Synapse Elimination in the Corticospinal Projection During the Early Postnatal Period

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Synapse elimination in the corticospinal projection during the early postnatal period. J Neurophysiol 95: 2304–2313, 2006. First published November 2, 2005; doi:10.1152/jn.00295.2005. In corticospinal synapses reconstructed in vitro by slice co-culture, we previously showed that the synapses were distributed across the gray matter at 6–7 days in vitro (DIV). Thereafter, they began to be eliminated from the ventral side, and dorsal-dominant distribution was nearly complete at 11–12 DIV. The synapse elimination is associated with retraction of the corticospinal (CS) terminals. We studied whether this specific type of synapse elimination is a physiological phenomenon rather than in vitro artifact. The rat studied whether this unique synapse elimination occurs in vitro by means of slice co-cultures of rat sensorimotor cortex and spinal cord (Takuma et al. 2002). We reported that synapses were widely formed in the spinal gray matter at postnatal day 7 (P7). These negative fEPSPs reversed to positive in the most ventrolateral part at P8. Reversal extended to the more mediodorsal area at P10, indicative of progressive synapse elimination in the ventrolateral area. To verify that regression of the axons in vivo paralleled the changes in spatial distribution of fEPSPs as observed in vitro, corticospinal axons were anterogradely labeled. Redistribution of the labeled terminals closely paralleled the fEPSP distribution, being present in the ventrolateral spinal cord at P7, decreased at P8, further deceased at P10, but unchanged at P11. Furthermore, double immunostaining for labeled terminals and synaptophysin observed under a confocal microscope suggests that corticospinal fibers at P7 possess presynaptic structures in the ventrolateral area as well as the dorsomedial area. These findings suggest that corticospinal synapses are widely formed in the spinal gray matter at P7, are rapidly eliminated from the ventrolateral side from P8 to P10, a time-course very similar to that observed in vitro, and are associated with axonal regression.

INTRODUCTION

Knowledge of CNS development is derived mainly from intensive studies of sensory systems including the visual, barrel cortex, and olfactory bulb (Brunjes and Greer 2003; Foeller and Feldman 2004; Fox 2002; Katz and Crowley 2002; Lin and Ngai 1999; Purves and Lichtman 1985; Sanes et al. 2000). Relatively little is known about motor system development, however, particularly synapse formation in the corticospinal (CS) projection, a major efferent pathway from the cerebral cortex. Clarification of processes in CS synapse formation will facilitate an understanding not only neuronal circuit formation as well as the development of control of voluntary movement.

Rat CS axons enter the spinal cord at postnatal day 0 (P0) (Porter and Lemon 1993; Stanfield 1992), reach the lower cervical cord by P3, and extend to the end of the spinal cord by the end of the second postnatal week (Donatelle 1977; Gribnau et al. 1986; Jones et al. 1982). Almost all of the axons pass transiently through their target segments and then grow more caudally. After a “waiting period” of 2–4 days, they branch at the target segment and enter the spinal gray matter (Joosten et al. 1987; Kuang and Kalil 1994). Excess axons distal to the branch points are eliminated (O’Leary and Terashima 1988). Little is known, however, about what happens after CS axons enter the spinal gray matter or how synapses are formed.

Previously, we obtained CS synapse formation in vitro by means of slice co-cultures of rat sensorimotor cortex and spinal cord (Takuma et al. 2002). We reported that synapses were widely formed in the spinal cord at 7 days in vitro (DIV) and that synapses on the ventral side were eliminated by 11–12 DIV (Ohno and Sakurai 2005; Ohno et al. 2004). Axon reflex examinations and double labeling with retrograde tracers suggested that synapse elimination is induced by axon branch elimination rather than neuronal cell death. Furthermore, this elimination is activity- and N-methyl-D-aspartic acid (NMDA)–dependent, because it was blocked by the Na-channel blocker TTX and by the NMDA antagonist D-2-amino-5-phosphonovaleric acid (N-APV) (Ohno and Sakurai 2005; Ohno et al. 2004). Although in vitro studies are suitable for investigating the cellular and molecular mechanisms of developmental plasticity, in vivo studies are needed to understand the functional implications of plasticity, including its effect on movement.

