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The development of the lateral spinothalamic tract in rats and its response to lesion

Miya, Dorene Yone, Ph.D.

City University of New York, 1993

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THE DEVELOPMENT OF THE LATERAL SPINOTHALAMIC TRACT IN RATS AND ITS RESPONSE TO LESION

By

DORENE Y. MIYA

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

THE DEVELOPMENT OF THE LATERAL SPINOTHALAMIC TRACT IN RATS AND ITS RESPONSE TO LESION

by

Dorene Y. Miya

Adviser: Professor Gordon A. Barr

The aim of these experiments was to examine the maturation of the ascending lateral spinothalamic tract (LSTT) in fetal, neonatal and young rats and to examine the LSTT response to lesion relative to its development. In experiment 1, WGA-HRP and green fluorescent microspheres were injected into the lateral ventrobasal nucleus of the thalamus in rats on fetal day 18 or 19, the day of birth and postnatal day 10. The results show that the LSTT projects to the ventrobasal thalamus by the earliest day examined, fetal day 18. WGA-HRP and microspheres both labeled cells contralateral to the injection in the ventromedial dorsal horn and superficial lamina of the lumbar spinal cord in all age groups examined. Postnatal animals also showed labeling in the neck of the dorsal horn. In fetal animals only, WGA-HRP faintly labeled a tract in the contralateral lateral funiculus of the dorsal horn. At this age, faint labeling was also seen in the ipsilateral medial lemniscus and most lateral portion of the contralateral principal sensory trigeminal nucleus.

Di-I was used as a postfixation anterograde tract tracer in experiment 2 and showed that the LSTT had developed at least to the level of the cervical spinal cord by fetal day 17, the earliest age examined.

Experiment 3 examined the anatomical and behavioral consequence of lesions that are made to the spinal cord at different developmental stages of the LSTT. Two age groups of animals (fetal day 17 and postnatal day 12 or 14) underwent a lateral hemisection of the thoracic spinal cord. Both groups of animals underwent behavioral testing approximately 72 hours after surgery and again at postnatal day 47. At the end of the survival period, all animals were injected with WGA-HRP into the ventrobasal thalamus. Morphologically, both groups showed major dorsal horn disorganization caudal to and at the site of lesion. Behaviorally, both groups were also similar in their severe impairment in responding to noxious tests and no major differences were noted. Replication with WGA-HRP is necessary to make conclusions as to the plasticity of the LSTT in developing animals.

Acknowledgements

I will never think of this dissertation as complete until this last page has been written, and because of that, I never though that it would be. But now, that it's all tested, lesioned, written and revised I need to thank a few people before I can truly close this chapter.

I would like to thank many of the people that I have gotten a chance to work with over the last 7 years who made it very bearable, always providing me with encouragement and friendship. I would especially like to thank Dr. Harry Hughes who I think really understood how truly hard it was for me but never let me quit. I will always be grateful to him for that.

To the gifted professors that I met in New York who have always been there to help me and to push me, I would like to thank-you all. I would like to especially thank the members of my committee, Dr. H. Phillip Zeigler, Dr. James Gordon, Dr. Marion Murray and Dr. William Smotherman. I would also like to thank Dr. Sheila Chase for her support. May I be half as instrumental in inspiring someone else's career as you all have been in inspiring mine.

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Every minute of this thesis and anyother endeavor that I am proud to have accomplished is dedicated to Kanjiro Sumi. Thank-you.

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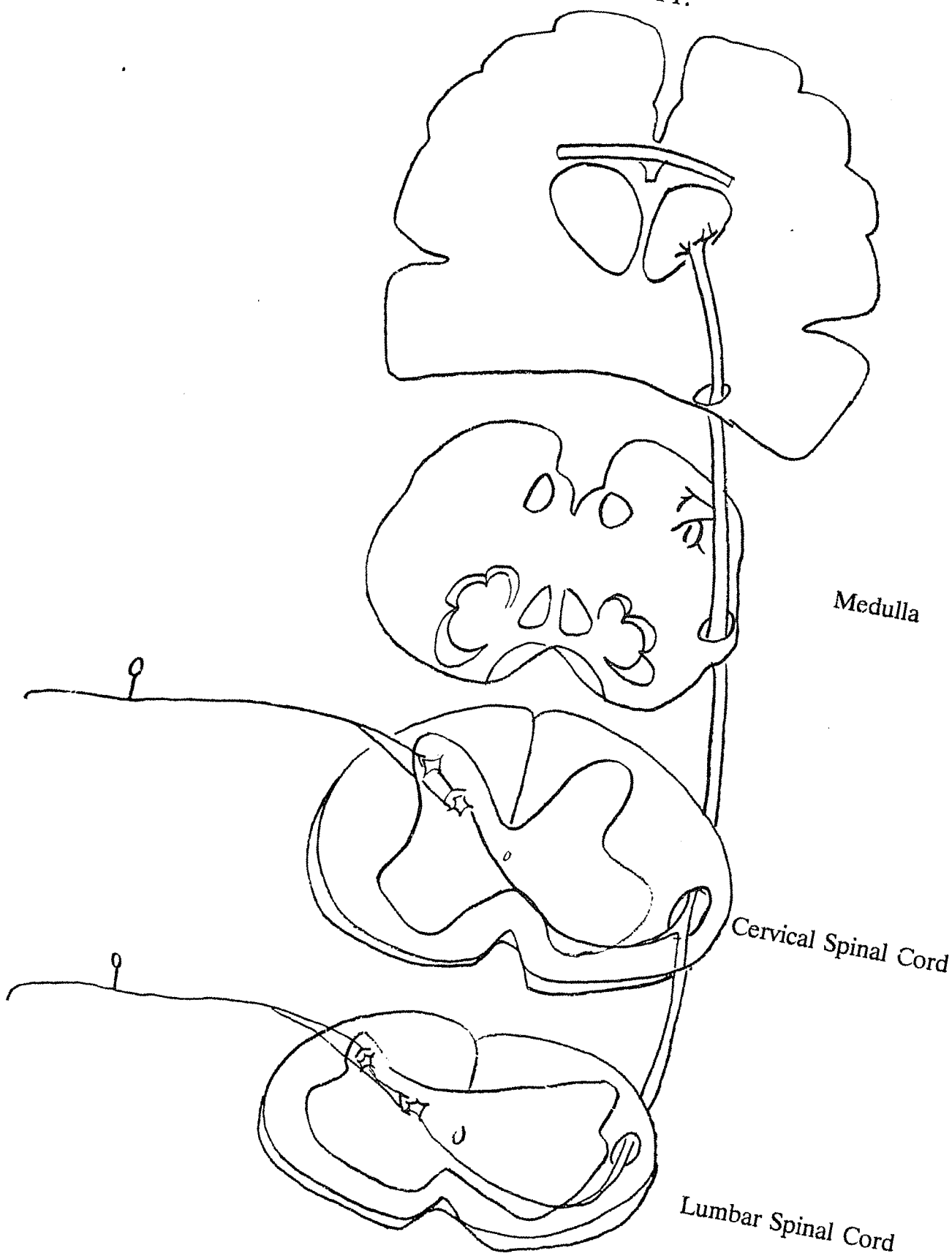
GENERAL INTRODUCTION

The overall goal of these experiments was to clarify the relationship between developmental status and spinal cord lesion. In order to accomplish this the lateral spinothalamic tract (LSTT) was used as the model system. First, its development in fetal, neonatal and young rats is described. Second, the effects of spinal cord lesion both before and after LSTT maturity can were assessed. It is the hypothesis of these experiments that anatomical plasticity is dependent on the developmental status of the system in question and not on the overall maturity of the animal. In general, understanding the development of any system will provide guides to understanding the organization of that system. In studying issues of anatomical plasticity, the developing animal as a model system may provide clues as to the role that ontogenetic factors may have in the regulation of functional CNS regeneration in the adult mammal.

The lateral spinothalamic tract (LSTT) was used as the model system in these experiments for several reasons: 1) in the adult rat, the anatomy and physiology of this system has been studied extensively and has been shown to be quite distinct, 2) it is a functionally significant system mediating pain nociceptive behavior, 3) the procedures and calibration of behavioral tests

Figure 1. Schematic Overview of the LSTT.



measuring pain nociception have been well established and studied by this lab, and 4) there has been little research on the development of ascending spinal systems in general, and no work on the development of the LSTT.

Descriptive overview of the ascending pain system

The organization of pain systems is very similar from species to species and consists of serial chains of neurons that link the periphery with the spinal cord, brain stem, thalamus and cerebral cortex (see Fig.1). Other aspects of sensation, such as touch and vibration, are processed in different but parallel systems.

Contact with the external environment occurs through specialized sensory receptors. For painful stimuli this occurs through specialized sensory receptors called nociceptors. There are three different types of nociceptors that respond to the three different types of damaging, noxious stimuli. Mechanical nociceptors respond to strong mechanical stimulation such as a pinch or a pinprick. Temperature nociceptors are activated by intense heat or cold stimuli. The third class of nociceptors, polymodal nociceptors, respond to both of the above stimuli as well as to noxious sensations caused by chemical stimlation (Burgess & Perl, 1973). All of the above nociceptors are bare nerve endings predominantly connected to axons of two fiber classes, Aδ and C. Both of these types of fibers are considered to

be of relatively fine diameter, with a slower conducting rate compared to the larger myelinated Aα or Aβ fibers that are associated with tactile sensation and proprioception.

Primary afferent fibers enter the spinal cord through the dorsal root ganglia at its dorsolateral margin. Once in the spinal cord, these dorsal root fibers carrying pain sensations pass into the zone of Lissauer which is located dorsal and lateral to the most superficial lamina of the dorsal horn. In adult animals, the medial and lateral boundaries of the zone of Lissauer are clearly indicated by the large, myelinated proprioceptive and tactile fibers. The fibers from the zone or tract of Lissauer ascend or descend one or two segments away from the cell bodies of origin and terminate in the upper two-thirds of the dorsal horn, mainly in lamina I-III, known respectively as the marginal zone and substantia gelatinosa. This marks the beginning of the LSTT. These second order fibers immediately cross in the spinal cord to the contralateral side and ascend in the anterolateral quadrant of the lateral white column.

On its ascent to the thalamus, the LSTT assumes a lateral position in the medulla, and some axons give off collaterals or terminate in the reticular formation. Known as the spinoreticular tract, this tract is important in the sensation of slow pain mediated by C fibers and in the relay of information

to the midbrain periaqueductal gray (PAG). The PAG, which surrounds the cerebral aqueduct is also known to be important as part of a descending pathway that modulates noxious information. As the fibers of the LSTT ascends through the pons and midbrain, they take on a more medial course before terminating in the ventral posterior lateral (VPL) nucleus of thalamus.

The anatomy of the lateral spinothalamic tract (LSTT)

The spinothalamic tract (STT) has been a subject of enduring interest to both basic and clinical scientists since Gowers (1878) first described its importance in the transmission of pain and temperature sensations in victims of spinal gunshot wounds. It was described experimentally by Mott (1895) as a long fiber bundle that ascends in the anterolateral quadrant (ALQ) of the spinal cord, terminating in the thalamus of the monkey. Surgical anterolateral cordotomy was used first by Spiller & Martin (1912) to alleviate chronic pain and they confirmed the importance of this area of spinal cord in pain transmission (Walker, 1940; White & Sweet, 1955; Kerr & Lippman, 1974).

Degeneration techniques demonstrated that the STT sends a sizeable portion of its fibers to nuclei within both the medial and lateral thalamus in a number of different species (Clark, 1936; Anderson & Berry, 1959;

Mehler, Feferman & Nauta, 1960; Bowsher, 1957; Lund & Webster, 1967; Mehler, 1969; Boivie, 1971; Rockel, Heath & Jones, 1972; Zemlan, Leonard, Kow & Pfaff, 1978; Boivie, 1979). Modern electrophysiological techniques, utilizing antidromic activation from the thalamus, allowed for the examination of the individual spinal cord cells of origin (Dilly, Wall & Webster, 1968). It was found that the STT arises from cells of laminae I, IV, V, VI and VII in the lumbar portion of the contralateral spinal cord in the monkey; in the cat lumbar cord the STT originates primarily from laminae I, VII and VIII and receives little contribution from laminae IV-VI. Giesler, Menetrey, Guilbaud & Besson (1976) demonstrated that the lumbar component of the rat STT more closely resembled that of the monkey than that of the cat.

Small injections of HRP were made to the ventrobasal complex (VPL and VPM) of the thalamus in the rat to identify the cells of origin of the lateral projecting spinothalamic tract (Giesler et al., 1979). Spinal cord neurons were labeled contralaterally in the superficial laminae (laminae I & II), a zone known to receive thermal and nociceptive inputs (Kuru, 1949; Willis & Coggeshall, 1978), and in the intermediate gray zone (IGZ) of lamina VII in all regions of the spinal cord. In upper cervical segments (C1-C3), cells were also encountered contralaterally in the internal basilar nucleus (IBN). Cells were also found in the contralateral lateral cervical nucleus

(LCN). This area is located in the dorsolateral funiculus, immediately ventral to the lateral aspect of the dorsal horn. In the cervical enlargement, cells were found contralaterally in nucleus proprius located at the neck of the dorsal horn, as well as in the superficial laminae. The thoracic segments had very little labeling, occurring in the nucleus proprius and IGZ. Many more cells were found in the lumbar enlargement than in the cervical enlargement. These cells were also contralateral to the injection and were located throughout the dorsal horn, intermediate gray zone and VMDH.

In summary, in the rat, the axons of the LSTT originate from neurons whose cell bodies lie in the dorsal 2/3 of the dorsal horn and deep gray matter from all segments of the spinal cord, but especially from the upper cervical and the lumbar regions.

