution of depth potentials**

Across all experiments, there were two penetration classes. 84% showed substantial changes in the form of the field...
potentials across depths. Figure 7 shows data from this penetration type. In the 4 wk old animal (Fig. 7, left), large field potentials—negative, positive, and late negative—during the surface volley and postsynaptic field response were recorded at all but the deepest depth, or >90% of the dorsoventral extent of the gray matter. The ventral border of the ventral horn was at 3,510 μm; thus the deepest recording—where the potential changed configuration and size—was at the ventral border of the gray and white matter. The dorsoventral distribution of the large initial negative and subsequent positive and negative potentials was more restricted by week 6 (middle), being eliminated both superficially and deeper. In the adult (Fig. 7, right), potentials recorded from the most superficial and deep sites were small and invariant. However, within the intermediate layers the potential increased substantially. This increase occurred between 2,000 and 3,500 μm, which was ~35% of the dorsoventral extent of the gray matter in this animal. The ventral horn border was 5,270 μm. Similar results were obtained in the second adult subjected to a laminar analysis, where there was a qualitative increase in the amplitude of the second positive potential (i.e., positive peak within the darkly shaded region in Figs. 6–8; measured between the baseline and peak). We chose this potential to measure because it was clearly present at all ages. However, other phases of the potential form of the surface response. These sites were present in 6-, 8-, 10-, and 14-wk-old kittens. Similar to the typical sites, field potential amplitude varied, being largest within the middle layers, and the dorsoventral distribution was more restricted in the older than younger kittens (e.g., compare 7, left and right). However, for a given age, these sites had a more restricted dorsoventral distribution than the more typical sites. These sites may simply have sparse CS terminations, resulting in a limited focus of activation, or have matured earlier, resulting in a more restricted distribution. Adjacent penetrations recorded the typical kind of potentials, suggesting local anisotropy in the distribution of CS terminations.

The results of the depth analysis show a dorsoventral restriction in both the volley and postsynaptic field potentials. Because the occurrence of the volley and postsynaptic field potentials were tightly correlated for both typical (i.e., Figs. 6 and 7) and atypical (i.e., Fig. 8) sites, the volley was not merely recording impulses in the CS tract at a distance. Rather, the potential was due to local conductances associated with CS axon terminals.

As animals grew older, there was a striking restriction in the dorsoventral distribution over which large potentials could be recorded. We quantified this by measuring the amplitude of the second positive potential (i.e., positive peak within the darkly shaded region in Figs. 6–8; measured between the baseline and peak). We chose this potential to measure because it was clearly present at all ages. However, other phases of the potential form of the surface response. These sites were present in 6-, 8-, 10-, and 14-wk-old kittens. Similar to the typical sites, field potential amplitude varied, being largest within the middle layers, and the dorsoventral distribution was more restricted in the older than younger kittens (e.g., compare 7, left and right). However, for a given age, these sites had a more restricted dorsoventral distribution than the more typical sites. These sites may simply have sparse CS terminations, resulting in a limited focus of activation, or have matured earlier, resulting in a more restricted distribution. Adjacent penetrations recorded the typical kind of potentials, suggesting local anisotropy in the distribution of CS terminations.

The results of the depth analysis show a dorsoventral restriction in both the volley and postsynaptic field potentials. Because the occurrence of the volley and postsynaptic field potentials were tightly correlated for both typical (i.e., Figs. 6 and 7) and atypical (i.e., Fig. 8) sites, the volley was not merely recording impulses in the CS tract at a distance. Rather, the potential was due to local conductances associated with CS axon terminals.

As animals grew older, there was a striking restriction in the dorsoventral distribution over which large potentials could be recorded. We quantified this by measuring the amplitude of the second positive potential (i.e., positive peak within the darkly shaded region in Figs. 6–8; measured between the baseline and peak). We chose this potential to measure because it was clearly present at all ages. However, other phases of the potential form of the surface response. These sites were present in 6-, 8-, 10-, and 14-wk-old kittens. Similar to the typical sites, field potential amplitude varied, being largest within the middle layers, and the dorsoventral distribution was more restricted in the older than younger kittens (e.g., compare 7, left and right). However, for a given age, these sites had a more restricted dorsoventral distribution than the more typical sites. These sites may simply have sparse CS terminations, resulting in a limited focus of activation, or have matured earlier, resulting in a more restricted distribution. Adjacent penetrations recorded the typical kind of potentials, suggesting local anisotropy in the distribution of CS terminations.