We studied whether this unique synapse elimination occurs in vivo in the rat spinal cord. Field potential recordings were used to measure synapse formation and spatial distribution. Only a few electrophysiological studies have been performed on neonatal or early postnatal rats. Moreover, there has been no clear description of field synaptic potentials recorded from rat spinal cord after CS tract stimulation. We therefore studied these potentials in detail. Our previous in vitro study (Ohno et al. 2004) showed that the synapse elimination is associated with the retraction of CS terminals. For comparison with the previous study, we studied the developmental changes of CS terminals by anterograde labeling using biotinylated dextran amine (BDA).

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METHODS

Animals and surgical procedures in electrophysiological experiments

All animal experiments were performed in accordance with the Ethics Committee Guidelines and the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80–23) revised in 1996. All efforts were made to minimize the number of animals used and their suffering.

Wistar rats were anesthetized at P7–P10 by intraperitoneal pento-barbital sodium (40–60 mg/kg; Dainippon Pharmaceutical Co.) injection. An additional one-third to one-half of the first dose was added as required. After tracheotomy, the rats were fixed in a stereotaxic apparatus and ventilated artificially. A hole (1 mm diam) was made in the interparietal bone, just anterior to the external occipital crest and 0.2–0.5 mm lateral to the midline to insert a stimulating electrode into the medullary pyramid. The spinal cord was exposed from C3 to Th1 by laminectomy, and the dura was removed. The exposed spinal cord was covered with mineral oil to retain moisture. Rectal temperature was maintained at 32–35°C using a heating pad. This decreased temperature reduced the heart rate to ~300 beats/min to prevent the ECG from obliterating the electrical response.

Stimulation and recording

The experimental setup is shown schematically in Fig. 1. The medullary pyramid was stimulated with a handmade concentric bipolar electrode, a 27-G stainless steel needle threaded with Teflon-coated Pt-Ir wire. The stainless steel needle was insulated except for the tip and used as an anodal electrode. The needle was inserted through the hole in the interparietal bones and penetrated the medulla oblongata to the ventral surface of the basal part of the occipital bone, after which it was gradually pulled back to the pyramid to obtain the maximum response. Constant current stimulation pulses of 500-μA amplitude and 100-μs duration were applied, which elicited maximal field potentials for all rats studied. Stimulation was time-locked with the ECG, and the frequency was adjusted to 2–3 Hz. The ratio of the number of stimulations to heart rate was 1.2–3.

Field potential recordings were made in the cervical cord (C6), with single barrel glass pipettes filled with 3 M NaCl (0.5 M) at 200-μm intervals dorsoventrally and on three tracks mediolaterally (200–300 μm apart) on the transverse plane of the spinal cord (Fig. 1). Signals were amplified with a unity gain amplifier (CEZ-3100, Nihon Kohden) and an oscilloscope (VC-10, high gain amplifier AVM-10, Nihon Kohden). Signal acquisition and storage were performed with an AD converter (Digidata-1200B, Axon Instruments) and a PC computer. One hundred responses at each recording site were averaged on the computer with a filter bandwidth of 20–1,000 Hz.

Parameters of response were measured with a Clampfit 8 (Axon Instruments) and analyzed with Excel (Microsoft, Redmond, WA) and statistics software (SAS Institute, Cary, NC). The peak amplitude at each recording site in each animal was normalized by the amplitude of the largest negative response. To compare measurements obtained during different developmental stages, the transverse plane of the spinal cord was divided into 15 sites: 5 in each dorsoventral column and 3 in each mediolateral row, forming a 3 × 5 lattice. Data for the same divisions were compared using Turkey’s multiple comparison test.

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Topical drug application

A mixed solution (pH 7.1, 286 mOsm) of an 1-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 30 mM), and an NMDA receptor antagonist, D-APV (30 mM), was applied at the recording sites through one barrel of a double-barrel glass pipette for 30 s by pressure ejection. The volume was estimated to be ~0.2 μl, which was measured by ejecting the solution into a Hamilton syringe. The other barrel was used for the recordings. In some experiments, CoCl2 (100 mM, pH 7.1, 284 mOsm) was applied in the same way.