The axons of the LSTT immediately cross in the spinal cord and ascend in the anterolateral quadrant (ALQ) of the cord, ascending through the lateral portion of the brainstem to the lateral ventrobasal complex (VPL) (Giesler, Menetrey, Guilbaud & Besson, 1976; Giesler, Menetrey & Basbaum, 1979; Peschanski, Mantyh & Besson, 1983). In addition, Giesler et al. (1981) found that, as the LSTT ascends through the cord, it assumes a more lateral position within the ALQ. At lumbar levels, axons are distributed throughout the ALQ; within the thoracic cord they are

distributed throughout the ALQ but are not found in such close proximity to the ventral horn. As they reach cervical regions, they are more tightly organized in the lateral most portion of the ALQ. Although the anatomy (and physiology) of the rat LSTT is similar to that of the monkey, axons destined for lateral and medial thalamic nuclei in the monkey are mixed and ascend within both the ventral funiculus and the ALQ (Mehler, 1969; Boivie, 1979).

In studying the differential origins of the LSTT and the medial projecting STT (MSTT), Giesler et al. (1979) noted that the lumbar cord cells that were labeled in the ventromedial dorsal horn were less densely filled with HRP than other labeled spinal cord cells after injections were made to either the ventrobasal or medial thalamus. This finding suggested that these neurons send axons that may bifurcate, giving off collaterals to other structures. In order to study the above possibility, Kevetter & Willis (1982, 1983) injected rats concurrently with Fast Blue or Nuclear Yellow into the medial (intralaminar) thalamus and with HRP into the ventrobasal complex of the same side. In another group of animals, injections were also made to the reticular formation. About 15-20% of the STT cells projected to both the medial and ventrobasal thalamus and most of these cells were concentrated bilaterally in the upper cervical segments and contralaterally in the ventromedial dorsal horn of the lumbar cord. To a much lesser

extent, Kevetter & Willis (1983) also found that a larger proportion of STT neurons that project to the medial thalamic structures, rather than to the ventrobasal complex, send collaterals to the reticular formation. In agreement with the findings of Giesler et al., (1979), it was concluded that the MSTT and the LSTT are largely distinct projections in the rat and that the LSTT is probably a direct pathway arising from the spinal cord dorsal horn and terminating in the ventrobasal thalamus.

Harmann, Carlton & Willis (1988) injected Diamidino Yellow and Fast Blue into the ventrobasal thalamus and periaqueductal gray (PAG), respectively, in order to study the distribution of LSTT neurons that project to both areas. An average of 1.4% of all LSTT neurons send collaterals to the PAG. These double-labeled cells were observed in the area of the VMDH (lamina V) and in the marginal zone (lamina I) of only the cervical and lumbar segments of the spinal cord. There was a shift in the concentration of double-labeled cells from lamina I in cervical levels to lamina V in lumbar levels.

The thalamic target of the LSTT, the ventrobasal complex, is also the target of the tactile sensing dorsal column-medial lemniscal system (Lund & Webster, 1967; Feldman & Kruger, 1980). In order to determine if there is an overlap of terminations between these two ascending pathways or if

there is a segregation of terminations, Ma, Peschanski & Besson (1986), used both tritiated leucine and WGA-HRP in a double-labelling study. It was found that the areas of termination for both the dorsal column-medial lemniscal pathway and the LSTT do overlap in the lateral portion of the ventrobasal complex (VPL). The overlap appears to be somatotopically organized, indicating that the same area of the ventrobasal complex receives somatic inputs from the same part of the body through both pathways.

Another tract of that terminates in the ventrobasal complex is the trigeminothalamic tract. Although this tract, which originates from the principal trigeminal nucleus, ascends within the medial lemniscus, studies have shown that it terminates in the medial aspect of the ventrobasal complex (VPM) (Belford & Killackey, 1978, 1979).

In summary, the LSTT originates from spinal cord cells in the marginal zone (lamina I) and dorsal nucleus proprius (lamina V), intermediate gray zone (lamina VII) and ventromedial aspect of the dorsal horn from all levels, but especially from upper cervical and lumbar segments. The axons immediately cross in the spinal cord within 1-2 segments and ascend in the ALQ as a diffuse bundle, becoming more tightly organized to the outer edge of the ALQ as they ascend to cervical levels. As this tract travels

through the lateral aspect of the brainstem, a few collaterals are given off to the reticular formation and lateral PAG, before terminating in the lateral portion of the ventrobasal complex, coincident with the terminal region of the dorsal column-medial lemniscal system.

Physiology of the lateral spinothalamic tract

Because of clinical demonstrations that an anterolateral cordotomy induces hypoanalgesia in the opposite side of the body in humans, numerous data have confirmed that the fiber tracts contained in this portion of the cord are necessary for the sensori-discriminative aspects of pain sensation (White & Sweet, 1955). Also, because it has been known from the classical studies of Poggio & Mountcastle (1960) that there are neurons in the posterior nuclear group of the thalamus that respond to noxious stimulation, the LSTT has been suspected as the major pathway in the transmission of noxious messages to the brain.

The involvement of the rat's LSTT in the pain-signaling system was first suggested by the electrophysiological experiments of Giesler, Menetrey, Gulbaud & Besson (1976). Antidromic activation from the thalamus (Dilly et al. 1968) was used to identify the neurons at the origin of this tract in the lumbar portion of the spinal cord. They found cells located in the superficial layers (lamina I & II) and neck of the dorsal horn (lamina IV-

V) that responded to noxious stimuli. Cells that responded to non-noxious stimuli, such as a touch to the paw with a camel-hair brush, were also identified but were also powerfully activated by noxious stimuli. These results are very similar to several reports in which the antidromic activation technique was used to identify and study the cells of origin to the primate LSTT (Trevino, Coulter & Willis, 1973; Applebaum, Beall, Foreman & Willis, 1975; Foreman, Applebaum, Beall, Trevino & Willis, 1975).

In other electrophysiological studies (Guilbaud, Peschanski, Gautron & Binder, 1979; Peschanski, Guilbaud, Gautron & Besson, 1980), the response of the ventrobasal thalamic neurons to noxious mechanical and thermal stimuli were studied. In the rat, ventrobasal neurons activated by noxious mechanical stimuli are also responsive to noxious thermal stimuli. By plunging the tail of a rat into a temperature-controlled hot water bath, Peschanski et al. (1980), found that 70% of the identified neurons encoded stimulus intensity by frequency of discharge. Some units also responded to a stimulus increase by the recruitment of other cells for temperatures up to 60°C. Response thresholds varied between 40° and 50°C. However, some ventrobasal neurons are clearly activated by non-noxious stimuli. This result, first discovered by Giesler et al., (1976) as mentioned above and again by Guilbaud, Peschanski, Gautron & Binder (1980), is probably due to the lack of segregation of LSTT and dorsal column-medial lemniscal

afferents in the ventrobasal complex. In order to separate the behavioral and electrophysiological correlates of these two somatosensory tracts, the following studies were undertaken.

Peschanski, Briand, Gautron & Guilbaud (1985) studied the electrophysiological responses to noxious mechanical (pinches) and thermal (50°C water bath) stimuli, as well as to non-noxious (brushing, pressure) stimuli applied to the hindpaw in the ventrobasal complex of the rat thalamus, both before and after lesions were made to various areas of the spinal cord. This study specifically examined the crossed LSTT, the uncrossed spinoreticular tract that also travels in the anterolateral quadrant, the uncrossed spinocervical tract and the dorsal column-medial lemniscal system that is uncrossed and ascends in the dorsolateral and dorsal portion of the cord, respectively. They found that lesions of the dorsal and dorsolateral portion of the cord failed to eliminate the ventrobasal neuronal response to noxious stimulation. In contrast, unilateral lesions of one anterolateral quadrant eliminated the ventrobasal responses to both mechanical and thermal stimuli of the contralateral hindpaw, strongly indicating that the LSTT is necessary for neuronal responses to noxious stimuli in the ventrobasal complex.

Peschanski, Kayser & Besson (1986) next examined the behavioral response

to noxious mechanical stimuli after systematically lesioning various quadrants of the spinal cord. Threshold latencies to vocalize and to withdraw from a noxious mechanical stimulus applied to the hindpaws were analyzed both before and after cervical cord lesions of various quadrants. Results indicated that only anterolateral quadrant lesions significantly increased the latencies for both responses to the noxious stimuli. It was also shown that the spinal pathway responsible for these results was mostly, if not completely crossed. Taken together, these two studies strongly indicate the direct, crossed LSTT pathway in the transmission of noxious mechanical and thermal stimuli to the rat ventrobasal complex.

Anatomical evidence, previously cited, demonstrated that some LSTT neurons do send collaterals to the PAG (Harmann et al., 1988). This is an area known to play an important role in pain perception and pain modulation (Mayer, Wolfe, Akil, Carder & Liebeskind, 1971; Mayer & Liebeskind, 1974; Rhoades & Liebeskind, 1978) and provides an anatomical mechanism by which noxious stimuli can activate neurons not only in the thalamus, but also in the PAG.

There have been a few studies, however, that conclude that the rat LSTT does not play a role in pain transmission based on LSTT cell counts and organization. In these two quantitative studies (Granum, 1986; Kemplay &

Webster, 1986), injections of HRP in the rat thalamus labeled fewer than 1000 neurons in the spinal cord. In both studies, the majority of cells were found in segments C1-C4. Because of this they concluded that in the rat, the STT is small and unlikely to play a role in transmitting nociceptive information to the thalamus and instead, the upper cervical cord served as a pain sensation relay to more rostral targets. In contrast, more recent papers (Lima & Coimbra, 1988; Burstein, Dado & Giesler, 1990) have quantitatively reexamined the STT cells of origin and have found that the number of cells contributing to this system is 10 times greater than previously reported and that these cells extend throughout the spinal cord (Kevetter & Willis, 1983; Harmann et al., 1988). Also, because Peschanski et al. (1986) systematically lesioned the different areas of the spinal cord, and found that only damage to the ALQ produced electrophysiological and behavioral deficits in response to noxious stimuli, it is likely that the LSTT has a significant role in the perception of pain stimuli.

Anatomical and Physiological Development of the Ascending Pain System

Although the dorsal horn cells of the ascending pain system mark the beginning of the LSTT, the maturation of a number of other components that are peripheral to and part of this system are necessary before the animal can respond to pain. Many of these other components have already been studied developmentally by others and allows for a more complete

picture as to the maturation of this behaviorally important system that mediates feelings of pain.

Prenatal studies have concentrated on the neurogenic period of spinal cord neurons (Nornes & Das, 1974; Altman & Bayer, 1980; Pendergrast & Beal, 1986; Nandi, Beal & Knight, 1990) and primary afferents to the spinal gray (Windle & Baxter, 1936; Smith, 1983; Fitzgerald, 1987). Long tract neurons from the superficial dorsal horn (lamina I and II) complete neurogenesis by FD-13 (Nandi et al., 1990). Ascending tract neurons from laminae III, IV, V and X and the nucleus dorsalis proliferate on FD-14. On the other hand, fine-diameter Aδ and C afferents grow into the L4/L5 spinal cord at FD-19 when they reach the reach the white matter overlying the dorsal horn and begin to penetrate lamina I. Twelve hours later at FD-19.5, terminals are seen in the outer portion of lamina II and by FD-20 they are increasing in density and have reached the inner portion of lamina II. By birth, they have reached the density in lamina I and II that is found in the neonate and young rat (Fitzgerald, 1987).

Peptides such as substance P (SP) and somatostatin, are located in fine-diameter afferents. The release of SP from neonatal rat spinal cord has been shown to be mediated by a calcium-dependent mechanism (Otsuka & Konishi, 1974) and electrophysiologically, SP has been shown to have an

excitatory effect on neurons of the neonatal rat spinal cord (Konishi & Otsuka, 1974). It has also been demonstrated in the cat spinal cord that neurons responsive to SP administration are also responsive to noxious stimuli (Randic & Miletic, 1977). Therefore, because the above findings indicate SP as one of the primary transmitters contained in and released by the nociceptive system, SP and somatostatin histochemical studies have been used to investigate the neurochemical development of Aδ and C fibers into the dorsal horn.

Semba, Shiosaka, Hara, Inagaki, Sakanaka, Takatsuki, Kawai & Tohyama 1982) investigated the ontogeny of the peptidergic system of the rat spinal cord and found that SP is present in lamina I and II of the dorsal horn just before birth, at FD-20. By postnatal day 1 (PD-1), SP containing cells are visible in the dorsal root ganglion and weakly stained SP fibers are concentrated in lamina I. By PD-8, the staining pattern is comparable to that of an adult.

The fluoride-resistant acid phosphatase (FRAP) marks a specific population of unmyelinated C afferents. Schoenen (1978), found FRAP to be present in dorsal root ganglion cells as early as FD-15, but not present in spinal cord lamina I or II of the fetal rat. FRAP activity in the dorsal horn is first present 12 hours after birth (Fitzgerald & Gibson, 1984).

However, Pignatelli et al. (1989) found that the terminal endings of afferents in lamina II, present at birth, do not show FRAP reactivity until PD-2. FRAP activity in the dorsal horn does not reach adult intensity until PD-6 or 7 (Fitzgerald et al., 1984).

