THE JOURNAL OF COMPARATIVE NEUROLOGY 467:375–388 (2003)

Expression of L1 Decreases during Postnatal Development of Rat Spinal Cord

ALIN AKOPIANS, STEVE A. RUNYAN, AND PATRICIA E. PHELPS*
Department of Physiological Science, University of California at Los Angeles, Los Angeles, California 90095-1527

ABSTRACT

L1 is a cell adhesion molecule that is highly expressed on developing axons and is associated with neurite outgrowth, guidance, and fasciculation. In this study we systematically examined L1 expression at all spinal levels across eight postnatal ages to detect regional and developmental differences. We observed striking changes in the developmental pattern of L1 expression between birth (P0) and adult ages, with intense L1-immunopositive axons prevalent throughout the funiculi at P0 compared with predominantly L1-immunonegative funicular axons in adults. At all ages and spinal levels examined, some L1-positive dorsal root afferents entered the spinal cord, coursed in Lissauer's tract, and projected into the superficial dorsal horn and the dorsal columns, as well as across the dorsal commissure. Additional L1-positive axons were detected consistently around the perimeter of the spinal cord, in the dorsolateral funiculus, and adjacent to the central canal. While specific L1-labeled axons were detected at all ages, a pattern of segmental variation was observed within animals, with the highest levels of L1 expression detected in lumbar and sacral segments and the lowest in cervical spinal cord. The pattern of L1 immunoreactivity was compared to that of the growth-associated protein GAP-43 and the results indicated colabeling of most axons. These observations demonstrate that L1 is expressed on immature axons well into postnatal development, possibly until they have completed their differentiation. Furthermore, the L1-positive axons that continue to be detected in adults are likely to be either unmyelinated or sprouting axons. J. Comp. Neurol. 467:375–388, 2003.

Indexing terms: cell adhesion molecule; corticospinal tract; development; dorsal horn; GAP-43

Cell adhesion molecules (CAMs) are cell-surface molecules that have been reported to play important developmental roles in cell adhesion, neurite outgrowth, axon fasciculation, and guidance (Jessell, 1988; Lemmon et al., 1989). Many CAMs, such as L1, are members of the immunoglobulin superfamily and are integral membrane glycoproteins (Jessell, 1988; Appel et al., 1993; Brummendorf and Rathjen, 1993; Kamiguchi et al., 1998b). L1 is a glycoprotein with a relative molecular weight of about 200 kd and a molecular structure containing six immunoglobulin (Ig)-like and five fibronectin type-III homologous repeats in the extracellular domain that are thought to participate in homophilic interactions (Grumet and Edelman, 1988; Kamiguchi et al., 1998b). In addition, L1 binds with heterophilic interactions to other cell adhesion molecules such as TAG-1 (Rader et al., 1996) or F3/contactin (Brummendorf et al., 1993) and has a short, highly conserved cytoplasmic tail that interacts with various kinases such as calmodulin (Moos et al., 1988; Appel et al., 1993; Fransen et al., 1997; Kamiguchi et al., 1998b). L1 was initially identified in the mouse (Rathjen and Schachner, 1984) and has an equivalent molecule in rat known as NGF-inducible large external glycoprotein (NILE; Bock et al., 1985; Prince et al., 1991) as well as homologous molecules in a wide variety of species, including chick (Ng-CAM, G4), *Drosophila* (neuroglian), zebrafish (L1.1, L1.2),

Grant sponsor: National Institute of Neurological Disorders and Stroke; Grant number: 1R21 NS42000-01; Grant sponsor: Christopher Reeves Paralysis Foundation; Grant numbers: PA 1-0102-2, PAC1-0102-2; Grant sponsor: the National Science Foundation; Grant number: IBN-9734550.

*Correspondence to: Patricia E. Phelps, Dept. of Physiological Science, UCLA, Box 951527, Los Angeles, CA 90095-1527.
E-mail: pphelps@physci.ucla.edu

Received 27 March 2003; Revised 16 July 2003; Accepted 29 July 2003
DOI 10.1002/cne.10956
Published online the week of October 27, 2003 in Wiley InterScience (www.interscience.wiley.com).

and humans (L1 CAM) (Thiery et al., 1985; Moos et al., 1988; Bieber et al., 1989; Hlavin and Lemmon, 1991; Shiga and Oppenheim, 1991; Tongiorgi et al., 1995).

Previous studies have shown that mutations in the *L1* gene are linked to human nervous system syndromes. Features of *L1* mutations are generally described by the acronym "CRASH" (Corpus callosum agenesis, mental Retardation, Adducted thumbs, Spastic paraplegia, and Hydrocephalus) (Fransen et al., 1995). There have been more than 85 L1 mutations identified in all parts of the L1 molecule in CRASH patients (Kamiguchi et al., 1998a). One of the most severe phenotypes of the *L1* mutation is X-linked hydrocephalus, which is associated with corticospinal tract hypoplasia and absent medullary pyramids (Kamiguchi et al., 1998a; Graf et al., 2000). Descriptions of *L1* knockout mice have shown a strong resemblance to the human mutation and include severe pathfinding errors of the corticospinal tract resulting in spastic paraplegia (Cohen et al., 1997; Dahme et al., 1997). In addition, in vitro studies of neurons from L1-deficient mice exhibited reduced fasciculation and impaired outgrowth on an L1 substrate, findings supportive of a role for L1 in fasciculation (Dahme et al., 1997).

During embryonic development L1 has been localized on axons and axonal growth cones in a variety of species as they project to their targets in both the central and peripheral nervous systems (Joosten et al., 1990; Van Den Pol and Kim, 1993; Burden-Gulley et al., 1995). Perturbation studies of axonal growth and targeting have shown that antibodies to L1 block neurite extension and cause defasciculation of commissural axons (Fischer et al., 1986; Beasley and Stallcup, 1987; Lagenaur and Lemmon, 1987; Stoeckli and Landmesser, 1995). The presence of L1 on fasciculated axons during spinal cord development together with the results from L1 antibody blocking experiments (Stoeckli and Landmesser, 1995) are consistent with L1 having a role in axon outgrowth and fasciculation.

While intense L1 axon labeling is observed during embryonic spinal cord development (Stallcup et al., 1985; Beasley and Stallcup, 1987; Orlino et al., 2000; Tran and Phelps, 2000), the reported trend after birth is one of low expression on axons within the white matter (Stallcup et al., 1985; Beasley and Stallcup, 1987). However, Joosten (1991) reported the presence of L1 on late developing, descending corticospinal tract axons and suggested that L1 might play a role in their fasciculation (Joosten et al., 1990). We have confirmed these findings and in addition have observed striking changes in the pattern of L1 expression during postnatal spinal cord maturation. Based on these changes in L1 expression patterns we suspected that the L1-immunopositive axons at postnatal ages represented developing, sprouting, or unmyelinated fibers, whereas the more prevalent immunonegative axons were older, myelinated fibers. In this study we systematically examined L1 expression patterns at all spinal cord levels from birth to one year of age to detect regional differences and overall developmental expression patterns of L1. To evaluate the role of L1 as a marker of growing axons, we compared the pattern of L1 immunoreactivity with that of the growth-associated protein GAP-43.

MATERIALS AND METHODS

Animals and tissue preparation

Male and female Sprague-Dawley rats were caged together overnight for breeding. The following morning vaginal smears were performed to confirm insemination. The day of birth was designated postnatal day 0 (P0) and 3–4 animals were studied at each of the following ages: P0, P7, P14, P21, P28, P100, P180, and P365. The younger animals (P0–28) were deeply anesthetized with 18% chlorohydrate (0.30 cc / 35 g / rat), whereas older rats (P100–365) were anesthetized with ketamine (90 mg/kg) and xylazine (15–20 mg/kg). Animals at each age were perfused through the heart with 4% paraformaldehyde. This protocol was approved by the Chancellor's Animal Research Committee at UCLA. Spinal cords and brainstems were postfixed overnight at 4°C and then washed repeatedly in phosphate buffer. After dissection, tissues were cryoprotected in 30% sucrose before being blocked, embedded in OCT, frozen, and stored at −80° C.

L1 immunocytochemical procedures

Before immunocytochemical experiments, coronal and horizontal sections (40 μm) were cut using a cryostat and stored in 0.12 M Millonig's buffer (pH 7.4) containing 0.06% azide. While most L1-immunoreactive axonal patterns were visualized on coronal sections, horizontal sections were used to evaluate the longitudinal extent of the L1-labeled axon pathways. Axonal processes expressing the L1 antigen were identified using a monoclonal antibody (ASCS4; Sweadner, 1983) obtained from the Developmental Studies Hybridoma Bank (Department of Biological Sciences, University of Iowa, Iowa City, IA). Immunocytochemical methods were adopted from Tran and Phelps (2000) and Orlino et al. (2000). For comparative studies across different ages and segmental levels, spinal cord sections were processed together in the same immunoreagents.

Initially, free-floating sections were rinsed in Tris-buffered saline (TBS; 0.1 M Tris buffer containing 1.4% NaCl; pH 7.4) and followed by treatment in 0.3% hydrogen peroxide and 0.1% sodium azide for 30 minutes. Then sections were incubated in detergent (0.8% Triton X-100 in TBS) for 15 minutes, followed by 0.1% Triton X-100 with 1.5% normal horse serum for 1 hour. Sections were incubated with an avidin-biotin blocking solution (diluted 1:1 with TBS; Vector Laboratories, Burlingame, CA) for 15 minutes each, with 5-minute TBS rinses between each step. Subsequently, sections were incubated in ASCS4 (diluted 1:25) for 2 hours at room temperature and overnight at 4°C. The following morning sections were rinsed and incubated for 1 hour in rat adsorbed biotinylated horse antimouse IgG, (diluted 1:200; Vector Laboratories). Following a TBS rinse, sections were incubated in avidin-biotin-peroxidase complex (ABC; diluted 1:100; Vector Laboratories) for an additional hour. A buffer wash was followed by a rinse in acetate buffer before developing with DAB that was intensified with Ni-glucose oxidase and yielded a dark reaction product. Sections were washed in acetate buffer and mounted on slides. After drying, sections were either counterstained (see below) or dehydrated and coverslipped.