Anterograde labeling of corticospinal axon terminals

BDA (20%; MW 10,000; Molecular Probes, Eugene, OR) dissolved in PBS was used as the tracer. For the injection, we used glass pipettes with long-tapered shanks and bevelled tips to produce an orifice diameter of ~100 μm. Rats were anesthetized with isoflurane and mounted in a stereotaxic apparatus. A craniotomy was performed on the medial one-half of the frontal and parietal bone, and the sensorimotor cortex was exposed with the dura intact. The pipette containing BDA was lowered to a depth of 1 mm. After waiting for 1 min, a 0.5-μl injection was quickly made with a 10-μl Hamilton syringe, and the pipette was left there for 5 min before withdrawing it from the cortex. The injection was repeated at six separate sites, which varied from 0.7 to 1 mm to avoid surface blood vessels. Because the precise map of C5 neurons and their projection pattern in early postnatal rats is not known, the dye was widely injected into the area of sensorimotor cortex containing the C5 neurons projecting to C7 guided by the adult map (Paxinos and Watson 1998). To increase the number of labeled neurons, we injected the dye solution at a higher concentration and in a larger volume than usual (Veemana et al. 1992; Yang and Lemon 2003). The animals were returned to their dam’s cage after recovering from anesthesia.

Three days after injection, the rats were killed. Only the oldest group of rats (P11) were fixed 4 days after the injection. The animals were anesthetized with pentobarbital sodium (80 mg/kg) and perfused transcardially with PBS followed by a fixative [4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (PB)]. The brain and cervical spinal cord were removed and stored in the same fixative for 1–5 days. The C7 segment of the spinal cord was excised, and 50-μm serial sections were cut with a Mircroslicer (Dosaka EM, Kyoto, Japan). Free-floating sections were incubated overnight in peroxidase-conjugated avidin-biotin complex reagent (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) with 0.1% Triton X-100 (Sigma Chemical, St. Louis, MO) and 0.3% bovine serum albumin (Sigma Chemical) in PBS. After the sections were rinsed with 0.1 M PB, the reaction product was developed in saturated diamobenidine (DAB; Sigma Chemical), 0.1% glucose, 0.8% nickel ammonium sulfate, 0.004% ammonium chloride, and 0.5 units/ml β-D-glucose oxidase. After rinse, the sections were mounted on gelatin-coated slides, dehydrated, and covered with mounting medium. To quantitatively evaluate the spatial distribution of C5 axon terminals in the spinal cord, the numbers of labeled fibers crossing the dorsomedial and ventrolateral oblique lines (Fig. 6A) were counted in five slices/animal (n = 8) for each age group. Tukey’s multi-comparison test was performed to compare the results among ages. The labeled fibers were traced manually on the computer screen (Fig. 6).

FIG. 1. Experimental setup for stimulation and recording. Stimulating electrode was inserted in the medullary pyramid dorsally and the recording electrode in the spinal cord (C7) 200, 500, and 800 μm lateral to the midline. Recordings were made at 200-μm intervals ventrodorsally. In the transverse view of the spinal cord, solid lines represent electrode tracks; crosses represent recording sites.
Double-labeling of corticospinal axons and synaptophysin

The surgical methods, BDA injection, fixation, and sectioning were the same as described above with some exceptions. Tissues were fixed with 4% paraformaldehyde and maintained in the same fixative overnight. Free-floating sections of 30 μm thick were treated with 0.1% Triton X-100 in PBS overnight and then with 5% skim milk in PBS at room temperature for 1 h (Li and Strittmatter 2003). Thereafter, they were incubated with anti-synaptophysin (diluted 1:100; Chemicon, Temecula, CA) at 4°C for 2 h. The sections were washed and incubated in a solution of fluorescein-conjugated secondary antibody (diluted 1:100; goat anti-mouse IgG, Pierce, Rockford, IL) and Texas red conjugated Avidin (6.6 μg/ml; Molecular Probes) at 4°C for 1 h and mounted on gelatin-coated slides (Li and Strittmatter 2003). Sections were analyzed with confocal fluorescence microscopy (MRC-1024, BioRad, Hercules, CA) and its software (LaserSharp2000, BioRad). The co-localization of the terminals and synaptophysin signals were analyzed on a single optical section.