There have been very few studies that have investigated the physiological and behavioral changes that take place in the neonatal somatosensory system, in general, and even fewer studies that have investigated the physiological properties of the pain system in the first few weeks of life. The first *in vivo* investigation of the cutaneous afferent input in the neonatal dorsal horn was performed by Fitzgerald, (1985). Extracellular recordings of single dorsal horn cells were made in the lumbar (L4) spinal cord to both electrical and natural mechanical stimulation (brushing with a fine brush, touching with a wooden rod and pinching with fine-toothed forceps) of the skin of the hindlimb in rat pups aged 0 to 15 days old. In the first three postnatal days of life, receptive fields were mostly evoked by pinching of the skin and responses were found to be very large covering 14.2% of the total hindlimb area. By PD-15, the receptive field had decreased to only 3.6% of the total hindlimb area. Corresponding to this, from PD-0 to PD-3, pinching the receptive field resulted in long-lasting discharges which decreased in amplitude and duration with age. Responses corresponding to both Aδ and C afferents were recorded in the superficial

lamina (I-III) at PD-0, but in the deeper lamina, only Aδ fiber responses were recorded until PD-7 and PD-8 when C afferent responses were also first present. This indicates that the developmental responses of C fibers is much delayed compared to that of Aδ fibers and does not fully develop until the second postnatal week of life. It was proposed that because C fiber evoked responses were observed in the superficial dorsal horn at birth, the cells here receive monosynaptic inputs, whereas deep dorsal horn cells contribute to polysynaptic pathways that develops secondarily to the monosynaptic ones in the second week of life. The delayed maturation of C primary afferent responses also corresponds to data that specific C fiber evoked responses to the chemical irritant, mustard oil, also does not develop until PD-10 or PD-11 (Fitzgerald et al., 1984). As was discussed earlier, capsaicin is a specific neurotoxin that permanently destroys fine-diameter primary afferents and it has also been used to understand the development of these afferents. When the cutaneous afferent volley on the dorsal root was recorded in normal rat pups and in pups that had been treated with capsicin at birth, Fitzgerald (1988) found that no difference could be detected to electrical skin stimulation between the two groups at PD-2 or PD-5. It was only at PD-9 that a clear long latency burst of spikes was absent in capsicin treated pups, indicating strongly that the primary afferents are in a very immature state of development in the first postnatal week of life and that the behavior that they mediate may depend on the maturation of C fiber polysynaptic pathways.

All of the above developmental studies indicate that the maturation of the ascending pain system is an early event probably taking place prenatally in the rat. However, there is no direct information on the development of the LSTT, in any species and so the first goal of these experiments is to trace the anatomical development of this ascending, spinal cord system in fetal, neonatal and young rats.

Experiment 1: The Anatomical Development of the LSTT Using Retrograde Tract Tracers

Much attention has been focused on tracing the development of descending motor systems (Donatelle, 1977; Gilbert & Stelzner, 1979; Martin, Cabana, DiTirro, Ho & Humbertson, 1982; Schreyer & Jones, 1982; Leong, Shieh & Wong, 1983; Kalil, 1984; Shieh, Leong & Wong, 1984; Cabana & Martin, 1985) in order to better understand the maturation, connectivity and plasticity of the CNS in general. The organization of many ascending spinal pathways has been described for a number of species (Forehand & Farel, 1982; Martin, Culberson & Hazlett, 1983; Nornes, Hart & Carry, 1980); however there have been very few studies that have examined the development of ascending projections in the rat. Studies that have traced the development of ascending projections have shown that unlike many decending tracts that mature postnatally in the neonatal rat, much of the development of these ascending, sensory systems occurs prenatally (Bryz-Gornia & Stelzner, 1986; Asanuma, Ohkawa, Stanfield & Cowan, 1988). For example, by the day of birth, the efferents from the dorsal column nuclei, deep cerebellar nuclei and the inferior colliculus have already entered, and arborized extensively within their appropriate thalamic nuclei (Asanuma et al., 1988).

In Experiment 1, WGA-HRP and fluorescent green latex microspheres were used to determine the earliest age at which VPL thalamic injections would label the dorsal horn cells of origin of the LSTT. WGA-HRP has been demonstrated to be a far more effective retrograde tracer of the spinothalamic tract than HRP alone (Mantyh & Peschanski, 1983). The fluorescent latex microspheres, unlike WGA-HRP, show minimal diffusion from the injection site and appear to be an attractive alternative neuroanatomical tracer in mapping the projections of neonatal animals (Katz & Iarovici, 1990).

METHODS

Subjects

Male and female Long-Evans hooded rat pups born in our colony were used in this study. Animals were examined at the following ages: fetal days 18 and 19 (FD-18, FD-19) and postnatal days 0 and 10 (PD-0, PD-10). Five to eight animals were examined at each age, for each method used (WGA-HRP and microspheres). For postnatal subjects, rats were checked once in the morning and again in the early evening for the presence of pups, with the day of birth termed PD-0. For embryonic subjects, timed-pregnant females were checked daily by the vaginal smear method for the presence of sperm. Once sperm was detected, the male was separated from the

female and this day was termed FD-0.

Postnatal retrograde tracing methods

Postnatal animals (PD-0 and PD-10) were deeply anesthetized through the inhalation of methoxyfluorane and placed in an infant stereotaxic apparatus. With the aid of the stereotaxic atlas for developing rats (Heller et al., 1979), the VPL thalamus was unilaterally injected with either .1 μl of a 5% solution of WGA-HRP in distilled water or an undiluted sample of latex green microspheres (LumaFluor). A single injection was made through a polyethylene tubing (PE-10)-30 gauge needle assembly connected to a 0.5 μl Hamilton syringe. Animals were placed in separate tubs in an incubator until recovery from anesthesia and then returned to the mother in the homecage for a survival period of 48 hours. Pups were then reanesthetized by an intraperitoneal injection of sodium pentobarbitol (64 mg/kg) and perfused transcardially with phosphate buffered saline followed by either 2% glutaraldehyde/1% paraformaldehyde if injected with WGA-HRP, or a 4% solution of paraformaldehyde if injected with microspheres. The spinal cord and whole brain were removed and stored in a 30% sucrose solution. Transverse frozen serial sections of all brains and spinal cords were cut at 30 μm and either reacted in solution for the presence of WGA-HRP by the TMB method and counterstained with a 1% solution of neutral red or collected in phosphate buffered saline and mounted on gelatin coated slides if injected with the microspheres. These sections were

air-dried, dehydrated in xylene for 1 minute and coverslipped with Krystalon (Harleco). Sections were examined under a fluorescent microscope using wide-band filters for the presence of the green microspheres.

Fetal retrograde tracing methods

This strain of rat has a gestation period of approximately 21 days. All intrauterine procedures follow the method by Smotherman (1984). Dams were deeply anesthetized by an intraperitoneal injection of sodium pentobarbitol (64 mg/kg) and placed in a restaining device that secured the rat in an inclined position, exposing the ventral side. An incision was made between the 2nd and 3rd pair of teats through the layer of muscle that overlies the uterus. The entire restraining device was then placed in a double warmed saline bath (38°C) so that the caudal half was submerged into the saline. Both uterine horns were then carefully externalized into the saline bath. Prior to day 18 of gestation, the layer of amnion that surrounds the fetus is quite thick, making the sutures of the skull very difficult to visualize. Therefore a hypodermic needle was carefully used to extract between 0.1 and 0.3 cc of fluid before injection. All of the fluid was then replaced after injection. Hand-held pressure injections of 0.05 μl of WGA-HRP was made through all membranes enclosing the fetus, using the sutures of the skull as landmarks. Because the WGA-HRP and microspheres appeared to label the same population of cells, only the

WGA-HRP was used on fetal animals as it was found to be easier to inject reliably in the fetus. Once the needle was extracted, the puncture marks were not visible. This procedure was then repeated with all remaining fetuses. The uterus was then placed back under the muscle wall and the dam was brought out of the saline bath. The incision was closed by 2 layers of square-knot sutures. A caesarian section was performed after 48 hours on fetuses injected at FD-18. Birth was allowed to occur normally in animals injected on FD-19. All delivered fetuses were intracardially perfused, and the tissue processed as for postnatal animals.

Experimental rationale

This study was designed to study the possibility that the LSTT is an early developing system, established during the fetal period of the rat. The retrograde tracers, green fluorescent latex microspheres and WGA-HRP were both used to determine at what age the lumbar afferents have reached the VPL thalamus. Due to the spread of WGA-HRP beyond the injection site of the VPL thalamus (often filling the entire hemisphere), green fluorescent latex microspheres were chosen as a second retrograde marker to verify the results obtained with the WGA-HRP since they diffuse very little from the site of injection.

Procedure

Postnatal day 10 rat pups were initially used in this experiment. When the results indicated that cells of the lumbar dorsal horn, at this age, projected to the VPL thalamus, PD-0 and PD-1 pups were used. Although the results indicated that some lumbar cord cells have sent projections to the VPL thalamus, both qualitative and quantitative criteria show this projection to be at a less mature state of development than PD-10 animals but indicated the need to work in younger, fetal animals. All injection sites for animals injected with retrograde tracers were visually verified by histology. Only those animals in which the histology verified the injection to have penetrated the VPL thalamus are included in the experimental results.

RESULTS

Site of retrograde tracer application

In preliminary work, rats were sacrificed at several different survival times (24, 48 and 72 hours) following injections. No differences in labeling was observed with the different survival times, and the 48 hour time period was used for all animals.

The injections of fetal rats *in-utero* yielded a hit rate of about 30% in most litters. Most positive injections were anterior to the posterior commissure;

Figure 2. A representative transverse section from a PD-0 rat in which injection of WGA-HRP was made into the VPL thalamus. WGA-HRP injection resulted in the extensive spread of the reaction product across the ventromedial posterior nucleus of thalamus the midline. Survival time was 48 hours. (Magnification is approximately 60X).

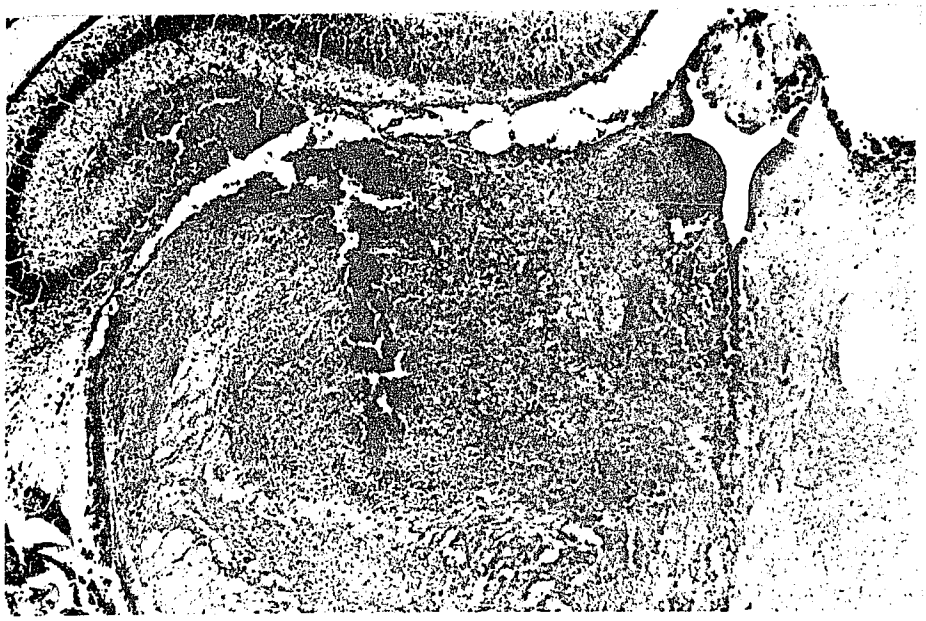
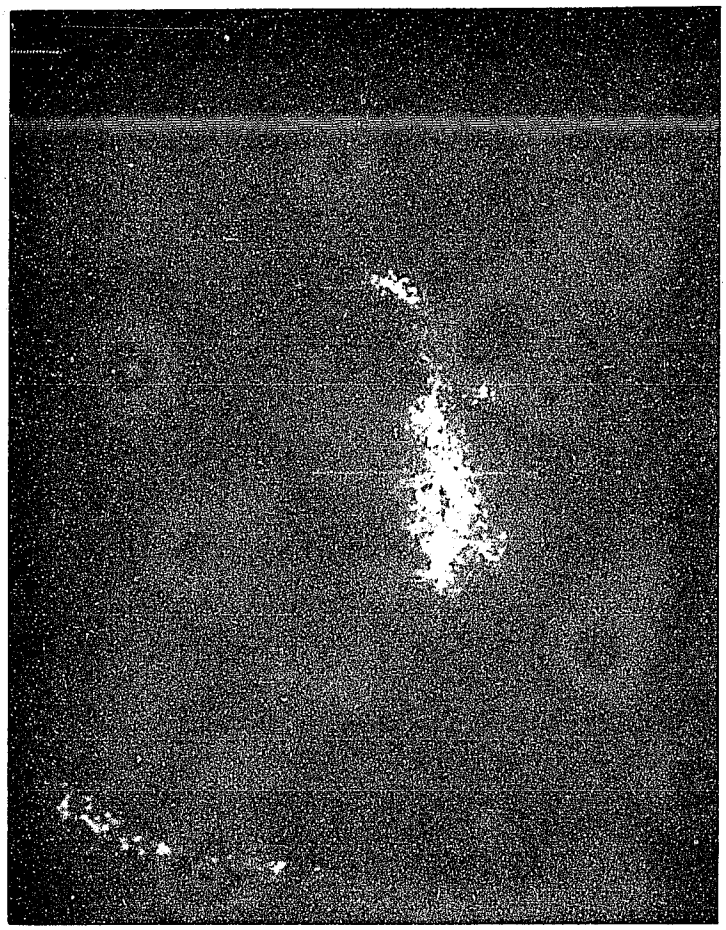


Figure 3. A representative transverse section from a PD-0 rat in which an injection of fluorescent latex microspheres was made to the VPL thalamus. The microspheres spread far less than the WGA-HRP and never spread to VPM or posterior thalamus. The survival time was 48 hours. Despite the diffusion differences in the two retrograde tracers, both yielded similar or identical results. (Magnification is approximately 150X).



however animals in which the injection penetrated more posterior levels of the VPL thalamus (at the level of the lateral geniculate bodies) were also included. In animals injected with WGA-HRP, the spread of diamino-benzidine (DAB) reaction product always extended across the ventromedial posterior thalamus group (VPM). Posteriorly, a halo of the DAB reaction product spread to the level of the medial geniculate body, and often times spread across the midline (Fig.2). In animals injected with microspheres, the fluorescent green label displayed minimal diffusion from the site of injection, never spreading to the VPM thalamus or to more posterior structures (Fig.3). However, despite the differences in the amount of injection diffusion, both tracers yielded similar or identical results such that for all postnatal age groups (PD-0, PD-10) and for fetal ages FD-18 and FD-19, cells were labeled in the contralateral dorsal horn of the lumbar spinal cord only. Because both tracers yielded similar results, the more problematic microspheres was not used in the fetal animals. All cell counts reflect this point with only WGA-HRP labeled cells being counted throughout the study. Neurons were never seen ventral to the central canal or in other segments of the spinal cord (cervical or thoracic).