A polyclonal anti-L1 antiserum (gift from Dr. Vance Lemmon of Case Western Reserve University, Cleveland, OH; Lemmon et al., 1989) was used to confirm the labeling

pattern of the monoclonal antibody for the lightly immunoreactive adult rat tissues. The pattern of L1 expression using either the polyclonal or monoclonal antibodies was identical (data not shown). Sections incubated with the polyclonal anti-L1 antiserum (diluted 1:40,000) were processed similarly to those described above, except that the buffer contained 0.1% bovine serum albumin (BSA), the ABC step was diluted 1:200, and the secondary antibody was biotinylated goat antirabbit IgG (diluted 1:400, Vectastain Elite Rabbit IgG Kit).

L1 and GAP43 double labeling procedures

A monoclonal mouse anti-GAP (GAP-43/B-50) that recognizes all known forms of GAP-43 (Chemicon International, Temecula, CA; clone 9-1E12, diluted 1:25,000) was used to identify growing axons (Schreyer and Skene, 1991). For double-labeling experiments the L1 immunoreactivity was detected as indicated above; however, L1 was localized with DAB containing 0.02 M imidazole yielding an amber-brown product. Sections then were temporarily slide-mounted, coverslipped with glycerin, and photographed. After thorough washing, GAP-43 immunocytochemical procedures were carried out on the same sections. Sections were processed similarly to those described above for L1 immunoreactivity, except that phosphate-buffered saline (PBS; 0.075 M PB containing 0.1% BSA; pH 7.3) was used and the hydrogen peroxide and detergent incubation steps were omitted. For double-labeling control experiments the sections were processed for L1 immunoreactivity as described above and then GAP-43 localization was carried out except that the primary antibody step was eliminated. In these control experiments no black DAB reaction product was seen overlying the amber-brown L1 immunoreactivity (data not shown).

Counterstaining procedures

In order to visualize the relationship between cell bodies and L1 axon staining, some L1-labeled sections were counterstained. Buffered cresyl echt violet (25% CEV; Cell Point Scientific, Gaithersburg, MD) stain was prepared by combining 100 ml CEV stain, 0.2 M acetate buffer, 0.2 M sodium acetate, and 0.2 M acetic acid and filtered. Slide-mounted sections were placed in CEV stain for 2 minutes, rinsed, dehydrated, and coverslipped.

All micrographs were photographed with an Olympus AX70 microscope using a Zeiss digital camera (AxioCam HRc) with Improvision Open Lab software. Plates were assembled in Adobe PhotoShop v. 7.0. Contrast, color balance, and brightness were adjusted if necessary.

RESULTS

During embryonic development L1 has been localized on axons and axonal growth cones as they grow toward their targets (Van Den Pol and Kim, 1993; Burden-Gulley et al., 1995). The present study focused on the temporal and regional patterns of L1 expression throughout postnatal development and subsequently compared them to axons identified with a well-established marker of axonal growth, GAP-43.

Overall L1 expression decreases from early to late postnatal ages

Although immunoreactive L1-labeled axons were observed across all ages and within all spinal segments examined, a striking overall decrease in L1 expression in fasciculated axonal tracts was apparent with increasing age. To directly compare the intensity of L1-immunoreactive axons during development, we processed sections from the same segment level (thoracic) of P0–365 animals together in the same immunoreagents. At P0 the white matter regions of the spinal cord were homogeneously filled with intensely L1-immunoreactive axons (Fig. 1A), whereas variations in the density of L1-labeled funicular axons was detected at all other postnatal ages (Fig. 1B–F). For example, the outer perimeter of the funiculi in P14 animals contained more numerous L1-labeled axons than the inner regions (Fig. 1C). In addition, the dorsolateral funiculi consistently contained large numbers of L1-positive axons at all postnatal ages (Fig. 1A–F). When compared to earlier postnatal ages a decrease in L1-labeling of the dorsal root fibers was detected at the adult ages. In general, fewer L1-labeled axons were detected in white matter as the postnatal age increased up to P100 (Fig. 1). Although the intensity of L1 expression varied somewhat between adult animals at the same age, the pattern of L1-positive axons observed at P365 was the same as that detected at P100 (Fig. 1E,F) and dramatically different from the pattern and intensity of L1 expression during the first postnatal month (Fig. 1A–D).

Variability in L1 expression in the neuropil was also found during postnatal development. At both P0 and P7 (Fig. 1A,B), there were considerably fewer L1-immunoreactive axons within the neuropil than were detected at P14–28 (Fig. 1C,D). In addition, a pattern of evenly dispersed axonal and punctate immunoreactivity was present at P0 and P7, whereas at older ages it was concentrated in the superficial dorsal horn. In the P14–365 thoracic sections studied, we found low L1 immunoreactivity in Clarke's nuclei and in somatic motor regions, compared to the higher levels of L1 expression in the surrounding neuropil regions (Fig. 1). By P100 the level of L1 immunoreactivity in the neuropil decreased from that seen in P14–P28 sections. Furthermore, the pattern of L1-labeling in the neuropil areas established by P100 was maintained through P365.

Developmental gradients from birth to adult

L1-immunoreactive axons were observed in specific regions of ventral and dorsolateral funiculi throughout the first month and in all segmental levels examined (Fig. 2A–D). We observed L1-labeled centrally directed primary afferent axons in the dorsal root entering the spinal cord, traveling through Lissauer's tract, entering into the dorsal gray matter, and coursing in the dorsal columns (Figs. 2A, 3E). Primary afferent axons are also known to project contralaterally by way of the dorsal commissure (Smith, 1983) and a number of L1-positive axons were detected in the *middle* and *posterior* dorsal commissural bundles during the first postnatal month (Figs. 1A–D, 3A), similar to observations in embryos (Ramón y Cajal, 1937; Orlino et al., 2000). Additional bundles of crosscut L1-labeled axons were observed scattered within the reticulated area of the dorsal horn during the first two postnatal weeks (Fig. 1A–C).

More L1-positive axons are found in caudal than rostral spinal cord. To visualize L1 expression at different segmental levels, cervical, thoracic, lumbar, and sacral spinal cord sections were compared within animals across all postnatal ages. Relatively few differences were

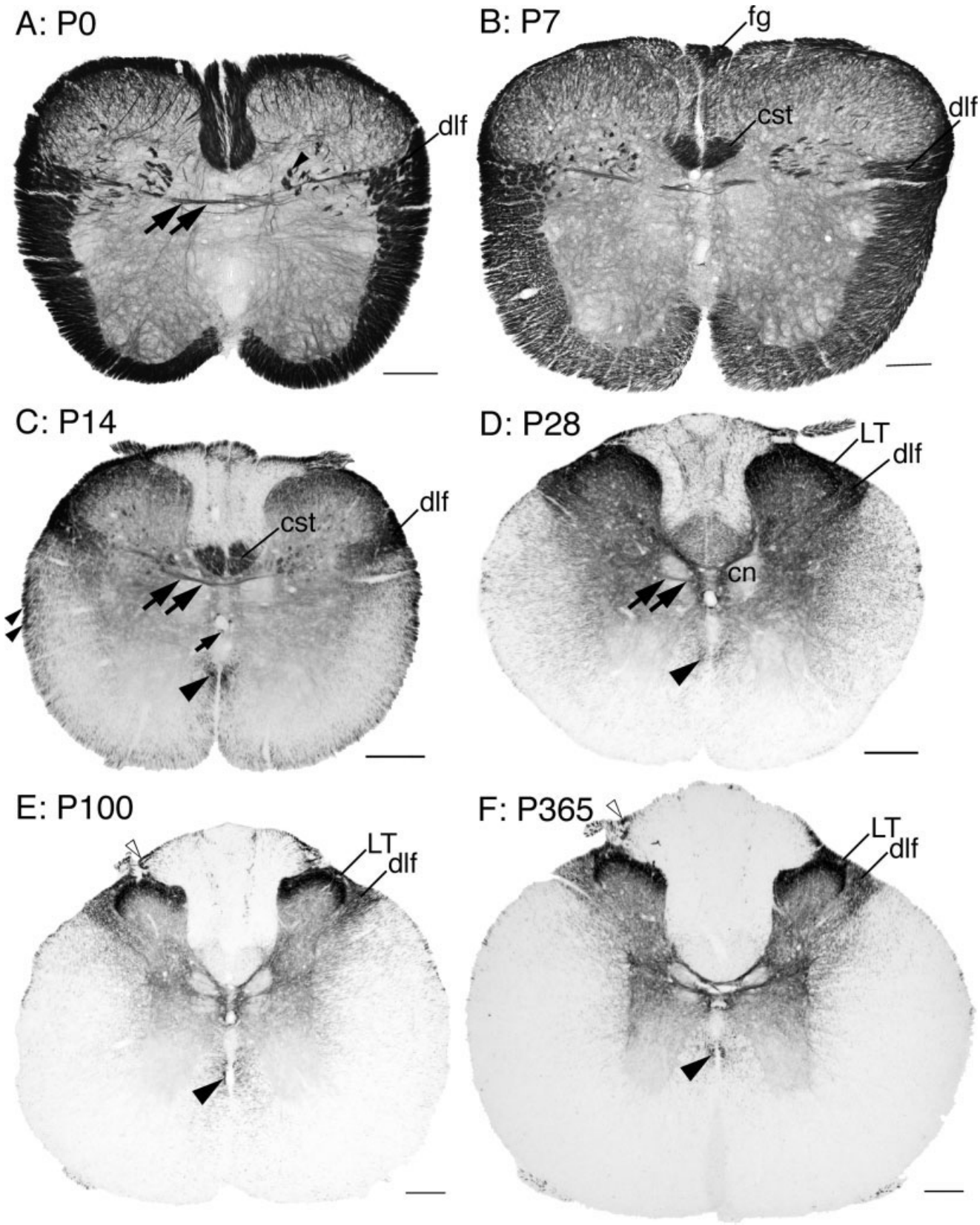
A: P0
dlf
B: P7
fg
cst
dlf
C: P14
cst
dlf
D: P28
LT
dlf
cn
E: P100
LT
dlf
F: P365
LT
dlf

Figure 1

observed in either the pattern or intensity of L1-labeling between P0 and P7 segments, with the exception that the corticospinal tract was not detected at the sacral level of P7 animals (data not shown). By P14, the overall intensity of L1 immunoreactivity varied between segmental levels with the strongest labeling in sacral sections (Fig. 2A–D). In both lumbar and sacral P14 sections there were substantially more immunoreactive preterminal-like axons in the gray matter and L1-positive axons in white matter than were observed at more rostral levels (Figs. 2A–D, 3F). This pattern continued through P28, with the white matter areas of lumbar and sacral sections also containing more immunoreactive axons than cervical sections.