The results of the depth analysis show a dorsoventral restriction in both the volley and postsynaptic field potentials. Because the occurrence of the volley and postsynaptic field potentials were tightly correlated for both typical (i.e., Figs. 6 and 7) and atypical (i.e., Fig. 8) sites, the volley was not merely recording impulses in the CS tract at a distance. Rather, the potential was due to local conductances associated with CS axon terminals.

As animals grew older, there was a striking restriction in the dorsoventral distribution over which large potentials could be recorded. We quantified this by measuring the amplitude of the second positive potential (i.e., positive peak within the darkly shaded region in Figs. 6–8; measured between the baseline and peak). We chose this potential to measure because it was clearly present at all ages. However, other phases of the potential form of the surface response. These sites were present in 6-, 8-, 10-, and 14-wk-old kittens. Similar to the typical sites, field potential amplitude varied, being largest within the middle layers, and the dorsoventral distribution was more restricted in the older than younger kittens (e.g., compare 7, left and right). However, for a given age, these sites had a more restricted dorsoventral distribution than the more typical sites. These sites may simply have sparse CS terminations, resulting in a limited focus of activation, or have matured earlier, resulting in a more restricted distribution. Adjacent penetrations recorded the typical kind of potentials, suggesting local anisotropy in the distribution of CS terminations.

The results of the depth analysis show a dorsoventral restriction in both the volley and postsynaptic field potentials. Because the occurrence of the volley and postsynaptic field potentials were tightly correlated for both typical (i.e., Figs. 6 and 7) and atypical (i.e., Fig. 8) sites, the volley was not merely recording impulses in the CS tract at a distance. Rather, the potential was due to local conductances associated with CS axon terminals.

As animals grew older, there was a striking restriction in the dorsoventral distribution over which large potentials could be recorded. We quantified this by measuring the amplitude of the second positive potential (i.e., positive peak within the darkly shaded region in Figs. 6–8; measured between the baseline and peak). We chose this potential to measure because it was clearly present at all ages. However, other phases of the potential form of the surface response. These sites were present in 6-, 8-, 10-, and 14-wk-old kittens. Similar to the typical sites, field potential amplitude varied, being largest within the middle layers, and the dorsoventral distribution was more restricted in the older than younger kittens (e.g., compare 7, left and right). However, for a given age, these sites had a more restricted dorsoventral distribution than the more typical sites.
tential also showed the same dorso-ventral changes, as can be seen in Figs. 6–8. Figure 9 plots the average values (across the 10 penetrations in each animal) of the potential, measured at 500-μm intervals. Because there was over a 50% increase in the size of the gray matter during development (e.g., at PW4, the gray matter was 2.8 mm but 4.3 mm in the adult), we normalized the depth measurements to the dimensions of the gray matter for each animal. The first recording (i.e., 0%) was at or close to the dorsal border of the gray matter, whereas the deepest recordings were at or close to the ventral border (100%). For a given animal, the largest potentials at any age were within the deeper laminae of the dorsal horn and the intermediate zone. This corresponds approximately to depths between 40 and 60%. Only in the oldest kitten (13 wk) and the adult was the focus of activation sharply restricted. This redistribution could reflect both the elimination of branches and local growth. This redistribution could not, however, be explained by the age-dependent increase in the size of the spinal cord. Although the gray matter was only 2% larger at week 13 than week 10, there was a qualitative change in the distribution of field potential amplitude, from broad to focused. In contrast, the area of the gray matter was 18% larger in the adult than the week 13 animal, yet the distributions of field potential amplitudes had a similar focus in the intermediate zone. These results show that there is a developmental restriction in the distribution of physiological effects of CS axon terminals.