Fetal rats

The first age at which label was observed in the dorsal horn was the earliest age, FD-18, but only when the placement of tracer was to the more

Figure 4. Photomicrographs of cells labeled in the spinal cord of FD-18 animals after *in-utero* injections of WGA-HRP into the VPL thalamus. *A,B.* Labeled cells found in the lateral portion of the superficial lamina (lamina II). *C.*Labeled cell located in the medial aspect of the superficial lamina. All processes were oriented toward the contralateral funiculus. *D.*A few labeled cells were found in the ventromedial dorsal horn (lamina VI). These cells exhibited fewer, shorter processes and a larger cell body as compared to those found in the superficial lamina. All labeled cells were small, and very faintly labeled. (Magnification is approximately 1500X)

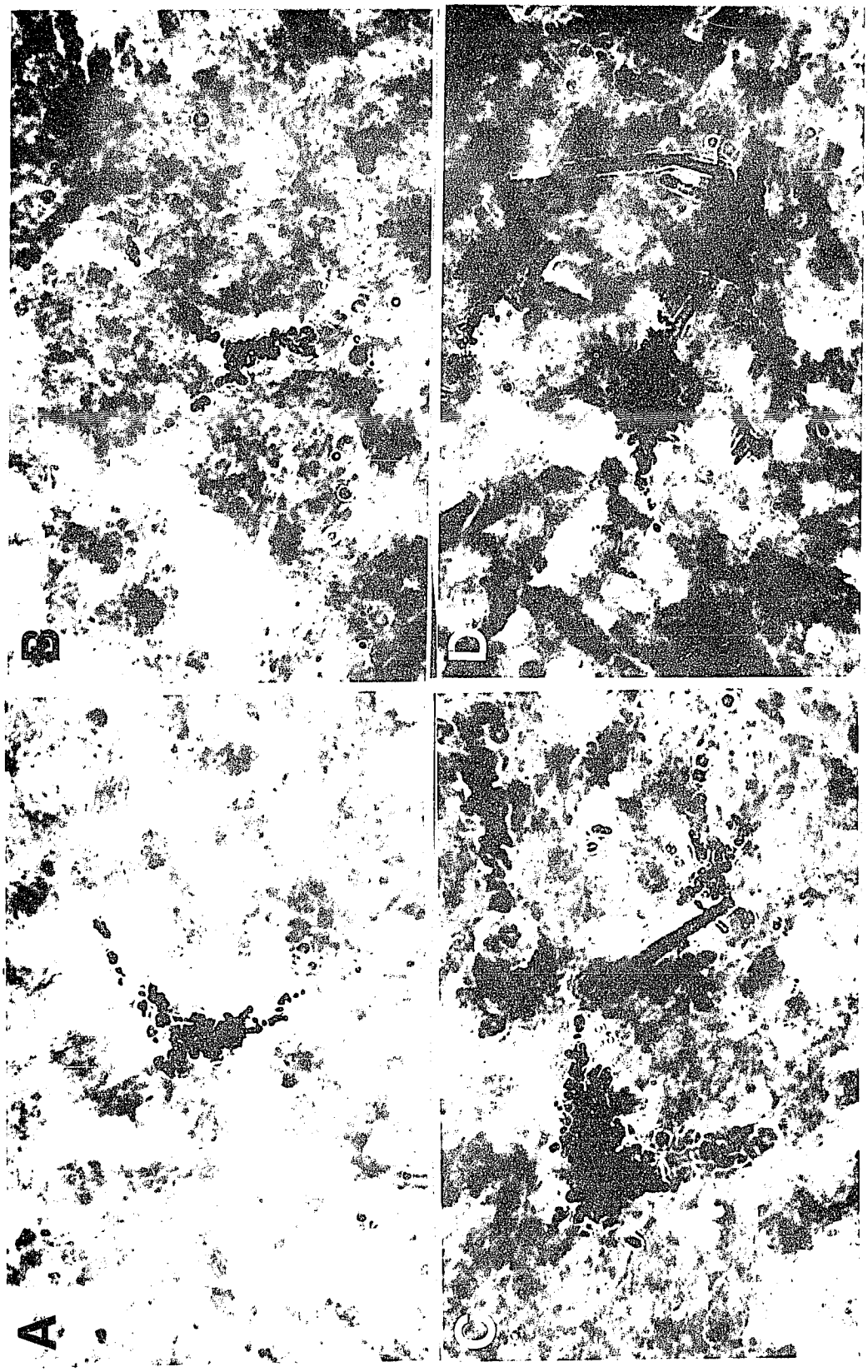

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Figure 5. Labeled tract of axons in the lateral funiculus ipsilateral to the injecion, but contralateral to the labeled spinal cord cells. This tract was not labeled in older groups of animals. (Magnification is approximately 300X)

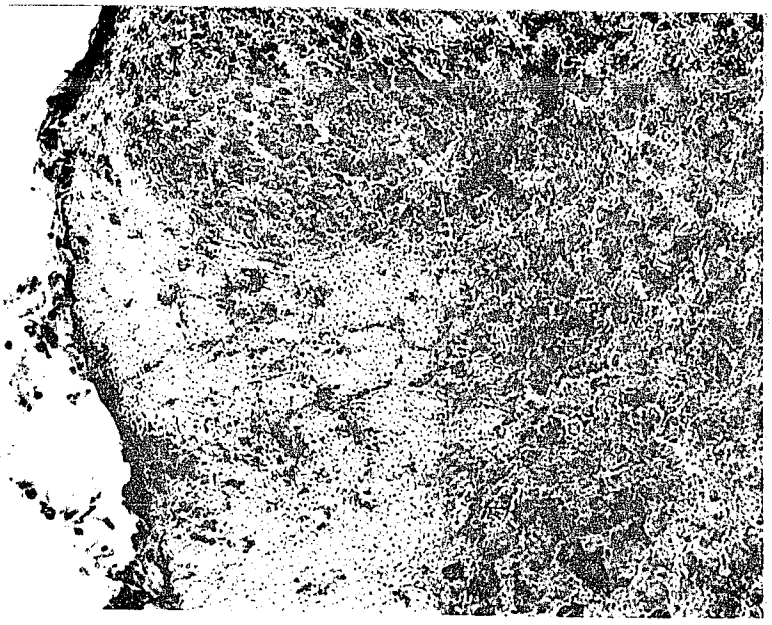
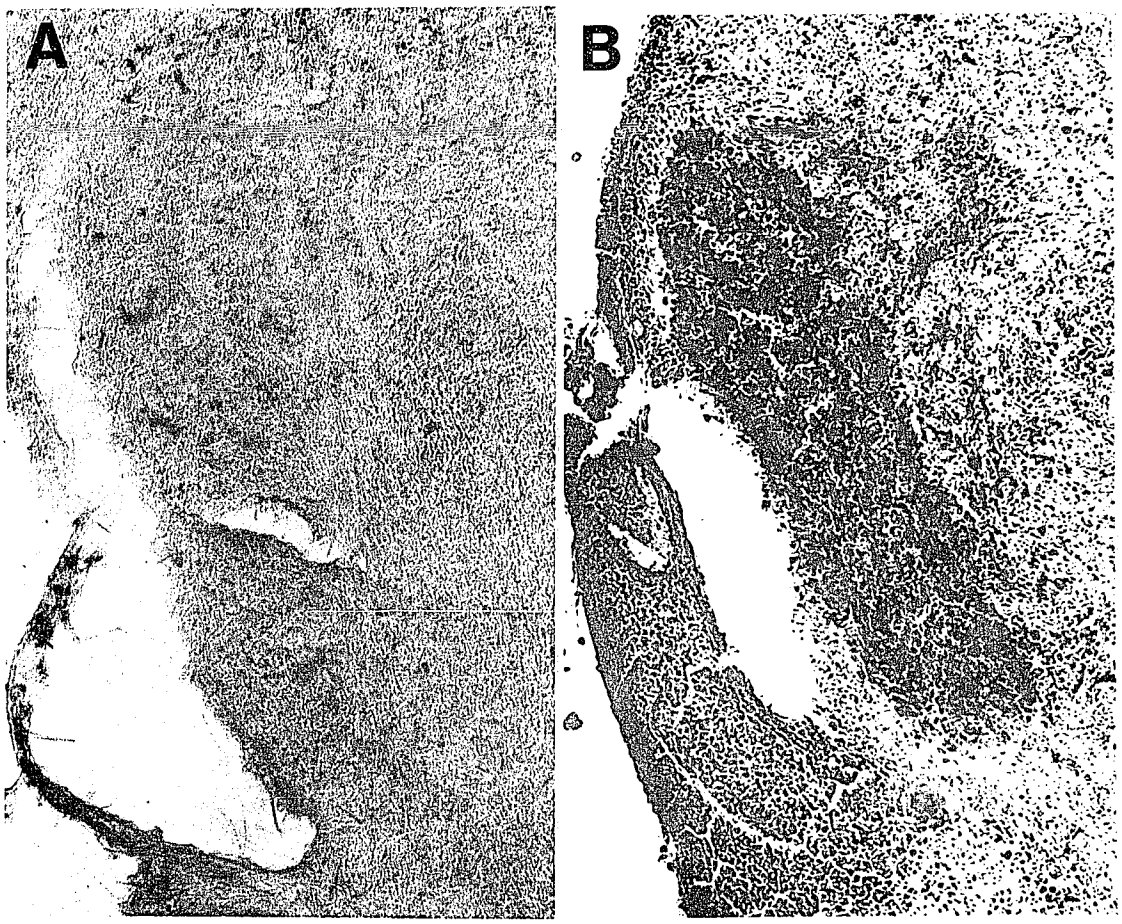


Figure 6. Photomicrographs of the contralateral principal sensory trigeminal nucleus (PrV) after injections of WGA-HRP into the VPL thalamus. *A.* On day 18 of gestation, the PrV nucleus is very lightly labeled. Arrows indicate the faint border of the nucleus. *B.* By the day of birth, the PrV nucleus is densely stained throughout its length. (Magnification is approximately 150X)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B

Table 1: Individual counts of labeled cell bodies found in the lumbar area of the contralateral dorsal horn after injection of WGA-HRP into the VPL thalamus at 18 days of gestation.

FETAL DAY 18 (FD-18)

ANIMAL #	**SUPERFICIAL (lamina II,III)**	**VMDH (ventromedial dorsal horn, lamina IV,V)**	**NECK AREA (lamina V)**
219-1	7	4	0
219-3	13	0	0
219-4	5	0	0
213-3	5	2	0
213-6	13	0	5
TOTAL	*43 ≈ 80%*	*6 ≈ 11%*	*5 ≈ 10%*
MEAN ± SD	**8.6 ± 4.1**	**1.2 ± 1.8**	**1.0 ± 2.2**

lateral regions of the VPL thalamus, and at more anterior levels of the nucleus. Other sites, including more medial and posterior regions of the VPL thalamus, that labeled cells in older animals, did not do so in these fetal animals. Similar to the pattern of labeling observed in neonatal animals, in fetal animals labeled neurons were confined to the lateral and medial aspects of the superficial lamina (lamina II) (Figs.4a,4b,4c) where a majority of the cells were located and in lamina IV and V of the ventromedial dorsal horn (VMDH) (Fig.4d). Fine processes were seen from soma only in the superficial lamina and these were oriented toward the contralateral lateral funiculus. All cells were faintly labeled displaying small cell bodies. For a summary of the amount and location of labeled cells at this age see Table 1. At FD-18 only, a tract of axons was densely labeled in the lateral funiculus of the lumbar cord (Fig.5); this tract was not seen in more rostral regions of the spinal cord. In addition to the labeling of the cells and tract of the LSTT, the contralateral principal sensory trigeminal nucleus, known also to send projections to the VPL thalamus, was very faintly labeled (Fig.6a).

The pattern of labeling in the FD-19 age group was identical to that seen in the FD-18 animals with the exception that no spinal cord tract labeling was ever seen and that the sensory medial lemniscus, which sends afferents to the ipsilateral VPL thalamus, was faintly labeled.