Overall, only low levels of L1-expression were detected in adult spinal cord, and in a substantially different pattern than seen during embryonic or early postnatal ages. All four segmental levels exhibited a consistent pattern of L1-expression in each of the P365 female rats examined. L1-labeled preterminal-like axons were concentrated in the superficial dorsal horn, primarily lamina I and II, with comparatively little L1 immunoreactivity within the ventral horn (Figs. 2E–H, 3E). L1-labeling in the superficial laminae appeared to be derived, at least in part, from afferent axons that enter through the dorsal root (Figs. 2E, 3E). In addition, fasciculated bundles of immunoreactive axons coursed through the dorsal commissure into the contralateral dorsal horn at each level (double arrows, Fig. 2F–H).

Although a similar pattern of immunoreactivity was seen at different segmental levels, distinct variations within this common motif were observed. Generally, the intensity of L1-positive axons throughout the gray matter of lumbar (Fig. 2G) and sacral (Fig. 2H) spinal cord was greater than in the more rostral levels (Fig. 2E,F). Higher regions of L1 expression in the caudal spinal cord were widespread, including the ventral gray matter, suggesting that the caudal-to-rostral gradient of L1 expression detected by P14 was maintained in adults.

In contrast to the gray matter, L1 was not detected on axons in most parts of the dorsal, lateral, and ventral funiculi in P100–365 adults. However, there were three areas where individual L1-positive funicular axons formed distinct, highly ordered patterns. L1-positive axons were detected within the dorsolateral funiculus at all levels (Fig. 2E–H). L1-labeled axons also were detected in the midline of the ventral funiculus with progressively more axons found in caudal than rostral segmental levels (Fig. 2). Finally, L1-positive axons coursed along the outer edge of the dorsal funiculus, in the area adjacent to the dorsal root entry zone (open arrowhead, Figs. 2E, 3E).

While the general pattern of L1 expression was similar across adult ages, the intensity of L1 immunoreactivity varied between animals of the same age. That is, at the same segmental spinal cord level, and under the same experimental conditions with sections incubated in the same immunoreagents, we found variation in the intensity of L1-expression between adult animals.

Many L1-positive axon bundles course longitudinally. While the pattern of L1-immunoreactive axons was examined on coronal sections, horizontal sections were used to evaluate the longitudinal extent of these fiber tracts. L1-labeled axons coursed long distances rostrocaudally in the lateral funiculus (Fig. 3A), as well as in the dorsal corticospinal tract (Fig. 3B) and in Lissauer's tract. In addition, the periodicity of the L1-positive dorsal commissural axons was evident in horizontal sections (Fig. 3A).

From P7 on, distinct bundles of crosscut L1-labeled axons were observed around the outside of the central canal ependymal cells (Fig. 3F). When examined in horizontal sections, small bundles of L1-labeled axons coursed rostrocaudally directly adjacent to the ependymal cells (Fig. 3C). In addition, bundles of L1-immunoreactive axons were detected near the midline of the ventral funiculus in all segmental levels except cervical (Figs. 2, 3F). While the origin of these axons is not known, L1-labeled axons coursed between the region surrounding the central canal and the midline of the ventral funiculi (arrows, Fig. 3F), suggesting that these axons may comprise the same or a closely related pathway.

L1-positive axons in the neuropil. Based on observations that the density of L1 immunoreactivity on axons and preterminal-like structures was lower in the ventral gray matter containing the somatic motor neurons and in Clarke's nuclei than in adjacent gray matter regions, we counterstained P14 L1-immunoreactive sections to determine the relationship between L1-labeled axons and these two groups of large neurons. In counterstained thoracic sections (Fig. 4C), L1-immunoreactive dorsal commissural axons coursed through a less densely immunoreactive region identified as Clarke's nucleus (cn, Fig. 4C). Counterstained sections in the upper lumbar spinal cord also contained large cells in the somatic motor columns that were surrounded by L1 immunoreactivity (Fig. 4A,B). By comparing counterstained and noncounterstained sections, our data suggest that the decreased levels of L1

Fig. 1. Comparison of L1 expression in rat thoracic spinal cord at P0 (A), P7 (B), P14 (C), P28 (D), P100 (E), and P365 (F). All sections were incubated together in the same immunoreagents. To compare the pattern and intensity of staining across all ages, the DAB reaction was optimized for the adult sections. **A:** Intensely immunoreactive L1-positive axons fill the white matter in the P0 funiculi yet low levels are detected in the gray matter. Bundles of L1-labeled axons cross in the posterior commissure (double arrows), and others are seen as cross cut axons in the reticulated area (small arrowhead) of the dorsal horn. **B:** Compared to P0, lower levels of L1 expression are detected in the P7 funiculi except for the intensely labeled corticospinal tract (cst), the fasciculus gracilis (fg), and the dorsolateral funiculus (dlf). **C:** Overall, L1 expression decreased substantially within funicular axons by P14 except for axons in the corticospinal tract (cst), the dorsolateral funiculus (dlf), the midline of the ventral funiculus (arrowhead), and around much of the perimeter of the spinal cord (double arrowheads). A few L1-labeled axons are found adjacent to the central canal (single arrow) and a prominent bundle of L1-labeled axons project through the dorsal commissure (double arrows). **D:** By P28 less L1 is detected in the white matter and more is seen in the dorsal and intermediate gray matter. Clarke's nuclei (cn) can be identified by its low levels of L1-labeling compared with the surrounding neuropil. L1-positive axons continue to be detected in Lissauer's tract (LT), the dorsal commissure (double arrows), and midline axons in the ventral funiculus (arrowhead) at this and subsequent ages. **E:** By P100, L1 expression is low in all but the dorsolateral funiculus (dlf), Lissauer's tract (LT), the dorsal funiculus adjacent to the dorsal root entry zone (open arrowhead), and in the ventral funicular midline (arrowhead). **F:** The pattern and intensity of L1 expression in the P365 spinal cord was similar to that detected at P100 (E). Scale bars = 100 μm in A,B; 200 μm in C–F.