RESULTS

Synaptic responses evoked by pyramid stimulation

The tip of the stimulating electrode was confirmed to be in the medullary pyramid by passing DC current (100 μA, 7 s) through it at the end of the experiment, and the lesion was later identified in frozen cresyl violet–stained sections (10 μm, n = 7, Fig. 2C2).

A single stimulation evoked a negative field potential that was recorded in the dorsomedial area adjacent to the ventralmost part of the dorsal column where the rat CS tract is located. A typical negative response recorded from one recording site of one animal at P10 is shown in Fig. 2A. Each trace (black), i.e., the response evoked by a single stimulation were all nearly constant: onset latency (14.3 ± 0.88 (SD) ms), peak latency (19.1 ± 0.85 ms), amplitude (−20.6 ± 3.9 μV), rate of rise (−8.08 ± 3.2 mV/s), and half-width (4.63 ± 1.06 ms). These steady responses
over 100 stimulations clearly matched monosynaptic responses. The smallest stimulus intensity that evoked a response was 100 μA. The relationship between stimulus intensity and amplitude of response is shown in Fig. 2B. Responses disappeared when the stimulating electrode was moved outside of the pyramid (600 μm dorsal) or an electrolytic lesion was made within the pyramid by DC current (Fig. 2, C and D). There was no unit activity observed in any recordings.

Fiber volleys of the CS tract were not clearly observed from C3 to C7. Therefore the conduction velocity (CV) was estimated by measuring the time differences in the first positive peaks of the response recorded at the site at which the largest negative peak was observed at C7 and segments 3 or 5 mm rostral to C7, which corresponded to C3 or C5, and at the same laterality and depth as points at C7. The estimated CV ranged from 0.85 to 1.21 m/s from P7 to P10.

Application of a mixed solution of CNQX and D-APV or Co2⁺ solution at the site of a recorded negative response greatly reduced response amplitude (Fig. 2, E1 and E2); CNQX-APV, 63.2 ± 12.6% (difference between the amplitude before and after drug application/the amplitude before application, n = 3, P7); Co2⁺, 57.4 ± 20.3% (n = 4, P7). Ten minutes after the end of Co2⁺ ejection, the response was restored (Fig. 2E2). Blockade by CNQX and D-APV, however, showed little recovery for ≥60 min (Fig. 2E1). Because the responses were not affected by vehicle (Fig. 2E3), the effect of these antagonists was not produced by compression because of liquid volume, pH, or osmolality change, but by blocking of the glutamate receptors. Slow clearance of these drugs from nervous tissue, CNQX in particular, might explain little recovery of response during stable recording periods (Mizuno et al. 2001). The block by CNQX and D-APV, or Co2⁺ are evidence that the response represents synaptic activity. These findings, together with the constancy of the responses evoked by single pulse stimulations, indicate that the negative response is caused by monosynaptic fEPSPs produced by CS synapses.
Developmental redistribution of fEPSPs

At P7, only negative potentials (Figs. 3A and 4A) were evoked over the entire spinal cord. The negative potential with the largest amplitude was recorded in the dorsal area next to the ventralmost part of the dorsal column ($-11.9 \pm 4.9 \mu V, n = 7$; Fig. 3). Time differences between the peaks of the largest negative potential recorded in the dorsal part (31.4 ± 5.4 ms) and the most ventrolateral area (34.2 ± 6.6 ms) ranged from 1.2 to 7.9 ms (median 2.8 ms). The straight distances between these two points were from 720 to 1,170 µm. This delay primarily is considered to be caused by slow conduction through nonmyelinated intrasegmental fibers.

At P8, negative potentials disappeared in the most ventral side. Instead, positive potentials with shorter latencies were recorded that were nearly identical to the negative peaks recorded in the dorsal area; the latency for all the negative peaks were 23.5 ± 1.4 ms ($n = 91$ in 7 animals) and for the positive ones were 24.5 ± 1.7 ms ($n = 22$ in 7 animals; Figs. 3B and 4B). The coincidence of the negative and positive peaks and phase reversal along the dorsomedial-ventrolateral axes suggest that the negative response corresponds to an active current sink produced by corticospinal synaptic activity and that the concurrent positive potential is a passive current source. This indicates that synapses once formed in the ventrolateral area are eliminated and that this area, in turn, provides current sources for the synaptic activity in the dorsomedial area.