Figure 7. Photomicrographs of cells labeled in the spinal cord of neonatal rats on the day of birth (PD-0) after injection of WGA-HRP into the VPL thalamus. *A,B.* Cells labeled in the lateral portion of the superficial lamina. In postnatal animals, fewer labeled cells were here with fewer processes than in fetal animals. Cells found in this area usually had a characteristic angular shaped, thin soma. *C,D.* Spinal cord cells labeled in more central areas in the neck of the dorsal horn (lamina V). These cells were not labeled in fetal animals but in neonatal animals typically exhibited many process arborizations coming off of the round cell body. *E,F.* Cells labeled in the vetromedial dorsal horn also exhibited some processes and a large, round cell body. In all postnatal animals, the majority of labeled cells were found here and in the neck of the dorsal horn. These cells were usually the most densely labeled. (Magnification is approximately 1500X)

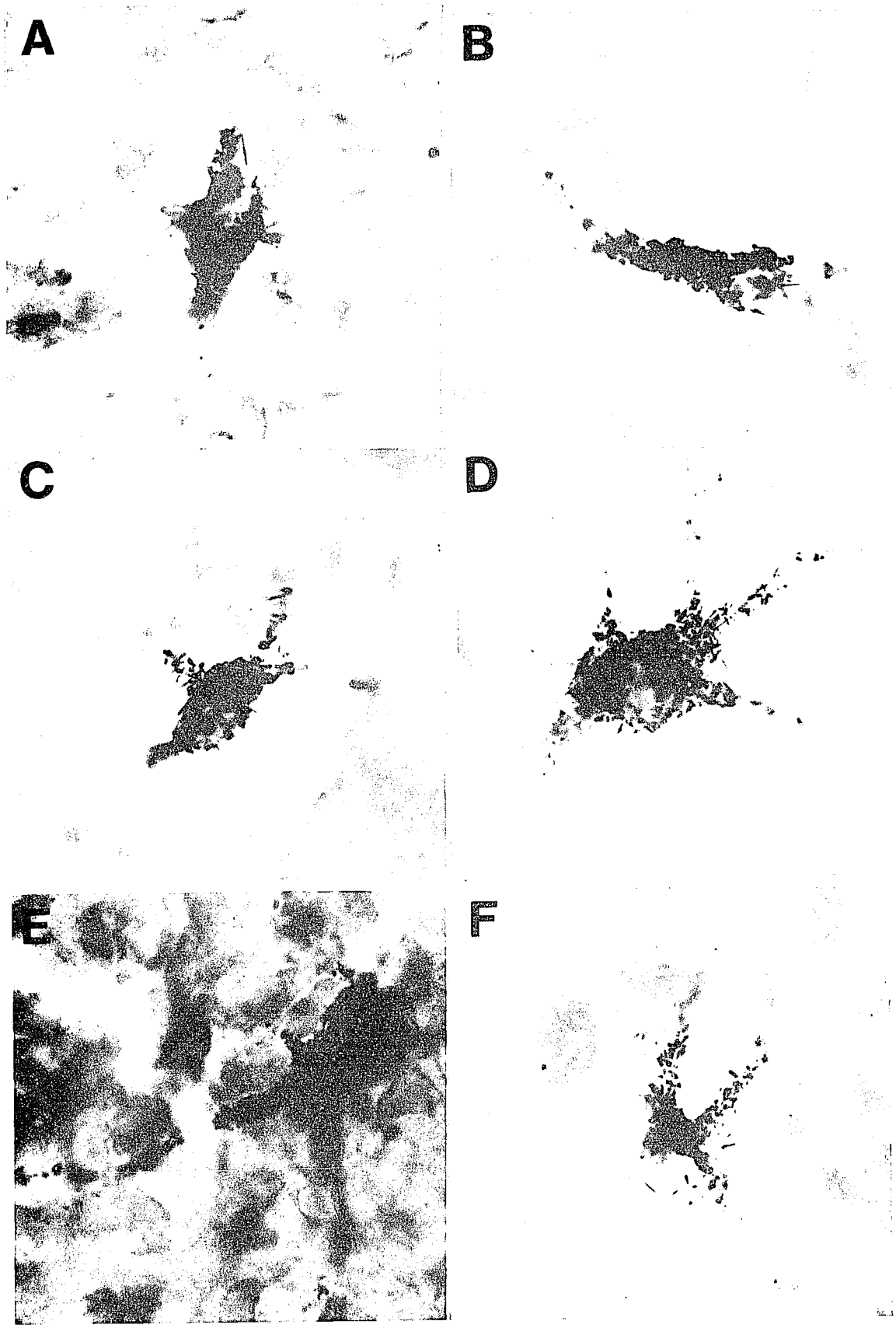
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Figure 8. Photomicrographs of cells labeled with fluorescent latex microspheres after injection into the VPL thalamus of neonatal rats. *A.* As with WGA-HRP, few cells were labeled in the lateral portion of the superficial lamina. *B.* Cells exhibiting larger soma were typically labeled in the central areas in the neck of the dorsal horn. *C,D.* Most cells displaying the fluorescent label were found in the ventromedial portion of the dorsal horn and were densely labeled similar to those labeled with the WGA-HRP.(Magnification is approximately 1500X).

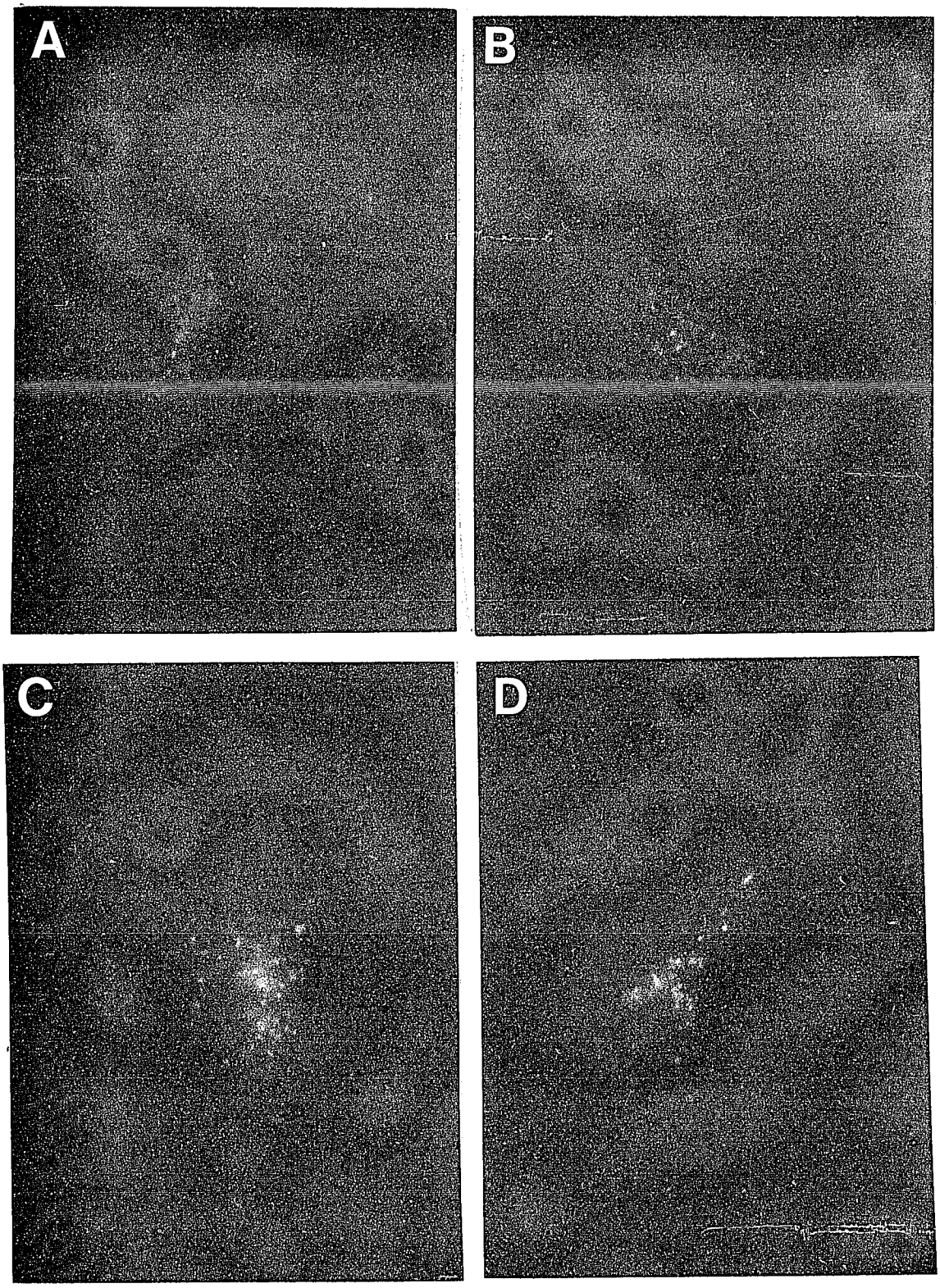
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Table 2: Individual counts of labeled cell bodies found in the lumbar area of the contralateral dorsal horn after injection of WGA-HRP into the VPL thalamus on the day of birth.

POSTNATAL DAY 0 (PD-0)

ANIMAL #	**SUPERFICIAL (lamina 2,3)**	**VMDH (ventromedial dorsal horn, lamina 4,5)**	**NECK AREA (lamina 5)**
R0-4	10	17	9
R0-9	5	17	4
R0-10	4	5	4
R0-12	3	9	1
R0-13	4	31	9
R0-16	2	9	7
TOTAL	*28 ≈ 20%*	*83 ≈ 58%*	*33 ≈ 23%*
MEAN ± SD	**4.7 ± 2.8**	**12.0 ± 10.7**	**5.7 ± 3.2**

PD-0 rats

In this group of animals, WGA-HRP or latex microspheres were injected in a similar region and level of the VPL thalamus as reported for fetal animals; however unlike fetal animals, cells were also labeled with more posterior and medial injections. By the day of birth, at PD-0, many differences from the fetal animals were observed in the pattern of spinal cord labeling. All cell bodies were densely labeled, showing larger somas. Specifically, labeled neurons were observed in the lateral aspects of the superficial layers of the dorsal horn (lamina II and III), although not in great numbers (Figs.7a,7b). In contrast to the fetal animals, fewer than 25% of all labeled neurons were in the superficial lamina. Also unlike fetal animals, processes were never seen from the angular, small cells of the superficial lamina. Cells were observed, for the first time, in central areas of the neck of the dorsal horn (Figs.7c,7d). All other neurons were located in the ventromedial region of the dorsal horn and surrounding the central canal (Figs.7e,7f). The larger neurons of the VMDH were seen in much greater numbers than in fetal animals and had processes that were horizontally oriented toward the contralateral side of the spinal cord. Injected latex fluorescent microspheres also labeled cells only in the contralateral superficial, central and ventromedial portions of the dorsal horn. (Figs.8a,8b,8c,8d). For a summary of the amount and location of labeled cells at this age see Table 2. By the day of birth, the ipsilateral

Figure 9. Photomicrographs of WGA-HRP labeled cells in the spinal cord of PD-10 rats after injection into the VPL thalamus. *A,B.* Typical neurons found in the lateral portion of lamina II. *C.* Cells labeled in the central area in the neck of the dorsal horn exhibited round cell bodies and several processes extending from it.(Magnification is approximately 1500X)

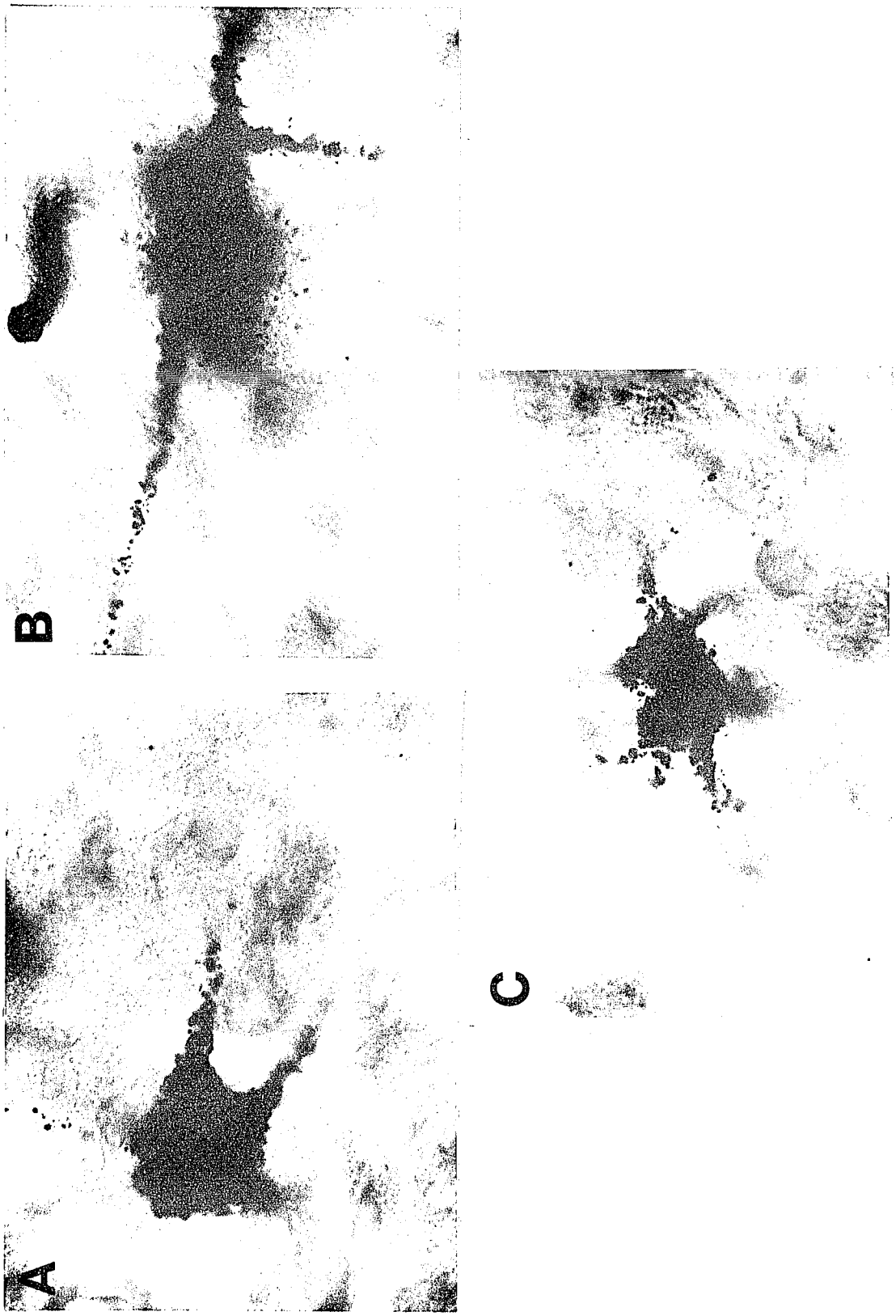
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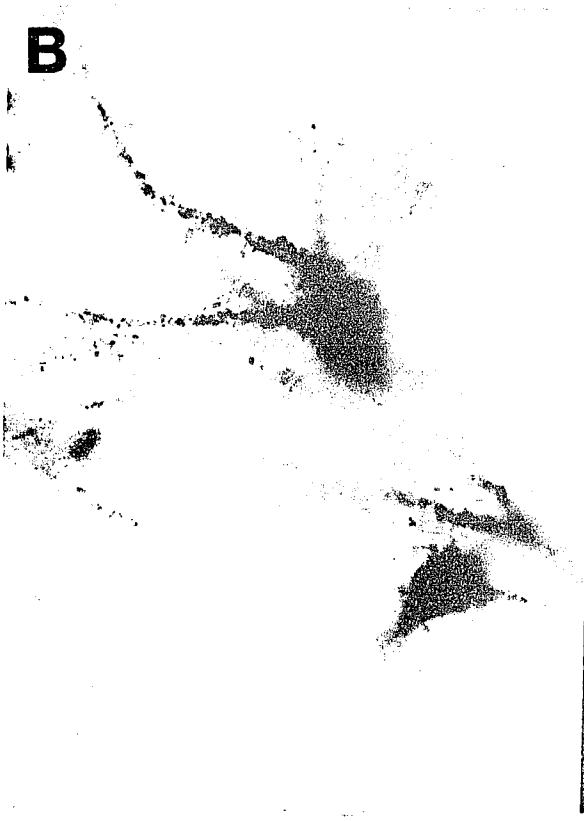
Figure 10. In a PD-10 rat, the majority of cells were found in the ventromedial dorsal horn. *A.* Typical cells could be found in pairs. (Magnification is approximately 1500x) *B,C.* Cells were often seen in clusters following thalamic injection with overlapping processes and densely labeled cell bodies. (Magnification is approximately 600X)