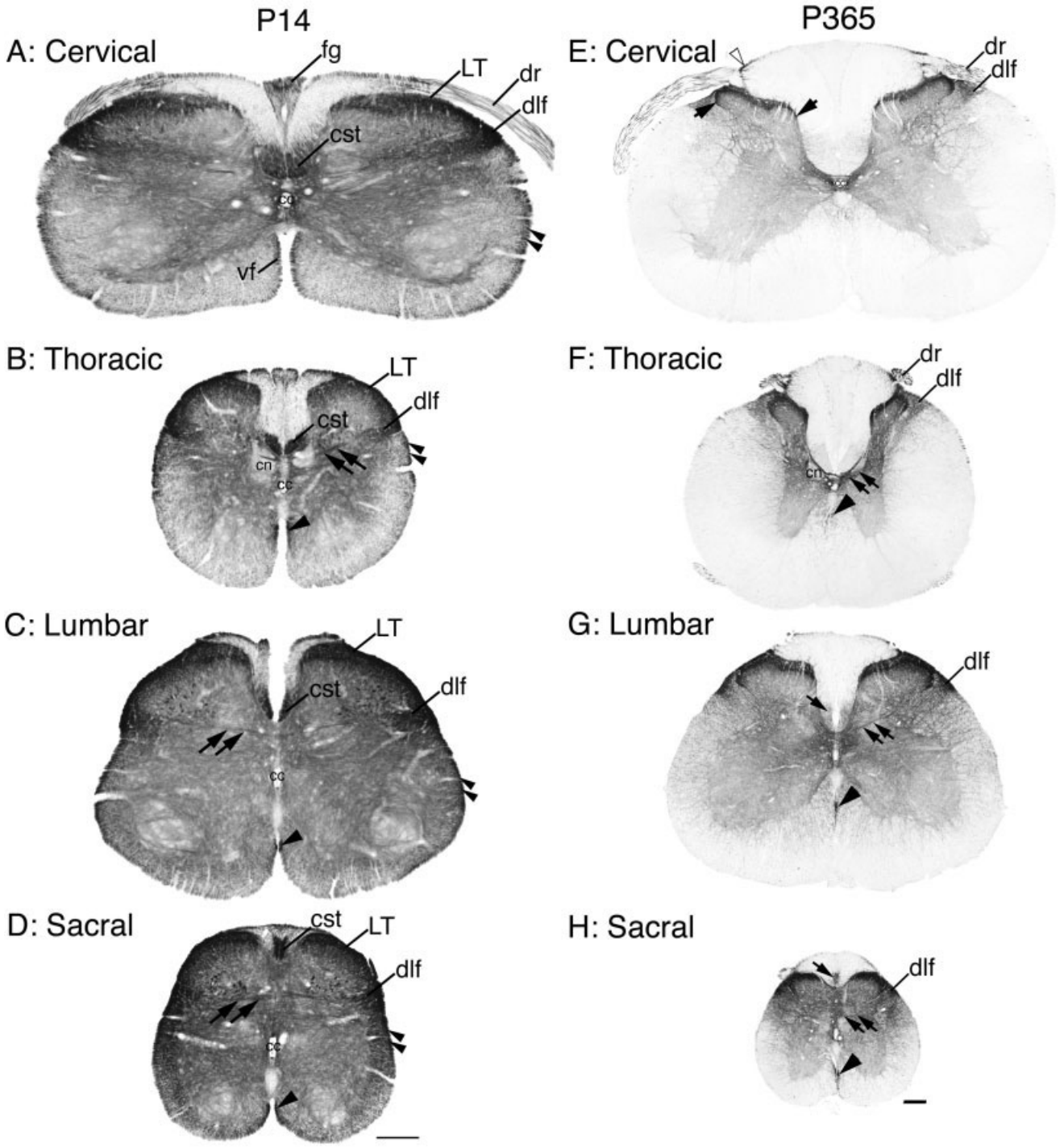

Fig. 2. Comparison of L1 expression patterns across segmental levels of P14 (A–D) and P365 (E–H) rat spinal cord: cervical (A,E); thoracic (B,F); lumbar (C,G); and sacral (D,H). At both ages L1 expression is highest in sacral and lowest in cervical sections taken from a single animal and processed in the same reagents. At all segmental levels L1-labeled axons are found in the dorsolateral funiculi (dlf), Lissauer's tract (LT), the dorsal commissure (double arrows), and adjacent to the ependymal cells of the central canal (cc). In all P14 levels, L1-labeled axons are found in the ventral, lateral, and dorsal funiculi, along the perimeter of the spinal cord (double arrowheads), in the corticospinal tract (cst), and in the fasciculus gracilis (fg). **A:** A unique feature of L1 expression in cervical sections is the individual L1-labeled axons in the midline of the ventral funiculi (vf) rather than the bundled axons seen at other levels. L1-labeled axons enter by way of the dorsal root (dr) into the dorsal columns. **B:** Distinct features in thoracic sections include a bundle of L1-labeling in the midline of the ventral funiculi (arrowhead), and the immunoreactive dorsal commissural axons (double arrows) coursing through Clarke's nucleus (cn). **C:** Lumbar sections include intensely labeled bundles of L1-positive axons in the midline of the ventral funiculi (arrowhead) and L1-positive dorsal commissural axons (double arrows). **D:** Sacral sections are characterized by heavy L1 immunoreactivity in both gray and white matter, numerous dorsal commissural axons (double arrows), increased labeling of L1-positive axons in the ventral funiculi (arrowhead), and the small size of the dorsal corticospinal tract (cst). **E:** In all P365 levels, L1 immunoreactivity in the gray matter is concentrated in the superficial dorsal horn (between short arrows in E only). L1-labeled axons are detected in the dorsal root (dr) and by the dorsal root entry zone (open arrowhead). **F:** In thoracic sections L1-positive axons are found in the midline of the ventral funiculus (arrowhead). Clarke's nucleus (cn) appears devoid of L1 immunoreactivity with the exception of L1-positive dorsal commissural axons (double arrows) that course through this region. **G:** L1-positive axons form a compact arrangement in the midline of the ventral funiculus (arrowhead) and a few are found in the midline of the dorsal funiculus (arrow). **H:** The neuropil in sacral sections displays the highest L1 expression of all four segmental levels. L1 immunoreactivity is seen in the midline of the dorsal (single arrow) and the ventral funiculi (arrowhead). Scale bars = 200 μm.

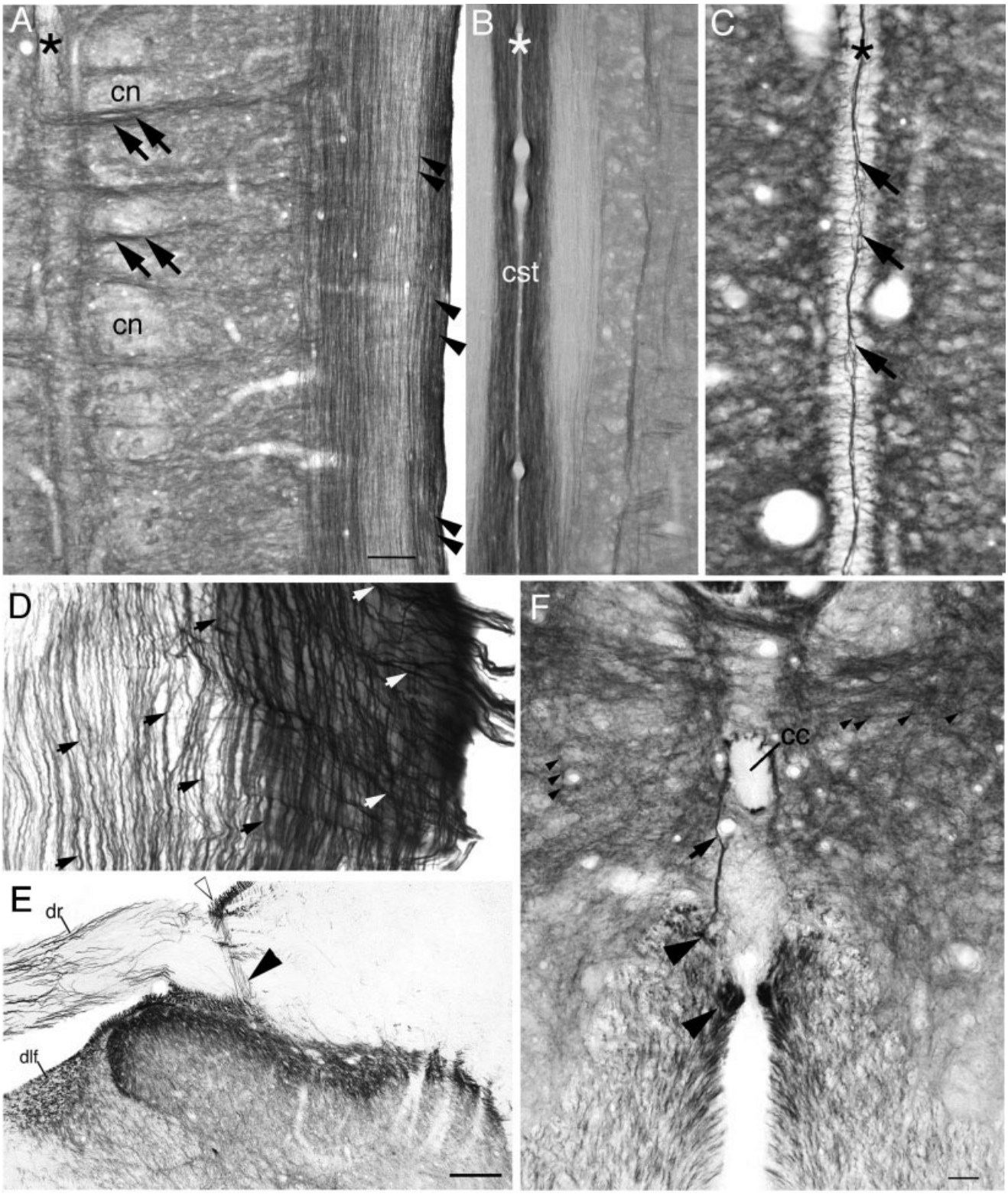

Fig. 3. Fasciculated bundles of L1-labeled axons characterize the P14 horizontal (A–D) and P365 (E) and P14 coronal (F) sections of spinal cord. Asterisks mark the midline of the horizontal sections (A–C) that are oriented with rostral towards the top. **A:** L1-labeled axons travel rostrocaudally within the lateral funiculi (double arrowheads) in this thoracic section, with the most densely immunoreactive axons along the outer edge. Periodic immunoreactive fiber bundles (double arrows) course through both the dorsal commissure and the immunonegative area of the Clarke's nucleus (cn). **B:** High magnification of the dorsal funiculus illustrates the axons of the corticospinal tract (cst) traveling caudally. **C:** A small bundle of L1-labeled axons (arrows) course rostrocaudally directly adjacent to the ependymal cells of the central canal (unlabeled area beneath fibers). **D:** Immunolabeled dorsal root axons enter the dorsal horn (white short arrows) and travel longitudinally (black short arrows) in the adjacent dorsal columns. **E:** High magnification of L1-labeled axons entering the P365 cervical spinal cord. L1-labeled axons enter through the dorsal root (dr) to project into the superficial laminae of the dorsal horn and along the outside of the dorsal funiculus (open arrowhead). Occasionally, L1-positive axons course between these two regions (large arrowhead). The dorsolateral funiculus (dlf) contains numerous L1-labeled axons. **F:** P14 L1-labeled axons (arrow) that course along to the central canal (cc) occasionally join immunoreactive axon bundles in the midline of the ventral funiculus (large arrowheads). Note the fine L1-positive axons and preterminal-like structures (small arrowheads). Scale bars = 100 μm in A,E; 50 μm in F (applies to B–D,F).