The distribution of positive potentials spread to a more dorsal area at P9, and negative potentials were limited more to the dorsal side than at P8 (Figs. 3C and 4C). Peak latencies of the all negative (23.9 ± 1.8 ms, $n = 88$ in 7 animals) and positive (24.0 ± 1.8 ms, $n = 39$ in 7 animals) responses were nearly the same as those at P8. Redistribution seemed nearly complete at P9. At P10, the response distribution was much the same as at P9 (Figs. 3D and 4D). Negative responses were elicited only in the dorsomedial area, with peak latencies (23.8 ± 1.9 ms, $n = 94$ in 7 animals) that were nearly coincident with the positive responses in the ventrolateral areas (24.9 ± 2.8 ms, $n = 71$ in 7 animals). Spatiotemporal changes in potential distributions from P7 to P10 suggest that CS synapses are eliminated from the ventrolateral side from P8 to P10 (Fig. 4E, see also Supplementary Figure1).

Developmental redistribution of CS terminals

To confirm our interpretation of the electrophysiological results indicating that ventrolateral synapses are eliminated, CS terminals were labeled anterogradely at P7, P8, P10, and P11 by injection of BDA to the sensorimotor cortex, which clearly stains axon terminals (Veenman et al. 1992). A schematic of the area of the injection sites on the sensorimotor cortex is shown in Fig. 5A. The cortical sections containing the injection sites are shown in Fig. 5B.

CS axons entered from the most ventral part of the dorsal column and extended radially to the gray matter (Fig. 6). At P7, these fibers had reached the end of the gray matter in the dorsal and ventral horns. Fiber density in the deep dorsal horn was greater than elsewhere. At P8, fewer fibers reached the ventrolateral part, whereas the density in the dorsal horn was even greater. Ventrolateral fibers further decreased at P10 and P11.

Varicosities were observed in the ventrolateral area (Fig. 7A). Furthermore, confocal microscopy of the double immu-
nostaining with synaptophysin and CS terminals (optical section: 0.295–0.734 μm thickness, 8–17 sections) revealed synaptophysin signals in the CS terminals in the ventrolateral area as well as in the dorsomedial area at P7 (27 double-labeling signals in 24 images from 12 sections, 30 μm thickness, 4 sections/animal, 3 animals; Fig. 7, B–F).

To quantitatively evaluate the number of terminals distributed in the ventrolateral and dorsomedial areas, we counted the fibers that crossed two drawn oblique lines (Fig. 6 A). The number of fibers crossing the ventrolateral line decreased significantly during P7–P10 (12.5 ± 3.0 at P7, n = 7; 7.8 ± 2.3 at P8, n = 7; 4.6 ± 2.0 at P10, n = 7; 4.6 ± 2.6 at P11, n = 7; P < 0.05, P7 vs. P8, P10, or P11; Fig. 8), whereas the number crossing the dorsomedial line did not significantly differ for any comparison (P7, 51.6 ± 16; P8, 49.6 ± 13.5; P10, 43.3 ± 10.8; P11, 47.5 ± 14.4; Fig. 8). The ratios of fibers crossing the ventral and dorsal lines therefore also decreased significantly (P7, 26.5 ± 8.2; P8, 16.2 ± 4.1; P10, 11.0 ± 4.6; P11, 10.6 ± 6.5; P < 0.05, P7 vs. P8, P10, or P11; Fig. 8).

Elimination of CS terminals in the ventrolateral area at P8 coincided with the potential reversal from negative to positive in timing and location. The decrease in the number of CS terminals in the ventrolateral area explains the change from negative to positive potentials in that area. Both the morphological and electrophysiological studies indicate that CS synapses are eliminated from the ventrolateral area from P7 to P10.

**DISCUSSION**

Postsynaptic responses in rat spinal cord evoked by CS tract stimulation have been studied in terms of cord dorsum potentials (Elger et al. 1977), intracellular EPSPs in motoneurons (Babalian et al. 1993; Bannister and Porter 1967), and both (Alstermark et al. 2004). Field excitatory postsynaptic potentials (fEPSPs) of CS synapses have not been studied except for the work by Alstermark et al. (2004), and there has been no such study in early postnatal rats. We therefore had to first identify the monosynaptic fEPSPs of CS synapses in early postnatal rats. fEPSPs were evoked consistently by stimulating the ventral and dorsal lines therefore also decreased significantly (P7, 26.5 ± 8.2; P8, 16.2 ± 4.1; P10, 11.0 ± 4.6; P11, 10.6 ± 6.5; P < 0.05, P7 vs. P8, P10, or P11; Fig. 8).