**DISCUSSION**

Our findings show that CS synapses are capable of activating spinal circuits from early postnatal ages at times when measures of CS function that are effective in mature cats, such as robust effects of motor cortex stimulation, indicate a lack of function in the neonate. In 4- and 5-wk-old animals, PT stimulation produced postsynaptic potentials from the superficial dorsal horn to the base of the ventral horn. This shows that CS terminals have the capacity to activate spinal circuits throughout the entire gray matter not only the motor laminae of the intermediate zone and ventral horn. In older kittens, the spatial activation territory became highly restricted dorsoventrally. This reduction parallels anatomical refinement (Li and Martin 2000–2002). We propose that CS synapses are not transmitting information for specifying muscle control at young postnatal ages, before the time when cortical stimulation in the cat produces motor effects (Bruce and Tatton 1980; Chakrabarty and Martin 2000). Although the lack of stimulation efficacy could be due to several factors, such as poor synchronization of descending signals or a reduced capacity of spinal neurons to integrate CS inputs, this nonetheless shows that the CS system cannot sufficiently drive spinal motor circuits to produce muscle contraction. We reason that if cortical burst stimulation (Chakrabarty and Martin 2000) or TMS (Flлемент et al. 1992; Olivier et al. 1997) fails to produce muscle contraction at young ages, then the naturally occurring discharges of CS neurons would be less able to do so because they are apt to be desynchronized by comparison. What then could the role be for CS synaptic activity? We propose that the capability of developing CS terminals to activate spinal neurons at this age is important for shaping early development of the topography of CS terminations. We have previously shown that CS neural activity during early postnatal life is important for the normal development of the topography of CS terminations (Martin et al. 1999) through an activity-dependent competition mechanism (Martin and Lee 1999). Sensory-motor cortex activity blockade disrupts development of the normal topography of CS terminations (Martin et al. 1999; Martin and Lee 1999) and prehension skills (Martin et al. 2000). With CS synapses capable of exciting spinal neurons during early postnatal life, activity during a brief postnatal period can have long-term consequences for constructing spinal motor circuits. A role in activity-dependent development does not necessarily preclude additional roles in other spinal functions. Indeed, as in the visual system where activity is presumably both shaping development and providing visual inputs to the brain (Goodman and Shatz 1993), CS activity may have important spinal functions in the neonate, such as providing excitability to segmental circuits to promote reflex and locomotor functions. Developing CS terminals may also play a role in refining the connections of other spinal systems. Recently Clowry and coworkers (Gibson et al. 2000) showed that early postnatal motor cortex lesion in the rat produced an increase in muscle afferent terminations in the ventral horn, suggesting interactions between developing cortical and spinal afferents.

**Relationship of changes in CS evoked responses to anatomical refinement**

All of our depth records showed a consistent association between the amplitude of the initial positive-negative phase, which is associated with presynaptic activation and the local postsynaptic potentials (both early positivity and late negativity). As the first negative potential grew in amplitude with

![FIG. 9. Change in amplitude of local postsynaptic field potential in relation to depth within the C6 gray matter. All depths are normalized to the depth of the ventral border of the ventral horn. Each data point is an average of recordings made at 10 locations within C6, each located ~500 μm apart. Amplitude scale in μV. Lines plot mean ± SE.](http://jn.physiology.org/Downloadedfrom)
depth, so too did the subsequent positive and second negative potentials. In a minority of sites in young and old animals, there were depths, both superficial and deep, where neither an early negative potential nor the late postsynaptic potential were recorded. This demonstrates that the extracellular potentials that we recorded were very local. If not, then we would have recorded the CS tract volley at all depths.

For the most common sites (i.e., where early biphasic potentials were recorded; Figs. 6 and 7), we propose that the initial negative phase measures primarily action potentials and depolarization in preterminal and terminal axons. This is because the potential was brief—consistent with summation of synchronous action potential currents—and negative—consistent with a local inward current (i.e., sink). The distribution of the amplitude of the potential along the dorsoventral axis correlated well with refinement of CS terminations examined anatomically. At 4 wk, CS terminals have an extensive dorsal-ventral termination pattern, being present from the superficial dorsal horn to the base of the ventral horn, including the motor nuclei (Li and Martin 2000, 2002). This is similar to the distribution of large-amplitude potentials shown in Figs. 6 and 7. By 6 wk, there is substantial anatomic refinement, so that terminations within the superficial dorsal horn and the motor nuclei are eliminated (Li and Martin 2000, 2001). In the present study, many sites in older kittens showed a restricted distribution of pre- and postsynaptic field potentials along the dorsoventral axis (e.g., Fig. 7). However, overall physiological refinement appeared to be incomplete at this age (e.g., Fig. 9) because there was further refinement into week 10. The distribution of the large potentials along the dorsoventral axis at week 13 and the adult was highly refined (e.g., Figs. 7–9) because the large increase in the relative size of the potentials was present only in the intermediate zone. This increase is likely to be partly due to the increase in local branching and presynaptic site development that occurs later in development (Li and Martin 2001, 2002). It is interesting that large potentials were recorded deep into the ventral horn only in the 4- and 5-wk-old animals. We reported that there are CS terminations within the motor nuclei in immature animals (weeks 4–5) (Li and Martin 2000), although these terminations are not maintained into maturity.