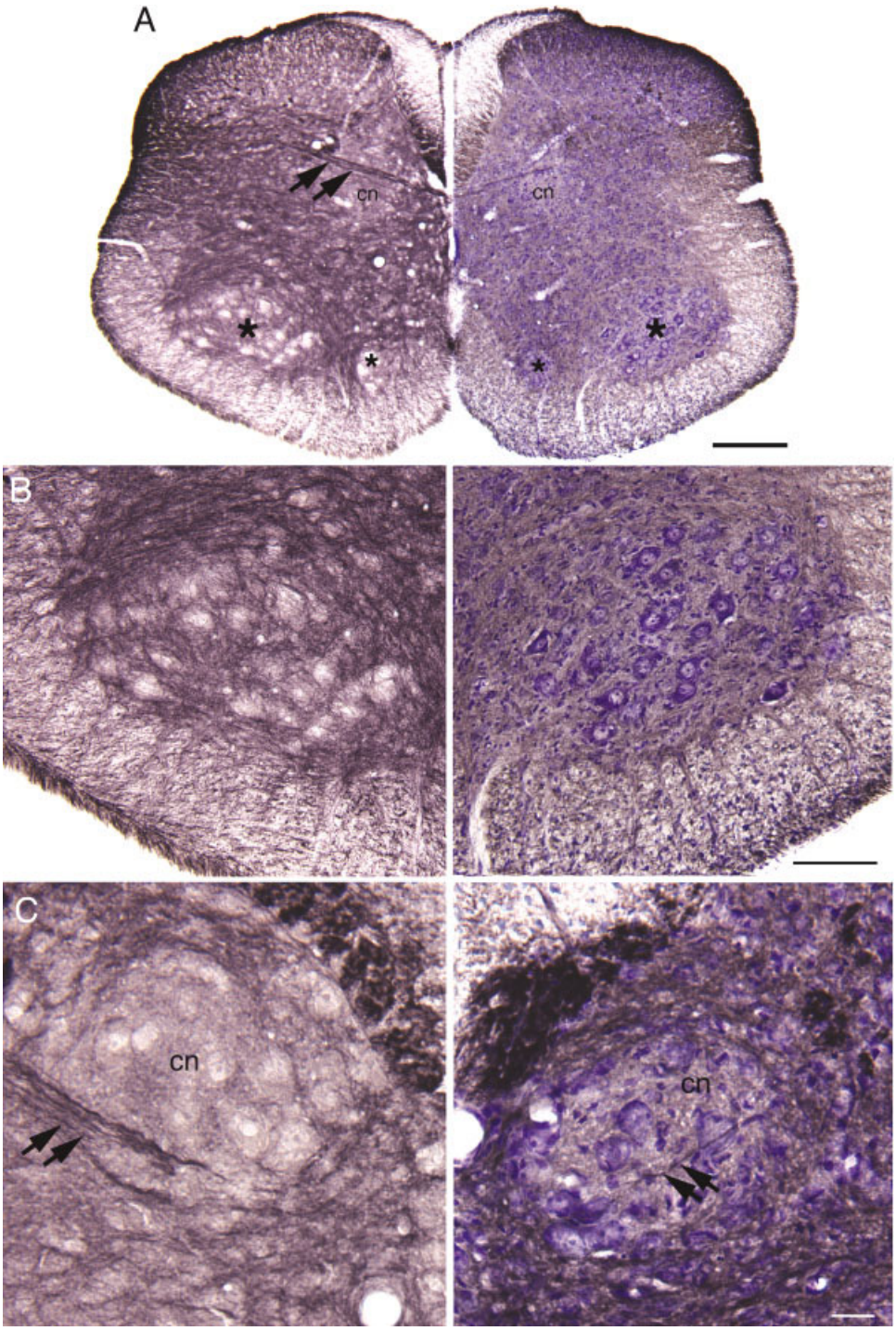

Fig. 4. L1-labeled axons in upper lumbar (A,B) and thoracic (C) sections of P14 rat spinal cord without (A–C; left image) and with (A–C; right image) subsequent counterstaining to identify neuronal cell bodies. **A:** The density of L1 expression appears less in medial and lateral motor columns (asterisks) and Clarke's nucleus (cn) than in the adjacent gray matter regions. **B:** High magnification of A showing the immunonegative regions in the L1 only section (left image) compared to the counterstained lateral motor column showing distinct cell bodies (right image). **C:** High magnification of Clarke's nucleus (cn). In the L1-only section large cell bodies appear to occupy the immunonegative region of Clarke's nucleus. Immunoreactive dorsal commissural axons (double arrows) project through Clarke's nucleus (cn) identified in the counterstained preparation. Scale bars = 100 μm in A; 200 μm in B; 50 μm in C.

expression observed around motor neurons and in Clarke's nuclei were due to space occupied by the large somata.

Corticospinal tract axons express L1 during outgrowth

Donatelle's (1977) tracing study described the projection of the corticospinal axons into the rat caudal spinal cord by P9 and Joosten et al. (1990) established that L1 immunoreactivity identified the corticospinal tract in early postnatal rat spinal cord. To further examine the specificity of L1 immunoreactivity for the developing corticospinal tract, we analyzed the P14 rat brainstem at the level of the decussation of pyramids. Corticospinal tract axons were identified based on their strong L1 expression (Fig. 5), their midline location in the medulla, and their unique pattern at the decussation (Fig. 5B,D). After crossing, the

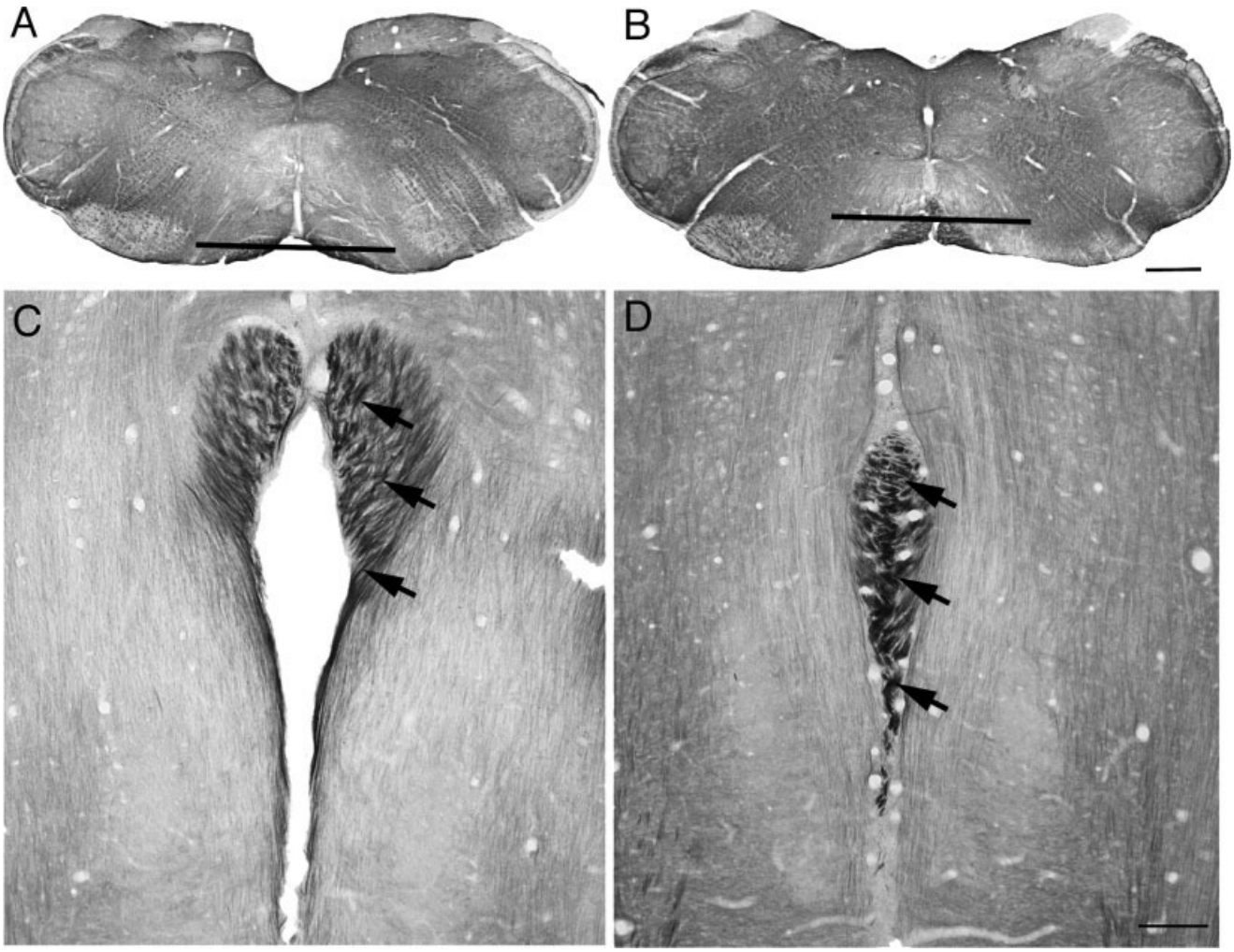

Fig. 5. L1-positive corticospinal tract detected in P14 coronal (A,B) and horizontal (C,D) brainstem sections at the level of the medullary pyramids. The lines in A and B mark the approximate area of horizontal sections in C and D, respectively. **A:** Distribution of L1 in a coronal section of the medulla with dense labeling in the ventral brainstem. **B:** A coronal brainstem section caudal to A shows intensely labeled L1-immunoreactive axon bundles in the ventral midline. **C:** L1-immunoreactive corticospinal axons (arrows) prior to crossing in the pyramidal tract of the medulla. **D:** A brainstem section dorsal to C shows L1-labeled fibers as they interdigitate during the pyramidal decussation (arrows). Scale bars = 400 μm in A,B; 200 μm in C,D.

fibers of the corticospinal tract could be followed toward the L1-labeled portion of the dorsal columns.

In P0 spinal cord sections the dorsal columns contained homogenous L1-labeled axons (Fig. 1A), while at older ages the L1-immunoreactive corticospinal axons could be distinguished in the ventral portion of the dorsal columns (Fig. 1B–D). Corticospinal tract axons were not detected at P0 but were strongly immunopositive at P7 in all but sacral spinal segments (data not shown). By P14, L1-labeled corticospinal tract axons were detected at all spinal cord levels (Fig. 2A–D). In addition to the corticospinal tract, the fasciculus gracilis in P7–21 sections was L1-immunoreactive and located in the dorsal part of the dorsal columns (Fig. 1B–D). At older postnatal ages (P21–365), the intensity of L1 expression in both the corticospinal tract and the fasciculus gracilis decreased considerably (Fig. 1D–F).