A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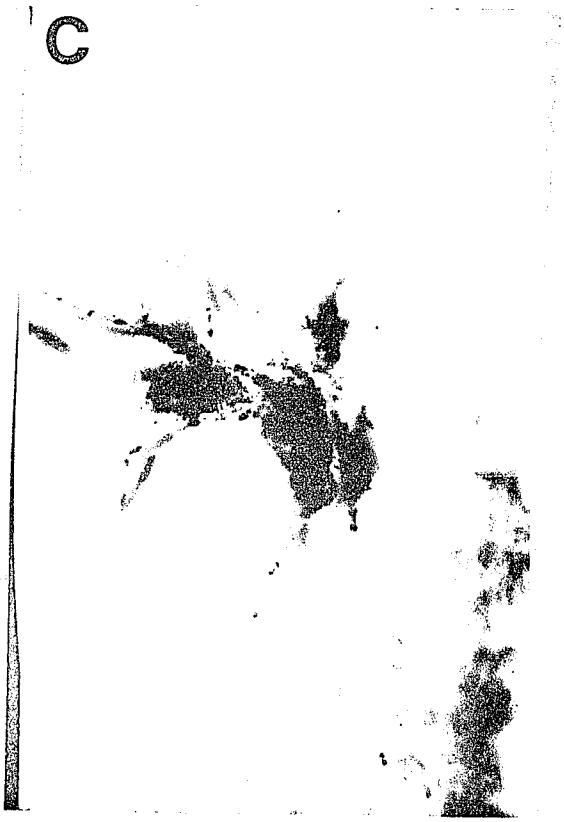

C

Table 3: Individual counts of labeled cell bodies found in the lumbar area of the contralateral dorsal horn after injection of WGA-HRP into the VPL thalamus on postnatal day 10.

POSTNATAL DAY 10 (PD-10)

ANIMAL #	**SUPERFICIAL (lamina 2,3)**	**VMDH (ventromedial dorsal horn, lamina 4,5)**	**NECK AREA (lamina 5)**
R10-35	0	27	16
R10-36	0	24	10
R10-37	1	30	15
R10-38	0	28	3
R10-39	0	23	4
TOTAL	*1 ≈ 1%*	*132 ≈ 73%*	*48 ≈ 26%*
MEAN ± SD	**8.6 ± 4.1**	**26.4 ± 2.9**	**9.6 ± 6.0**

medial lemniscus was more darkly labeled and the entire contralateral principal sensory trigeminal nucleus was very densely stained (Fig.6b). No difference was seen in labeling between regions of this nucleus. Axonal labeling in the lateral funiculus was also never observed in any postnatal animal.

PD-10 rats

Similar to the PD-0 group, injections made to any region of the VPL nucleus resulted in spinal cord labeling. By PD-10, neurons of the more superficial layers were no longer limited to the most lateral aspects of the dorsal horn as was seen in younger animals and cells were observed in more central areas of the superficial lamina (Figs.9a,9b). Cells were again observed in central areas of the neck of the dorsal horn (Fig.9c). In general, by 10 days of age, a greater number of labeled neurons were observed with extensive process arborization, especially in the VMDH. These latter cells were densely labeled, with much larger somas than in younger animals (Figs.10a,10b,10c). For a summary of the amount and location of labeled cells at this age see Table 3. No differences were seen in labeling between the PD-0 and PD-10 age groups in respect to the medial lemniscus and trigeminal nucleus. Although in all postnatal animals, the pattern of labeled cells was similar using either the WGA-HRP or the fluorescent green microspheres, the microspheres were more successful at

Figure 11. Photomicrographs of cells labeled following latex microsphere injection into the VPL thalamus of PD-10 rats. *A.*Few cells were labeled in the superficial portion of the dorsal horn. *B.* Representative cells labeled in the ventromedial dorsal horn. Note that the green microspheres were more successful at labeling cells in the neonatal group than in the PD-10 group. (Magnification is approximately 1500X)

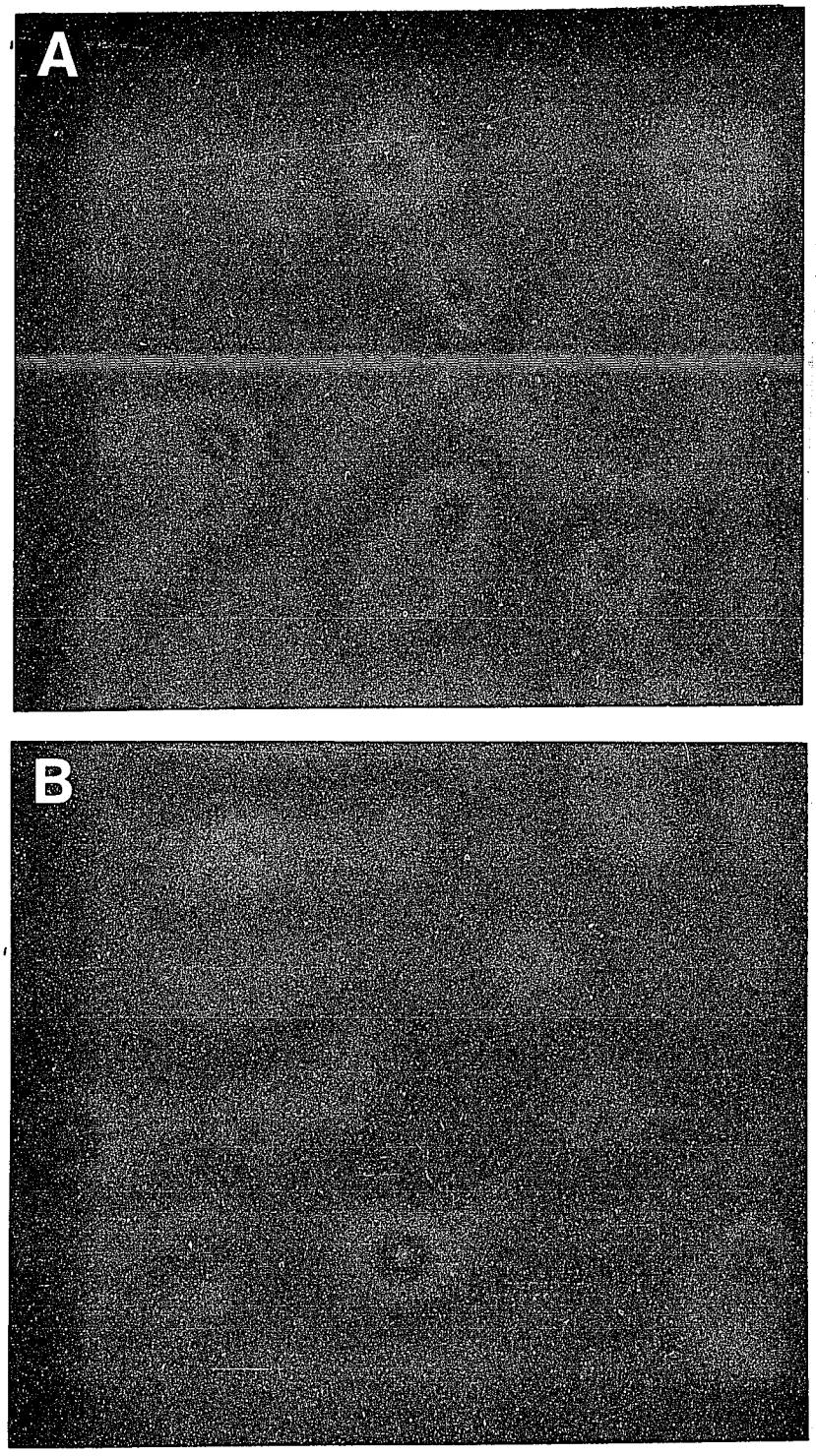
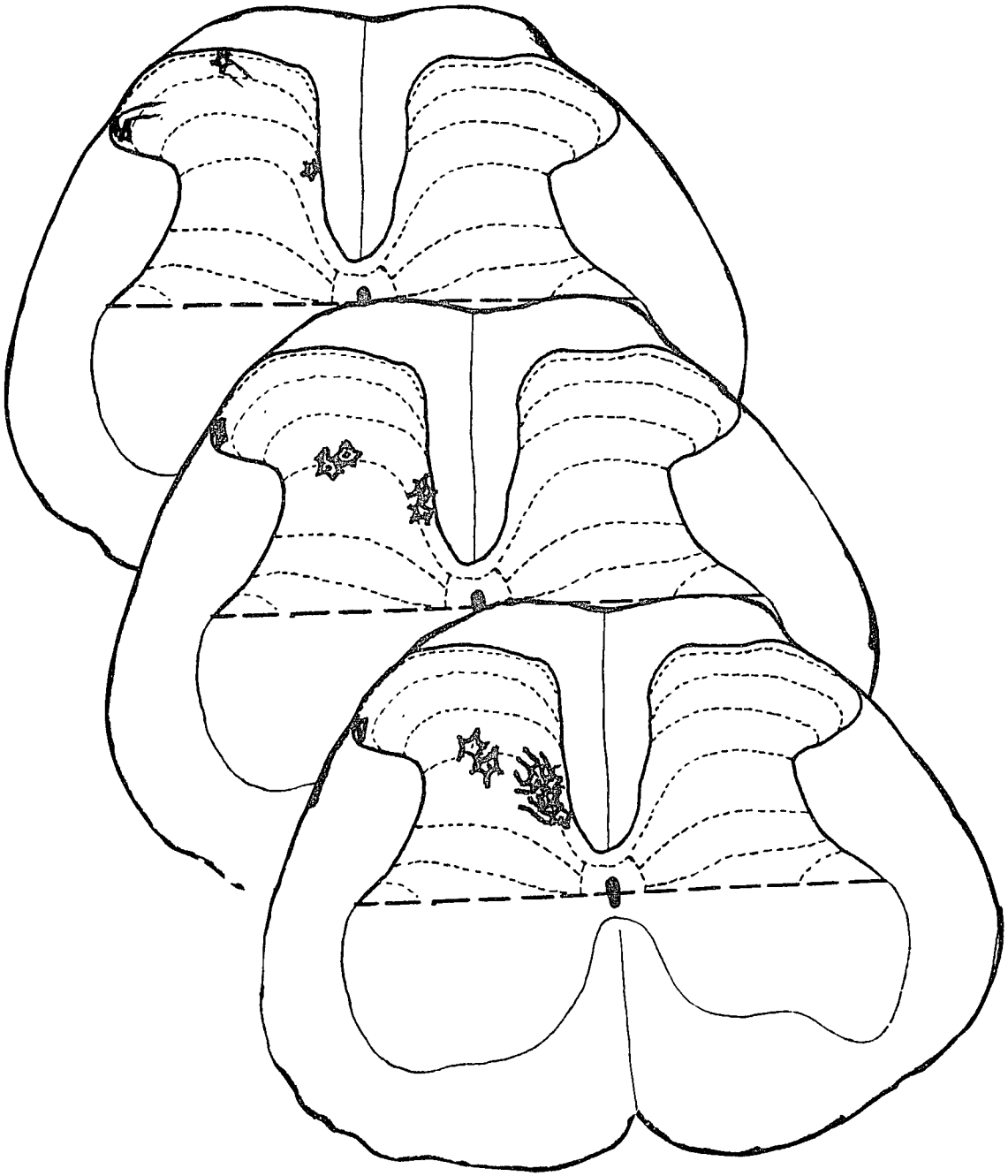

A
B

Figure 12. Schematic comparison of the location and quantitative changes of LSTT spinal cord cells in the developing rat. Pictured from top to bottom is a lumbar spinal cord section representing a typical *FD-18, PD-0 and PD-10 section (respectively)* in terms of both quantity and distribution of labelled cells.



densely labeling somas and processes in the PD-0 group (See Fig.8) than in the PD-0 animals (Figs.11a,11b).