Role of myelination in maturation of CS function

Traditionally, the importance of myelination of corticospinal axons has been stressed as key to development of cortical motor function. Myelination is essential for rapid and synchronous activation of spinal neuronal populations, which are certainly important for proper CS function. While adequate myelination is a requirement for functional development, else spatial and temporal summation cannot take place, it is not sufficient. Development of the CS terminal—axon branching and presynaptic density—is obviously key. In the cat, we have observed a good correlation between development of limb motor control and CS axon terminal morphology (e.g., Li and Martin 2001). Prior to PW8, which is before topographic specificity is achieved, kittens have impoverished forelimb motor skills. After week 8, skill is noticeably greater; this is that age that we can begin to train kittens to reach (unpublished observations; Martin et al. 2000). Clearly this improvement is not due to CS myelination, which is well developed by week 4 (Oka et al. 1985). Presynaptic corticospinal site development has not yet been examined in the primate.

In the present study, we were surprised that the CS volley remained well synchronized throughout the cervical cord at all ages. As shown in the averages in Fig. 1B, for a 5-wk-old animal, there was no noticeable prolongation in the duration of the volley with conduction distance. Similarly, surface and depth recordings (e.g., Figs. 4 and 7) revealed little shortening with age. We were also struck by the difference between the age dependence of volley and postsynaptic latency (Fig. 5). While the postsynaptic latency showed a progressive decrease, volley latency was more abrupt. Between PW5 and -13 there was little change in volley latency despite a large increase in conduction distance. This is reminiscent of the findings of Eyre and colleagues (Eyre et al. 1991) using TMS in humans, showing constancy of central conduction delay during development. This phenomenon is also present in the monkey, using TMS (Olivier et al. 1997). Constancy of central conduction delay is thought to be a way for the supraspinal motor systems to better predict the time at which their control signals act at the cord while the body is growing (Eyre et al. 1991).

Role of development of intrinsic spinal circuits in maturation of CS postsynaptic responses

Development of the spinal postsynaptic responses reflect both the efficacy of CS synaptic transmission and the response of spinal target neurons to this input. Unfortunately little relevant information is available about development of the spinal interneuronal circuits that develop CS terminals engage. However, the spinal cord is quite mature at 4 wk because young kittens are capable of a variety of limb and postural reflexes and can locomote (Levine et al. 1980), albeit clumsily. Motoneurons have developed their mature electrophysiological characteristics and limb muscle has mature properties (Bagust et al. 1973, 1974; Buller et al. 1960). This mature state of the spinal cord (and skeletal muscle) reflects the fact that the brain stem motor pathways and primary afferent fibers have developed their spinal terminations before this age. Despite relative maturity of the spinal cord during the time that the CS system is refining its spinal terminations, local circuits may continue to be capable of plastic changes, especially synaptic strength (Iriki et al. 1990). However, more needs to be learned about spinal motor circuit plasticity before we can partition changes due to the CS synaptic action and intrinsic spinal neurons.

Distinct early CS developmental periods for refining axon terminations and for steering motor output

Our findings point to a nonmotor function for CS activity during early postnatal development. Interestingly, data in the human also suggest that CS terminals can activate spinal neurons before development of the sorts of distal skills that are generally attributed to CS control. Eyre and colleagues (2000, 2001) have recently reported that TMS evoked motor responses in preterm and term infants before the usual 1–2 yr of age (e.g., Koh and Eyre 1988; Nezu et al. 1997). Surprisingly, they found an early time window, <3 mo of age, during which TMS thresholds were slightly lower than in older neonates (Eyre et al. 2001). Afterwards, threshold decreased with age. Using an immunological marker (GAP-43) on autopsy mate-
rial, they proposed that CS terminals were present on motoneurons at preterm ages (Eyre et al. 2000). However, both our present findings and those of Eyre and colleagues, showing spinal activation at early CS developmental stages, should not be taken to mean that the CS system is poised to deliver control signals to the spinal cord for producing muscle contraction at very young ages. On the contrary, PT electrical stimulation and TMS produce highly synchronous motor system activation. Natural output from motor cortex is much less synchronous and therefore much less effective in exciting spinal circuits and motor output. Nevertheless the ability to weakly excite spinal neurons (but without muscle contraction) could lead to increased synaptic strength, through Hebbian or other forms of associative plasticity (Bailey et al. 2000).

During early postnatal development, CS terminals—like those of some developing sensory systems and the neuromuscular junction (Goodman and Shatz 1993)—undergo activity-dependent refinement (Martin et al. 1999). Through pruning and accretion, the optimal topography and density of CS presynaptic sites becomes gradually specified (Li and Martin 2001, 2002). This process could lead to enhancing synaptic strength sufficiently for the CS system to effectively control particular spinal circuits.

We thank S. Edgley and J. Nielsen for advice recording corticospinal field potentials, X.-L. Wu for histology and help during experiments, M. Choy for help with data analysis, and Dr. Mo Osman and G. Asfaw for veterinary care.

DISCLOSURE

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

REFERENCES