L1-positive axons are double-labeled with GAP-43 in postnatal spinal cord

To investigate the role of L1 in postnatal axonal growth and sprouting, we compared the expression of L1 immunoreactivity to that of GAP-43, a well-established marker of axonal growth and regeneration (Skene, 1989; Schreyer and Skene, 1991). The pattern of L1-labeled axons was similar to the pattern of GAP-43 expression in both P28 and adult (P365) spinal cord (Fig. 6). L1 and GAP-43 positive axons were concentrated in the superficial dorsal horn as well as the region of the corticospinal tract in P28 thoracic spinal cord (Fig. 6A,B). In addition, the L1-labeled axons within the fasciculus gracilis and cuneatus also expressed GAP-43 (Fig. 6C,D). Some dorsal commissural fibers and the crosscut axons that coursed next to the ependymal cells of the central canal (single arrows)

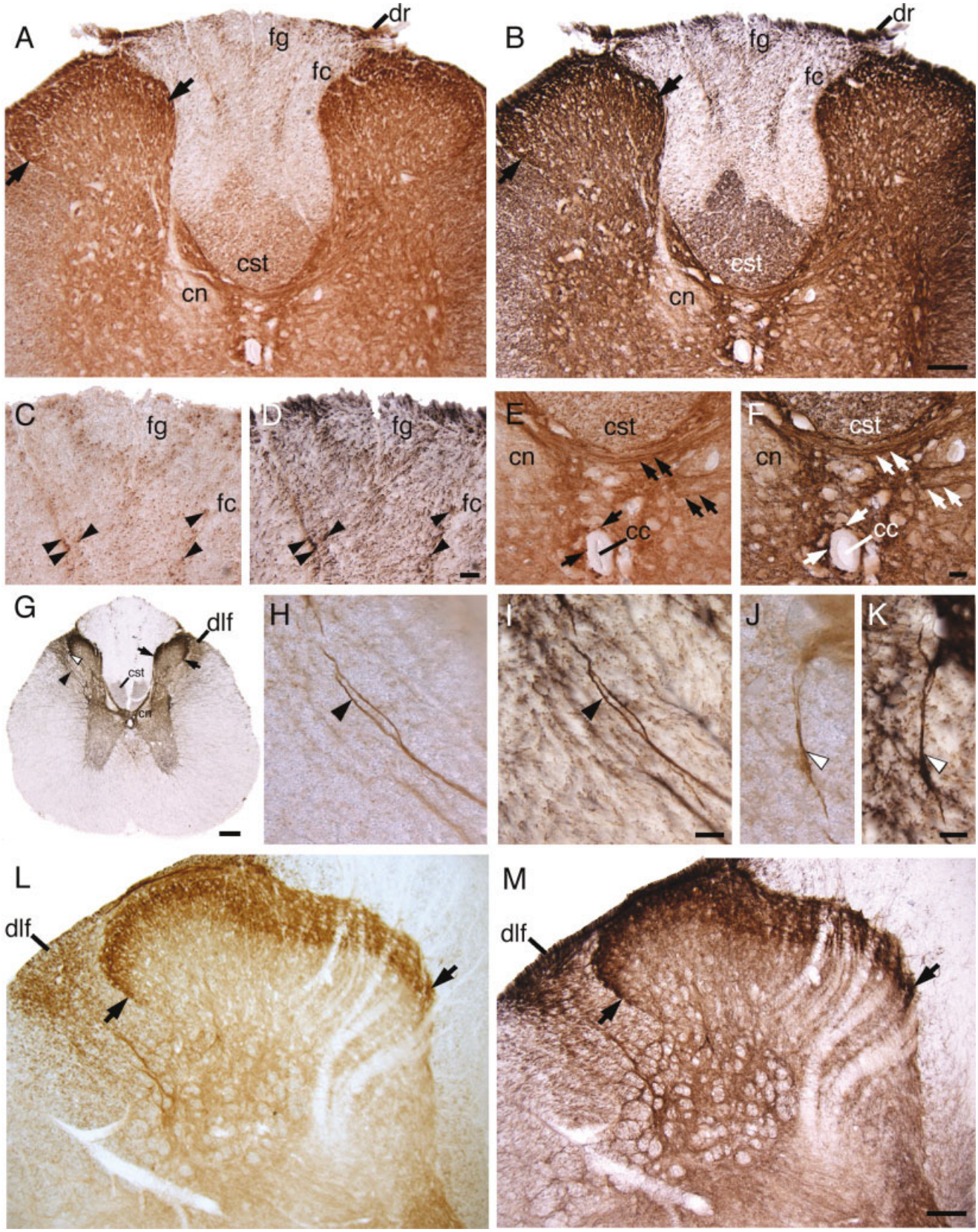
A
dr
fg
fc
cst
cn
B
dr
fg
fc
cst
cn
C
fg
fc
D
fg
fc
E
cst
cn
cc
F
cst
cn
cc
G
dlf
cst
cn
H
I
J
K
L
dlf
M
dlf

Figure 6

were colocalized with L1 and GAP-43 immunoreactivity (Fig. 6E,F).

When comparing L1- and subsequent GAP-43-labeled axons in P365 thoracic and lumbar spinal cord the same axonal populations appeared to be immunopositive, although there were generally more GAP-43 than L1-immunoreactive fibers identified. In the adult sections numerous L1 and GAP-43 double-labeled axons were detected in the laminae I–II of the dorsal horn and in the dorsolateral funiculus (Fig. 6G,L,M). At high magnification axons in several parts of the dorsolateral funiculus expressed both L1 and GAP-43 antigens (Fig. 6G–K).

DISCUSSION

In this study we report a dramatic decrease in L1 expression in the spinal cord funiculi during postnatal development. Our results also demonstrated novel L1-positive axons in the dorsolateral funiculus, Lissauer's tract, the midline of the ventral funiculus, and around the central canal at all postnatal ages. In addition, we presented evidence for a segmental gradient of L1 expression in older postnatal and adult spinal cord, with higher L1 expression at caudal compared to rostral levels. Furthermore, we demonstrated that most L1-immunoreactive fibers are colocalized with GAP-43 immunoreactivity.

L1 and GAP-43 are expressed on growing axons during postnatal development

Electron microscopy studies of cultured hippocampal neurons during early stages of neurite extension have localized L1 in growth cones and then showed that L1 expression increased during neurite outgrowth (Van Den Pol and Kim, 1993). Similarly, our findings in early postnatal neuropil also showed high levels of L1 expression on axons and preterminal-like structures at ages when axons would be elaborating processes. L1-labeled axon staining continued to be prominent between P7 and P28 in neuropil areas that contained the late-arriving axons of the corticospinal tract (Brosamle and Schwab, 1997). Then from P28 to adult ages, L1 immunoreactivity in the gray matter decreased to low but constant levels. These observations support the idea that L1 is expressed on immature axons well into postnatal development, possibly until they have completed their differentiation.

During embryonic development, L1-positive axons were distributed homogeneously throughout the funicular regions, the dorsal root, and the dorsal and ventral commissures (Stallcup et al., 1985; Beasley and Stallcup, 1987; Orlino et al., 2000; Tran and Phelps, 2000). However, during postnatal development the intensity of L1 immunoreactivity in the funiculi decreased dramatically with increasing age. While all funicular axons appeared to be L1-positive in embryos, only a few specific populations maintained their L1 immunoreactivity in adults.

Based on studies to date of adult nervous system regeneration, axon processes that are regenerating have been shown to express cell adhesion molecules that are commonly seen only during development. For example, in the peripheral nervous system L1 has been shown to be involved during regeneration (Nieke and Schachner, 1985; Martini and Schachner, 1988; Remsen et al., 1990). In addition, Aubert et al. (1998) showed that L1 expression was upregulated on regenerating cholinergic axons during axonal elongation. Furthermore, olfactory epithelial neurons that are generated continuously throughout life and their ensheathing glia both express L1 (Miragall et al., 1989; Doucette, 1990).

In this study, the L1-positive corticospinal tract axons were first detected and then maintained well into postnatal development. Based on Donatelle's tracing study (1977), the corticospinal axons were found to project into the dorsal funicular region of the lower cervical segments of the spinal cord by P1, into the mid-thoracic segments by P3, into the upper lumbar segments by P5, and into the coccygeal segments by P9. Our data detected distinct L1-immunoreactive corticospinal axons in all but sacral segments of P7 animals and throughout all segmental levels by P14, findings consistent with L1 expression on these axons as they grow caudally. Our findings of L1 expression in the corticospinal tract are consistent with those in previous reports by Joosten et al. (1990) and Joosten (1991) and suggest that L1 may be involved in fasciculation of this late-arriving pathway. The loss of L1-labeling in postnatal white matter, the upregulation of L1 on regenerating axons, and the increase in L1 expression associated with the late-developing corticospinal tract are all consistent with the interpretation that L1 is expressed on growing axons until they have established their appropriate connections.