DISCUSSION

Injection of either WGA-HRP or fluorescent green latex microspheres into fetal, neonatal and postnatal animals provide an anatomical method of tracing the development of an ascending system. In the present study, these methods were used to study the developing LSTT. The results confirm that in the rat, the ascending LSTT is an early developing tract and is present in fetal animals as early as 18 days after conception but that changes in development occur in the density, pattern and quantity of labeled cells in the neonatal and postnatal rat. For a schematic summary of the labeled cells at the three age groups studied please refer to Figure 12.

Although the LSTT originates from cells of the spinal cord dorsal horn of all levels in the adult rat (Dilly et al., 1968; Willis et. al., 1978; Giesler et al., 1979; Granum-Robson, 1983; Kemplay et al., 1986) I found that in the developing rat only lumbar spinal cord cells were labeled indicating that at this time, they may develop earlier and contribute more significantly to the LSTT than do more rostral segments. However, because the function of the LSTT is the transmission of noxious messages to the brain (Guilbaud,

Peschanski, Gautron & Binder, 1980; Peschanski, Guilbaud, Gautron & Besson, 1980; Peschanski, Briand, Gautron & Guilbaud, 1985; Peschanski, Kayser, Besson, 1986), this is unlikely since behavioral responses to painful stimuli can be elicited from the forepaws of newborn rats (Barr, Miya & Paredes, 1991). It is more likely, in agreement with other investigators (Smith, 1983), it may also be likely that the fine-diameter thickness of afferent systems in general and of prenatal animals, especially, makes it very difficult to label with WGA-HRP or microspheres.

The fine-diameter Aδ and C primary afferents that carry noxious and tactile sensations from the periphery terminate on cells in the dorsal 2/3 of the dorsal horn in the adult rat (Giesler et al., 1976), but especially in the superficial lamina of the substantia gelatinosa. Fitzgerald (1987) found these Aδ and C afferents to grow into the lumbar spinal cord (L4-L5) at FD-19 when they reach the white matter overlying the dorsal horn and begin to penetrate lamina I. Twelve hours later at FD-19.5, terminals are seen in the outer portion of lamina II and by FD-20 they have increased in density, reaching the inner portion of lamina II. It is not until birth that they have reached the density in the superficial lamina that is found in the neonate and young rat. Neurons from lamina I and II that contribute axons to long ascending tracts have been found to complete neurogenesis early by FD-13 (Nandi, Beal & Knight, 1990) while ascending tract neurons from lamina III, IV, V and X (immediately surrounding the central canal)

complete neurogenesis later by FD-15.

All of the above studies, like the present experiment, illustrate a general developmental principal of target guidance where the influence of the target cells in establishing appropriate axonal connections is necessary for normal pattern development. It seems likely, in the immature pain system as in other sensory systems that normal development depends on the primary sensory inputs. For example, neonatal treatment of rats with capsaicin, a neurotoxin which destroys unmyelinated primary sensory afferents, resulted in a failure of descending brain stem inhibition to develop (Cervero & Plenderith, 1985). In the immature ascending pain system, the delicate balance of target-axon and axon-target interaction is exemplified many times. For example, lamina I-V and X cells are complete by FD-15 and may guide the growth of the primary afferents that starts later, at FD-19 and in the present study, we have shown that by the time many of the primary afferents are reaching the dorsal horn, a proportion of these cells have sent axons to the VPL thalamus. Also, by 5 days after the early neurogenesis of lamina I and II cells of the dorsal horn, some of these neurons have already terminated in the VPL thalamus.

Although in the fetal animal, cells of the VMDH were also labeled, the smaller proportions of the soma relative to the same cells labeled in the

neonatal animal, the absence of dendritic arborizations and the faintness of label indicate that although the axons of these cells have reached the VPL thalamus, they may continue to develop and mature in the final three days of gestation.

It was in only the FD-18 group of animals that the spinal cord portion of the LSTT was labeled in the lateral quadrant of the lumbar cord. The explanation for this finding is not known. It is possible that this the immaturity of this tract at this early age limits its ability to transport WGA-HRP; this seems unlikely in view of the clear ability of growing axons to transport tracers to their growing tips as demonstrated in other systems (Scott, 1982; Sretevan & Schatz, 1984). It is also possible that the above result is due to a combination of tract immaturity and the fine diameter of pain system axons.

In tracing the development of other systems, different patterns of organization have been found. For example, by using an orthograde tracer it was found that ascending inputs to the thalamus from the dorsal column nuclei, deep cerebellar nuclei and the inferior colliculus overshoot their target with extraneous projections that correctly retract by the end of the first month of life (Asanuma, Ohkawa, Stanfield & Cowan, 1988). Spatial segregation and somatotopic arrangement was found in the development of

the rubrospinal tract (Shieh, Leong & Wong, 1983). In the present study the results indicated a possible spatial segregation whereby in fetal animals, only injections of tracer made to the anterior region of the VPL thalamus resulted in the labeling of spinal cord cells. This finding was quite different in postnatal animals where injections could be made to any part of the VPL thalamus in order to label spinal cord cells. This may indicate that the neurons of the superficial dorsal horn that are the first to project to the VPL thalamus do so to the anterior VPL thalamus.

Because the present study utilized only retrograde tracer methods that indicated the LSTT is a prenatally developing tract that continues to mature to at least PD-10 in the rat, the goal of the second study was to utilize an anterograde tract tracer in order to visualize the exact location in the spinal cord that these projections have ascended to in the developing animal. A technique was also necessary that could be accurately used in fetal animals younger than FD-18.

Experiment 2: The Development of the LSTT Using an Anterograde Tract Tracer

An anterograde marker was used in this study to trace the location of LSTT afferents as they ascend through the spinal cord to the thalamus. Because the validity of Experiment 3 depended on the accuracy of timing spinal cord lesions to take place both during early and late developmental stages of the LSTT, it was important that an ascending marker be used. The carbocyanine compound 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Di-I) was chosen as the method of anterograde tract tracing primarily because it has the ability to label neuronal circuits in fixed tissue making the visualization of ascending projections in very young fetuses possible (Godement, Vanselow, 1987; Honig & Hume, 1989; McConnel, Ghosh & Shatz, 1989; von Bartheld, Cunningham & Rubel, 1990).

METHODS

Subjects

Male and female Long-Evans hooded rat pups born in our colony were used in this study. Animals were examined at the following ages: FD-17, FD-18, PD-0 and PD-10. Three to five animals were examined at each age.

For all subjects, the detection and dating of timed-pregnant females is the same as in Experiment 1.

Postnatal and fetal anterograde tracing methods

The anterograde tracer Di-I was used in postnatal and fetal animals. Because it can be used in perfused animals, a FD-17 group of animals was included, which was not possible with the retrograde methods. Glass micropipettes (1 μl) were pulled by hand over a flame and sectioned into 1 mm length "sticks" and soaked overnight in a 2% solution of Di-I in ethyl alcohol. Animals were either immersed in (FD-17) or perfused with a 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). A laminectomy was performed at the level of the lumbar enlargement and the Di-I coated "sticks" were placed in the region of the lateral funiculus. Rats were placed back into the fixative for a period of 4-8 months at a temperature of 37°C. In order to minimize background diffusion and leakage of label out of the membrane, the brain and spinal cord was sectioned by vibratome. Before sectioning, the whole brain and spinal cord were removed and embedded in a 10% gelatin solution. Transverse, sections of 80 μm were taken of the brain and spinal cord. Some longitudinal sections were also taken of the spinal cord. All sections were floated into .1M phosphate buffer and then mounted on gelatin-coated slides but not permanently coverslippped in order to reduce the spread of label (von Bartheld, Cunningham & Rubel, 1990). Sections were

Figure 13. Photomicrograph of a coronal section of the spinal cord after application of the anterograde tract tracer, Di-I. Just rostral to the site of Di-I application in all animals, diffusion was often seen across the entire half of the spinal cord. This taken from a FD-18 rat. (Magnification is approximately 60X)

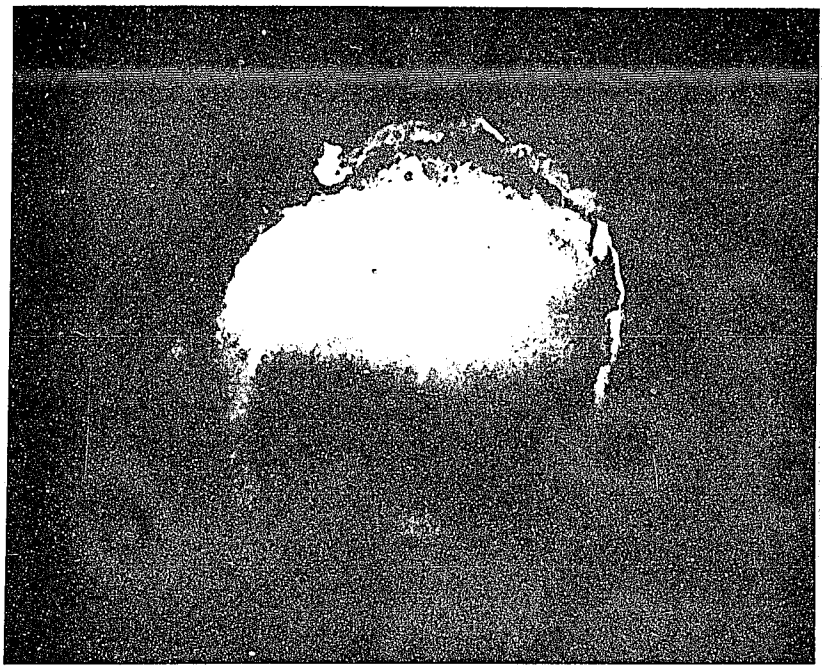
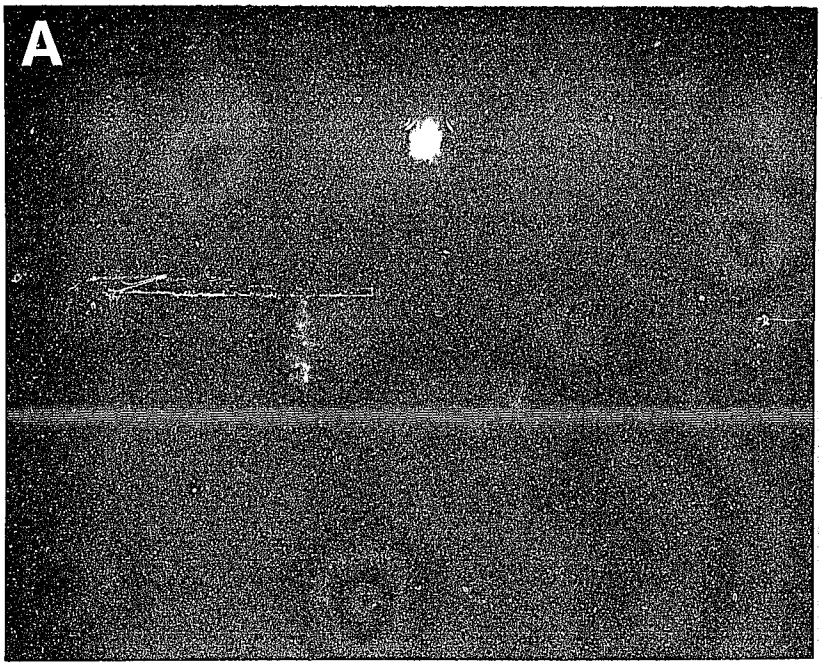
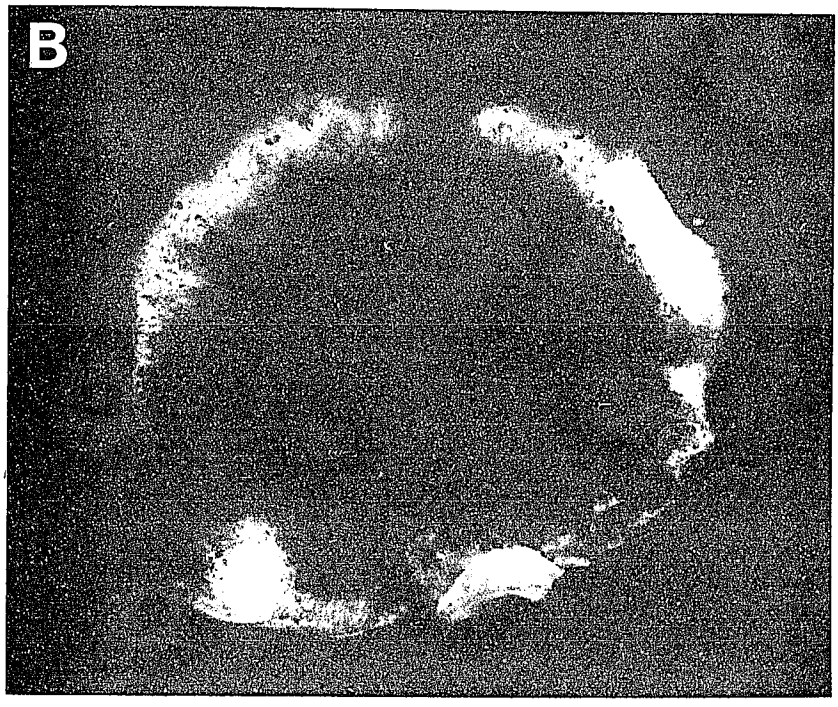


Figure 14. Di-I was injected into the lumbar portion of the lateral spinal cord of perfused rat fetuses for a period of up to 8 months. *A.* Label was found in the cervical cord at the earliest age tested, FD-17, concentrated bilaterally in the anterior lateral quadrants (as indicated by arrows) and in the dorsal columns. *B.*In a PD-1 rat, the fluorescent label had become more restricted in the anterior lateral quadrants and less dense in the dorsal columns at the level of the cervical cord. (Magnification is approximately 60X)


A


B

viewed under a fluorescent microscope using rhodamine filters.