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P10 (23.8 ± 1.9 ms, n = 94, the average for all the negative fEPSPs in 7 animals) was, however, sixfold longer than in adults (~4 ms; Alstermark et al. 2004); this might be because CS fibers are largely unmyelinated at P10 (Gorgels et al. 1989; Stanfield 1992).

The CV of the CS tract in the adult rat is 4–19 m/s (Alstermark et al. 2004; Bannister and Porter 1967; McComas and Wilson 1968; Mediratta and Nicoll 1983; Porter and Lemon 1993; Stewart et al. 1990). Although CS fiber volley is generally necessary for measuring CV of the CS tract, it could not be recorded under our conditions, which might be caused by unmyelinated CS fibers (Gorgels et al. 1989; Stanfield 1992). The activation of CS fibers was not synchronous enough to set up a fiber volley in early postnatal stage. Furthermore, even in adult rats, the CS fiber volley merges with following potentials at C7 (Alstermark et al. 2004) because of its poor synchronization. To estimate the CV, we measured the difference in the latencies of the first positive peaks of the response, which reflect the later phase of the presynaptic volley from two segments. The CV values estimated in our study (0.85–1.21 m/s) were 1/10th that of adult (Alstermark et al. 2004; Bannister and Porter 1967; McComas and Wilson 1968; Mediratta and Nicoll 1983; Porter and Lemon 1993; Stewart et al. 1990). In fact, neonatal CVs of the CS tract of postnatal monkeys and cats are 10 times slower than those of adult animals (Oka et al. 1985; Olivier et al. 1997). The CV values we estimated in early postnatal rat are consistent with the decreased CV in other species.
The areas of BDA injection sites on the cerebral cortex (Fig. 5A) were a little different among the ages in this study. Some previous mapping studies of the sensorimotor cortex projecting to the cervical enlargement were performed by retrograde labeling with horseradish peroxidase (HRP). The areas in which HRP was injected were much larger than the area we studied (Bates and Killackey 1984; Leong 1983; Uozumi et al. 1988). Microstimulation for mapping of the CS neurons (Neafsey et al. 1986) is limited to adult rats because it is impossible to evoke the response in early postnatal rats. The precise location of the CS neurons projecting to C7 in early postnatal rats is not known. The comparison of the injection areas at P7-P10 with that at P11 suggests that the area of the neurons projecting to C7 is an ~1-mm-square region as at P11. This was supported by the finding that the number of labeled fibers at P10 was almost the same as at P11, although the injected area at P10 was broader than at P11. In addition, injections outside of this small area did not contribute to labeling of the C7 terminals (data not shown). Furthermore, the area projecting to C7 appeared to change little during development, at least for the first 2 neonatal wk.

CS synapses were distributed widely throughout the spinal cord at P7 but eliminated in the ventrolateral area at P8 and restricted to the dorsal side up to P10. Regressive events during CS projection development have been studied, mainly by morphological methods (Alisky et al. 1992; Armand et al. 1997; Cabana and Martin 1985; Curfs et al. 1994; Joostven et al. 1987; Kuang and Kalil 1994; O’Leary and Terashima 1988; Stanfield et al. 1982; Theriault and Tatton 1989). Whether CS synapses are formed and function during development is not known. Functional CS synapses during development and their regression have been directly studied only in the cat (Meng and Martin 2003; Meng et al. 2004). There might be aspects in common with our findings, but the time-course is as long as several weeks and therefore might represent a different regres-
in vivo. An interesting future task will be to closely compare and at 7 DIV in slices prepared from P0 rats; elimination in vitro. CS synapses are most widely distributed at P7 in vivo and at 7 DIV in slices prepared from P0 rats; elimination begins at P8 in vivo and at the same point between 7 and 9 DIV in vitro. An interesting future task will be to closely compare the developmental events of these two conditions. Furthermore, in vivo studies should help us to understand the meaning of synapse elimination in the development of movement and behavior.

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