To further investigate a possible role of L1 in axonal growth and sprouting we colocalized L1-immunoreactive axons with a well-established marker of axonal growth and regeneration, GAP-43 (Skene, 1989; Schreyer and

Fig. 6. An overlapping pattern of L1- (amber brown chromagen; A,C,E,H,J,L) and GAP-43-labeled (black chromagen; B,D,F,G,I,K,M) axons are found in P28 (A–F) and adult (P365; G–M) spinal cord. **A,B:** L1 immunoreactivity was conducted first, temporarily coverslipped with glycerin and photographed (A). Subsequently, GAP-43 immunoreactivity was carried out on the same section (B) and had a similar pattern to L1. Enlargements of the dorsal columns (C,D) and central canal region (E,F) are illustrated below. L1- and GAP-43-labeled axons are seen in the dorsal root (dr), the fasciculus gracilis (fg), the fasciculus cuneatus (fc), the dorsal corticospinal tract (cst), and the superficial laminae of the dorsal horn (between short arrows). **C,D:** High magnification of L1 only (C) and L1/GAP-43 (D) double-labeled axons in the dorsal columns. Arrowheads delineate colocalized crosscut fibers. **E,F:** High magnification of L1 only (E) and GAP-43 double-labeled (F) fibers in the dorsal commissure (double arrows) and around the ependymal cells of the central canal (cc; single arrows). Double-labeled axons are detected in the dorsal commissure (double arrows) and the corticospinal tract (cst). **G:** This P365 thoracic spinal cord was double-labeled with L1 and GAP-43 immunoreactivity. The dorsal horn (between arrows) and the dorsolateral funiculus (dlf) contain numerous double-labeled axons. The corticospinal tract (cst) is primarily GAP-43 positive and Clarke's nuclei (cn) are devoid of immunoreactivity. The location of the axons enlarged in panels H–K are designated by the black (H,I) and white (J,K) arrowheads. **H,I:** High magnification of axons (black arrowhead) traveling through the dorsolateral funiculus in G are L1-positive (H) and double-labeled with GAP-43 (I). **J,K:** A group of fasciculated L1-labeled (J) and L1/GAP-43 colocalized (I) fibers (white arrowhead) are detected in the dorsolateral funiculus just lateral to the dorsal horn in G. **L,M:** L1-immunoreactive axons are concentrated in laminae I–II (L; between short arrows) and in the dorsolateral funiculus (dlf) in this lumbar section. After subsequent labeling with GAP-43 the same dorsal horn structures are colocalized (M). Scale bars = 100 μm in B (applies to A,B); 25 μm in F (applies to C–F); 200 μm in G; 25 μm in I, K (applies to H–K); 100 μm in L (applies to L,M).

Skene, 1991). Studies on early axonal development have localized GAP-43 in the growth cones (Gorgels et al., 1989) and shown that overexpression of GAP-43 in adult mice increases axonal sprouting (Aigner et al., 1995). In addition, mice deficient in GAP-43 displayed errors in axonal pathfinding (Strittmatter et al., 1995). Studies of GAP-43 development in spinal cord have reported that levels of expression decrease with increasing postnatal age (Kapfhammer and Schwab, 1994). The expression pattern of GAP-43 detected in this study was very similar to that previously published (Gorgels et al., 1987, 1989; Schreyer and Skene, 1991; Kapfhammer and Schwab, 1994). In addition, GAP-43-labeled axons were colocalized with those expressing high levels of L1 in the superficial dorsal horn and in the dorsolateral funiculus. Colocalization of L1 and GAP-43 immunoreactivity offers further support for the role of L1 as a marker for developing and sprouting axons.

L1 is expressed on unmyelinated axons during postnatal development

The remarkable loss of L1 expression in axons within the white matter suggests that L1 expression may be downregulated during postnatal development, or alternatively, that L1 expression is covered up by myelin, rendering it undetectable (Stallcup et al., 1985; Beasley and Stallcup, 1987; Prince et al., 1991). During development, L1 is expressed on peripheral nervous system axons prior to the appearance of any myelin constituents (Martini and Schachner, 1986, 1988). The pattern observed after peripheral nervous system axon injury recapitulated the sequence observed during development; that is, L1 was detected on growing and regenerating axons and expressed until Schwann cell processes had wrapped ~1.5 loops of myelin around the axons (Martini and Schachner, 1986, 1988). When the L1 expression pattern in adult spinal cord was compared with immunohistochemical studies of myelin constituents (Schwab and Schnell, 1989; Coffey and McDermott, 1997), the regions previously reported to be devoid of myelin were similar to those that maintained L1 immunoreactivity in adults (present results). Schwab and Schnell (1989) reported that the adult cervical spinal cord lamina II, the dorsal commissure, and Lissauer's tract all remained relatively free of myelin or were myelinated relatively late. Additionally, Chung et al. (1979) have shown that many peripheral afferents are unmyelinated fibers and that Lissauer's tract is predominantly a primary afferent fiber system. In addition, studies on the dorsal and dorsolateral funiculi of the rat spinal cord indicated that these regions contain large numbers of unmyelinated axons (Chung et al., 1987). These same regions continue to express L1 in the adult spinal cord and most likely the normal adult L1 expression represents unmyelinated axons. Postnatal developmental studies on expression patterns of L1 and GAP-43 immunoreactivity also have reported an inverse relationship between GAP-43 and myelin basic protein (Kapfhammer and Schwab, 1994).

Our data illustrated low levels of L1 immunoreactivity in the corticospinal tract at P28 and in adult ages. In addition, when comparing our data to expression of the myelin constituent data (Schwab and Schnell, 1989), a related pattern emerged. Schwab and Schnell (1989) indicated that myelin-specific antigens were detected in the dorsal corticospinal tract at around P10–11, and we noticed a slight decrease in L1 expression of the dorsal corticospinal tract by P14. Furthermore, the level of expression of myelin specific antigens continued to increase in the P14–28 dorsal corticospinal tract (Schwab and Schnell, 1989), while the level of L1 expression decreased (present results). These observations support the idea that L1 can no longer be detected on axons after myelination, a hypothesis that requires further investigation.

Gradients of L1 expression

Our data demonstrated a caudal-to-rostral gradient in the intensity of L1 expression in both the white and gray matter regions in adult spinal cord. The differences between L1 immunoreactivity in the cervical and lumbar levels were unexpected and suggest that these findings reflect major variations in the axonal organization between different segmental levels. One possible explanation for these differences is that the projection patterns of the dorsal root ganglion neurons vary substantially between rostral and caudal spinal cord. Certainly, distinctive features of L1 expression in the sacral spinal cord most likely reflect the unique visceral primary afferent distribution in the caudal spinal cord (Morgan et al., 1981; Nadelhaft and Booth, 1984; Grant, 1995).

Alternatively, this *caudal*-to-rostral gradient of L1 expression could be explained by the presence of a *rostral*-to-caudal gradient of myelination in developing postnatal spinal cord. Schwab and Schnell (1989) demonstrated a rostral-to-caudal gradient in the expression of the myelin-specific antigens on both ascending and descending fiber tracts. In addition, when examining the number of myelinated and unmyelinated axons in the dorsolateral funiculus, Chung and Coggeshall (1983) demonstrated that unmyelinated axons predominate over myelinated axons in the white matter region of sacral spinal cord. Together, these data correlate with the idea that myelination may cover up the L1 immunoreactivity on developing axons.

When examining the L1 expression pattern in the white matter regions, our data demonstrated increased L1-labeling along the perimeter in P7–21 spinal cord segments compared to the inner funicular regions. This outer-to-inner gradient suggests that new axons maybe added preferentially at the perimeter of fasciculated bundles as the white matter regions grow in size with age. Similar findings in the optic tract have also shown newly generated axons situated superficially (Colello and Guillery, 1992).

By P14, our data indicated a *dorsal*-to-ventral gradient of L1 expression with higher levels of L1 immunoreactivity concentrated in the superficial dorsal horn region compared to more ventral regions. The high levels of L1 maintained in the dorsal horn most likely originated from dorsal root ganglion cells for the following reasons. First, we detected L1-positive axons entering in the dorsal root and branching into the superficial dorsal horn and along the outer rim of the dorsal columns. Second, the high levels of L1 immunoreactivity on axons in the superficial dorsal horn, in Lissauer's tract, and in the dorsal commissure are consistent with known targets and pathways of primary sensory afferents (Smith, 1983). As discussed above, these dorsal spinal areas are known to contain numerous unmyelinated axons, reflecting the myelination gradient present in the postnatal rat spinal cord (Coffey and McDermott, 1997).

In conclusion, high levels of L1 expression characterized the early postnatal spinal cord, whereas adult levels were low and the specific L1-labeling appeared to correlate with areas containing unmyelinated axons. The temporal pattern of L1 expression in the late-arriving corticospinal tract as well as the high expression levels during development both suggest an interpretation that L1 is expressed continuously in newly growing axons. In addition, the colocalization of L1 and GAP-43 further supports the role of L1 during axonal growth. Thus, we believe that L1 may serve as an indicator of axon growth as well as axonal sprouting and regeneration. Ongoing experiments are currently addressing whether spinal cord axons will reexpress L1 following injury.

ACKNOWLEDGMENTS

We thank Marc D. Kubasak for help with perfusions, Dr. Tracy S. Tran for assistance with the early experiments, and Dr. Ellen Carpenter for helpful suggestions on the article.

LITERATURE CITED

Aigner L, Arber S, Kapfhammer JP, Laux T, Schneider C, Botteri F, Brenner HR, Caroni P. 1995. Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell 83:269–278.

Appel F, Holm J, Conscience J-F, Schachner M. 1993. Several extracellular domains of the neural cell adhesion molecule L1 are involved in neurite outgrowth and cell body adhesion. J Neurosci 13:4764–4775.

Aubert I, Ridet J-L, Schachner M, Rougon G, Gage FH. 1998. Expression of L1 and PSA during sprouting and regeneration in the adult hippocampal formation. J Comp Neurol 399:1–19.

Beasley LL, Stallcup WB. 1987. The nerve growth factor-inducible large external (NILE) glycoprotein and neural cell adhesion molecule (N-CAM) have distinct patterns of expression in the developing rat central nervous system. J Neurosci 7:708–715.

Bieber AJ, Snow PM, Hortsch M, Patel NH, Jacobs JR, Traquina ZR, Schilling J, Goodman CS. 1989. Drosophila neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1. Cell 59:447–460.

Bock E, Richter-Landsberg C, Faissner A, Schachner M. 1985. Demonstration of immunochemical identity between the nerve growth factor-inducible large external (NILE) glycoprotein and the cell adhesion molecule L1. EMBO J 4:2765–2768.

Brosamle C, Schwab ME. 1997. Cells of origin, course, and termination patterns of the ventral, uncrossed component of the mature rat corticospinal tract. J Comp Neurol 386:293–303.

Brummendorf T, Rathjen FG. 1993. Axonal glycoproteins with immunoglobulin- and fibronectin type III-related domains in vertebrates: structural features, binding activities, and signal transduction. J Neurochem 61:1207–1219.

Burden-Gulley SM, Payne HR, Lemmon V. 1995. Growth cones are actively influenced by substrate-bound adhesion molecules. J Neurosci 15: 4370–4381.

Chung K, Coggeshall RE. 1983. Numbers of axons in lateral and ventral funiculi of rat sacral spinal cord. J Comp Neurol 214:72–78.

Chung K, Langford LA, Applehaum AE, Coggeshall RE. 1979. Primary afferent fibers in the tract of Lissauer in the rat. J Comp Neurol 184:587–598.

Chung K, Langford LA, Coggeshall RE. 1987. Primary afferent and propriospinal fibers in the rat dorsal and dorsolateral funiculi. J Comp Neurol 263:68–75.

Coffey JC, McDermott KW. 1997. The regional distribution of myelin oligodendrocyte glycoprotein (MOG) in the developing rat CNS: an in vivo immunohistochemical study. J Neurocytol 26:149–161.

Cohen NR, Taylor JSH, Scott LB, Guillery RW, Soriano P, Furley AJW. 1997. Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. Curr Biol 8:26–33.

Colello RJ, Guillery RW. 1992. Observations on the early development of the optic nerve and tract of the mouse. J Comp Neurol 317:357–378.

Dahme M, Bartsch U, Martini R, Anliker B, Schachner M, Mantei N. 1997. Disruption of the mouse *L1* gene leads to malformations of the nervous system. Nat Genet 17:346–349.

Donatelle JM. 1977. Growth of the corticospinal tract and the development of placing reactions in the postnatal rat. J Comp Neurol 175:207–232.

Doucette R. 1990. Glial influences on axonal growth in the primary olfactory system. Glia 3:433–449.

Fischer G, Kunemund V, Schachner M. 1986. Neurite outgrowth patterns in cerebellar microexplant cultures are affected by antibodies to the cell surface glycoprotein L1. J Neurosci 6:605–612.

Fransen E, Lemmon V, Camp GV, Vits L, Coucke P, Willems PJ. 1995. CRASH syndrome: clinical spectrum of corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraparesis and hydrocephalus due to mutations in one single gene. Eur J Hum Genet 3:273–284.

Fransen E, Camp GV, Willems PJ. 1997. L1-associated diseases: clinical geneticists divide, molecular geneticists unite. Hum Mol Genet 6:1625–1632.

Gorgels TG, Oestreicher AB, de Kort EJ, Gispen WH. 1987. Immunocytochemical distribution of the protein kinase C substrate B-50 (GAP43) in developing rat pyramidal tract. Neurosci Lett 83:59–64.

Gorgels TG, Campagne MVL, Oestreicher AB, Gribnau AAM, Gispen, WH. 1989. B-50/GAP43 is localized at the cytoplasmic side of the plasma membrane in developing and adult rat pyramidal tract. J of Neurosci 9:3861–3869.

Graf WD, Born DE, Shaw DWW, Thomas JR, Holloway LW, Michaelis RC. 2000. Diffusion-weighted magnetic resonance imaging in boys with neural cell adhesion molecule L1 mutations and congenital hydrocephalus. Ann Neurol 47:113–117.

Grant G. 1995. Primary afferent projections to the spinal cord. In: Paxinos G, editor. The rat nervous system, 2nd ed. New York: Academic Press. p 61–66.

Grumet M, Edelman GM. 1988. Neuron-glia cell adhesion molecule interacts with neurons and astroglia via different binding mechanisms. J Cell Biol 106:487–503.

Hlavin ML, Lemmon V. 1991. Molecular structure and functional testing of human L1CAM: an interspecies comparison. Genomics 11:416–423.

Jessell TM. 1988. Adhesion molecules and the hierarchy of neural development. Neuron 1:3–13.

Joosten EAJ. 1991. Immuno-electronmicroscopic visualization of cell adhesion molecule L1 in adult rat pyramidal tract: localization on neuronal and oligodendrocytic processes. Brain Res 546:155–160.

Joosten EAJ, Gribnau AAM, Gorgels TGMF. 1990. Immunoelectron microscopic localization of cell adhesion molecule L1 in developing rat pyramidal tract. Neuroscience 38:675–686.

Kamiguchi H, Hlavin ML, Lemmon V. 1998a. Role of L1 in neural development: What the knockouts tell us. Mol Cell Neurosci 12:48–55.

Kamiguchi H, Hlavin ML, Yamasaki M, Lemmon V. 1998b. Adhesion molecules and inherited diseases of the human nervous system. Annu Rev Neurosci 21:97–125.

Kapfhammer JP, Schwab ME. 1994. Inverse patterns of myelination and GAP-43 expression in the adult CNS: neurite growth inhibitors as regulators of neuronal plasticity? J Comp Neurol 340:194–206.

Lagenaur C, Lemmon V. 1987. An L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension. Proc Natl Acad Sci U S A 84:7753–7757.

Lemmon V, Farr KL, Lagenaur C. 1989. L1-mediated axon outgrowth occurs via a homophilic binding mechanism. Neuron 2:1597–1603.

Martini R, Schachner M. 1986. Immunoelectron microscopic localization of neural cell adhesion molecule (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. J Cell Biol 103:2439–2448.

Martini R, Schachner M. 1988. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and myelin-associated glycoprotein) in regenerating adult mouse sciatic nerve. J Cell Biol 106:1735–1746.

Miragall F, Kadmon G, Schachner M. 1989. Expression of L1 and N-CAM cell adhesion molecules during development of the mouse olfactory system. Dev Biol 135:272–286.

Moos M, Tacke R, Scherer H, Teplow D, Fruh K, Schachner M. 1988. Neural cell adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature 334: 701–703.

Morgan C, Nadelhaft I, Groat WC. 1981. The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J Comp Neurol 201:415–440.

Nadelhaft I, Booth AM. 1984. The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study. J Comp Neurol 226:238–245.

Nieke J, Schachner M. 1985. Expression of the neural cell adhesion molecules L1 and N-CAM and their common carbohydrate epitope L2/HNK-1 during development and after transection of the mouse sciatic nerve. Differentiation 30:141–151.

Orlino EN, Wong CM, Phelps PE. 2000. L1 and GAD65 are expressed on dorsal commissural axons in embryonic rat spinal cord. Dev Brain Res 125:117–130.

Prince JT, Alberti L, Healy PA, Nauman SJ, Stallcup WB. 1991. Molecular cloning of NILE glycoprotein and evidence for its continued expression in mature rat CNS. J Neurosci Res 30:567–581.

Rader C, Kunz B, Lierheimer R, Giger RJ, Berger P, Tittmann P, Gross H, Sonderegger P. 1996. Implications for the domain arrangement of axonin-1 derived from the mapping of its NgCAM binding site. EMBO J 15:2056–2068.

Ramón y Cajal S. 1937. Recollections of my life — Santiago Ramón y Cajal. Cambridge, MA: MIT Press.

Rathjen FG, Schachner M. 1984. Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. EMBO J 3:1–10.

Remsen LG, Strain GM, Newman MJ, Satterlee N, Daniloff JK. 1990. Antibodies to the neural cell adhesion molecule disrupt functional recovery in injured nerves. Exp Neurol 110:268–273.

Schreyer DJ, Skene PJH. 1991. Fate of GAP-43 in ascending spinal axons of DRG neurons after peripheral nerve injury: Delayed accumulation and correlation with regenerative potential. J Neurosci 11:3738–3751.

Schwab ME, Schnell L. 1989. Region-specific appearance of myelin constituents in the developing rat spinal cord. J Neurocytol 18:161–169.

Shiga T, Oppenheim RW. 1991. Immunolocalization studies of putative guidance molecules used by axons and growth cones of intersegmental interneurons in the chick embryo spinal cord. J Comp Neurol 310:234–252.

Skene PJH. 1989. Axonal growth-associated protein. Annu Rev Neurosci 12:127–156.

Smith CL. 1983. The development and postnatal organization of primary afferent projections to the rat thoracic spinal cord. J Comp Neurol 220:29–43.

Stallcup WB, Beasley LL, Levine JM. 1985. Antibody against nerve growth factor-inducible large external (NILE) glycoprotein labels nerve fiber tracts in the developing rat nervous system. J Neurosci 5:1090–1101.

Stoeckli ET, Landmesser LT. 1995. Axonin-1, Nr-CAM, and Ng-CAM play different roles in the in vivo guidance of chick commissural neurons. Neuron 14:1165–1179.

Strittmatter SM, Fankhauser C, Huang PL, Mashimo H, Fishman MC. 1995. Neuronal pathfinding is abnormal in mice lacking the neuronal growth cone protein GAP-43. Cell 80:445–452.

Sweadner KJ. 1983. Post-translational modification and evoked release of two large surface proteins of sympathetic neurons. J Neurosci 3:2504–2517.

Thiery JP, Delouvee A, Grumet M, Edelman GM. 1985. Initial appearance and regional distribution of the neuron-glia cell adhesion molecule in the chick embryo. J Cell Biol 100:442–456.

Tongiorgi E, Bernhardt RR, Schachner M. 1995. Zebrafish neurons express two L1-related molecules during early axonogenesis. J Neurosci Res 42:547–561.

Tran TS, Phelps PE. 2000. Axons crossing in the ventral commissure express L1 and GAD65 in the developing rat spinal cord. Dev Neurosci 22:228–236.

Van Den Pol AN, Kim WT. 1993. NILE/L1 and NCAM-polysialic acid expression on growing axons of isolated neurons. J Comp Neurol 332:237–257.