RESULTS

Because Di-I was used as a post-fixation tracing method, a FD-17 age group of animals was the main focus in this portion of the study. In all age groups studied, Di-I was placed in the lateral area of the lumbar spinal cord. Labeling was never seen rostral to the cervical spinal cord. At the site of application, the spread of the Di-I covered the entire lateral half of the cord (Fig.13). In FD-17 animals, the fluorescence labeling had become restricted to three separate tracts at the level of the cervical cord; bilaterally in the anterior lateral quadrants and in the dorsal columns (Fig.14a). In PD-1 animals, at the level of the thoracic cord, labeling had become more restricted than in younger animals clearly showing a large bundle of fluorescence unilaterally in the dorsal lateral quadrant and two faintly labeled tracts in the anterior lateral quadrants (Fig.14b).

DISCUSSION

The use of Di-I in perfused fetal tissue provided an anatomical method of tracing the development of an early maturing ascending system. The results confirm that in the rat, the ascending LSTT is an early developing tract

that has ascended to at least the cervical level of the spinal cord as early as 17 days after conception, only 4 days after the early neurogenesis of lamina I and II cells of the dorsal horn. It is not known why the fluorescent Di-I label was never seen supraspinally but because the retrograde results of Experiment 1 show that cells are labeled in the lumbar spinal cord at FD-18, this is probably a limitation of the method, in our hands.

The anterograde tracer was used in this experiment in order to ascertain the approximate level of growth that the dorsal horn cell afferents had ascended to, and if possible, to find the age before any outgrowth was seen. It is probable that the earliest outgrowth of dorsal horn cell axons takes place soon after the neurogenesis of lumbar spinal cord neurons of lamina I-IV, X and of the VMDH is complete by FD-17.

Experiment 3: The LSTT Response to Spinal Cord Lesion

The overall goal of this experiment was to investigate the response of the LSTT to spinal cord lesion relative to its developmental status.

The definitions and issues of central nervous system plasticity

The rules that govern the success and failure of central nervous system (CNS) plasticity are not fully understood at the present time. The definition of plasticity, itself is not always agreed upon. In this thesis, plasticity is defined as the rerouting, regrowth or general pliablility of an axonal tract either through or around its normal path of development, following spinal cord lesion to its normal trajectory or to that of a neighboring system. The plasticity that occurs allows the axons to make appropriate terminations maintaining the neurons of origin to some degree. This anatomical plasticity will maintain the integrity of that system; thus the behavior that it mediates is also maintained to the same degree.

Actual, functional or lasting regeneration of mechanically injured CNS axons has not been demonstrated in the adult animal but have been found to regenerate abortively (Brown & McCouch, 1947; Barnard & Carpenter, 1950; Bernstein & Bernstein, 1971, 1973). A number of factors have been hypothesized to interfere with functional CNS regeneration in the adult.

These hypotheses include: (1) Lesioned axons cannot regenerate through a lesion site due to the formation of a heavy glial and/or connective tissue scar (Brown et al., 1947; Clemente, 1955; Lampert & Cressman, 1964; Lampert, 1967); (2) Lesioned axons cannot grow through the fluid interface following CNS interface (Kao, 1974); (3) Lesioned axons cannot regenerate due to an immediate vascular interruption (Barnard & Carpenter, 1950); (4) Axonal regrowth is inhibited following the formation of premature, inappropriate synapses (Bernstein & Bernstein, 1971).

However, it has been shown that axons may regenerate following a chemical lesion of the descending serotonergic spinal system of adult rats, but not after surgical lesions (Bjorklund, Nobin & Stenevi, 1984; Bjorklund & Stenevi, 1984). This suggests that the local environment prevents the regrowth of these axons. For example, in some mammalian species, it has been demonstrated that if axons in the spinal cord are provided with an alternate terrain that is conducive to axonal elongation, such as a peripheral nerve bridge, then axons are capable of regeneration after surgical lesion (David & Aguayo, 1981, 1985; Richardson, McGuinness & Aguayo, 1982; Richardson, Issa & Aguayo, 1984). Recently, it has been shown that injured dorsal root axons will regenerate several millimeters and form synapses into a transplant of embryonic spinal cord or brain tissue (Tessler, Himes, Houle & Reier, 1988; Itoh & Tessler, 1990a; 1990b). The

local environment has also been found to be an important factor in the regeneration of rabbit optic nerve axons. Although goldfish retinal ganglion cells readily regenerate severed axons and make appropriate and functional synapses (Attardi & Sperry, 1963), mammalian optic nerve injury leads to the death of most of the axotimized neurons (Kiernan, 1979; Grafstein & Ingoglia, 1982). This is probably due to the formation of a heavy glial scar at the site of injury (Grafstein et al., 1982; Misantone, Gershenbaum & Murrray, 1984). By delaying the formation of the glial scar through laser irradiation and by supplying soluble substances of conditioned medium obtained from regenerating fish optic nerves, it is possible to regenerate severed axons of the rabbit optic nerve. These axons have been shown to have grown 6 mm distal to the site of injury, 8 weeks postoperatively (Lavie, Murray, Solomon, Ben-Bassat, Belken, Rumelt & Schwartz, 1990). This strongly indicates that the conditions of the neuronal environment, both the physical substrate as well as neurotrophic factors influence regrowth after injury.

Although some axons are capable of plasticity if an alternate terrain is provided in some non-mammal species, most long axons ascending from and descending to the spinal cord will not regenerate after injury in adult mammals, even if the axons are provided with a favorable glial environment (Richardson, et al., 1984). In both descending (corticospinal) and ascending

(LSTT) axons of the mammalian spinal cord, this limited competence for axon regeneration has been correlated with the failure of axotimized neurons to re-induce certain factors that are available only during a limited period during development.

Factors of plasticity in the developing animal

Lesions in neonatal animals often result in greater plasticity than in adults and as a result the issue of neonatal plasticity and recovery of function is less controversial. Also, because the repair of injured tissue often mimics the original ontogenetic development, it is especially useful to understand the parameters and factors of axonal outgrowth during normal development and to apply these to the injured, adult CNS. This is the theoretical basis for embryonic tissue transplantation studies, to reintroduce factors to the mature CNS that will induce and mimic ontogenetic development. Some of these factors include: axonal proteins that are expressed transiently only during development, such as GAP-43, a major growth cone constituent (Richardson & Issa, 1984; Pate Skene, 1990); proteases associated with growing neurites of neonatal rat sympathetic and sensory neurons, such as calcium-dependent metalloprotease that is released from growth cones to open "channels" in the extracelllar matrix enabling growing axons in the peripheral nervous system to reach their targets (Pittmann, 1990); membrane proteins present in oligodendrocytes and CNS myelin, such as

NI-35 and NI-250, that exert a contact-mediated inhibitory effect on neurite growth after development is complete (Schwab, 1990); and a family of nerve growth neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), that are necessary for neuronal outgrowth and target finding (Barde, Baily, Leibrock, Hohn & Hofer, 1990). For example, there is considerable evidence that these polypeptide growth factors are secreted by target cells, bind to the receptors on axons and retrogradely transported back to the cell body where they activate genes whose expression leads to synthesis of proteins necessary for neurite outgrowth (Murray, 1990). NGF administration promotes sprouting from sensory neurons both *in vitro and in vivo* (Levi-Montalcini, 1982; Caroni & Grandes, 1990; Helper & Lund, 1990). Synthesis of growth-associated proteins is also stimulated by NGF, and increased synthesis of GAP43 is associated with central sprouting (Woolf, Reynolds, Molander, O'Brien, Lindsay & Benowitz, 1990; Wong & Oblinger, 1991).

In the newborn, other factors besides the above chemical differences may also be in part responsible for the increased plasticity demonstrated in neonatal systems. For example, debris absorption is faster in younger animals (Guillery, 1972; Schneider, 1973) and there is also less scar formation (Liu & Chambers, 1958; Hicks & D'Amato, 1970). Astrocytes

that develop at the PNS/CNS transition zone are thought to obstruct or inhibit dorsal root regeneration (Moyer, Kimmel & Winbourne, 1953; Pindzola & Silver, 1990; Liuzzi, 1990) and without an embryonic spinal cord transplant, axotimized dorsal root axons are unable to regenerate into spinal cord after the first postnatal week (Carlstedt et al., 1987). In fetal spinal cord transplant research, possible mechanisms for the increased plasticity seen in the adult hosts after transplantation are examined. Itoh, Sugawara, Kowada & Tessler (1992) have shown that embryonic day 14 spinal cord grafts transplanted into the lumbar enlargement of adult rats with severed L4 or L5 dorsal roots, promotes the regeneration of the severed dorsal roots into the transplant. The exact nature of this prolonged period of growth is unclear but it is thought that the primary afferent fibers grow into the transplant prior to the formation of the astroglial barrier that subsequently inhibits axon growth and that this barrier is in place by one month after transplantation.

The infant lesion effect

The infant lesion effect (ILE) refers to greater recovery and sparing of function when CNS damage is sustained neonatally, rather than in adulthood. Evidence for the ILE has been demonstrated in motor systems (Bregman & Goldberger, 1983a,b,c) and in sensory systems (Kalil & Reh, 1979; Carlson, 1984). These studies conclude that when lesions are made in

the neonatal nervous system, some developing pathways are able to survive and make appropriate connections in their terminal field that are not made after comparable lesions in the adult nervous system. Many times this plasticity is due to the growth of late-developing tracts of axons that were not themselves injured by the lesion. The damage is only to the trajectory of their future pathway allowing axons to reroute to a different location of the spinal cord that was not injured by the lesion. The formation of aberrant trajectories is only one mechanism that may be mediating the ILE; other compensatory mechanisms include active sprouting by either the axons of another system sharing the same terminal fields or the collaterals of the lesioned axons themselves, or the retention of developmentally exhuberant projections (Goldberger, 1986). An example of a spinal system that demonstrates the ILE is the descending corticospinal tract (CST).

An example of the ILE: CST development and plasticity

The growth of CST axons into the spinal cord was demonstrated in a study by Donatelle (1977) that also examined the temporal relationship between the development of this tract and of paw placing behavior. The most common type of paw placing reaction is elicited by gently touching the the dorsal surface of the animal's paw to the edge of a platform and timing the animal's reaction to lift the limb and place it on the platform. By using

both autoradiographic and silver degeneration techniques, Donatelle (1977) found CST axons in the contralateral dorsal funiculus of the lower cervical cord by postnatal day 1, in the mid-thoracic region by 3 days postnatal, in the lumbar cord by postnatal day 5, and in the coccygeal segments by 9 days of age. A close temporal relationship was found between the appearance of the hindlimb placing reaction and the appearance of the CST axons within the spinal gray at the appropriate level of the cord. More precisely, in a study utilizing the anterograde transport of horseradish peroxidase (HRP) injected into the cerebral cortex of fetal and neonatal mice, Schreyer & Jones (1982), found that labeled corticofugal axons traverse the diencephalon by fetal day 17.5, reach the pontine nuclei by fetal day 19.5 and the caudal limit of the medulla by fetal day 20.5, just before birth. Examining some general features of axonal growth, they also found that the CST demonstrates initial axonal overproduction followed by axonal diminution (Schreyer & Jones, 1988). This initial overproduction has been exemplified by sensory systems as well, such as the visual system (Innocenti, Fiore & Caminiti, 1977; Innocenti, 1981; Crespy, Leary & Cowan, 1984). Schreyer & Jones (1988) injected the retrogradely transported dye, Fast blue, into the cervical or lumbar segments of the spinal cord of newborn rats, and found that the first CST axons arrive from a circumscribed group of layer V pyramidal cells in the cortex. During the second and third weeks, the area sending axons to the upper cord

diminishes and retrogradely labeled cells disappear until the adult pattern of labeling is seen by the end of the third week. They concluded that this is probably due to the occurrence of axon elimination and not parent cell death. Inappropriate CST axons may arise from corticobulbar axons that may have extended beyond their normal targets in the pons and medulla and on failing to enter the spinal gray or make stabilizing connections die during the second and third postnatal weeks. In summary this system is relatively immature both functionally and anatomically at birth and because much is known about its anatomy in the adult and neonate, the effects of lesioning can be studied in a developmental paradigm.

Bernstein & Stelzner (1983) employed a midthoracic spinal cord "overhemisection" to cut the entire right hemicord and left dorsal funiculus in neonatal and weanling age rats (21 days postnatal), severing the pathways in which most CST axons are found in the mature rat. The remainder of the left hemicord was left intact to serve as an alternate axonal pathway for CST axons whose path was severed by the lesion. It is important to note that this lesion did not damage CST axons themselves in the neonate but severed the area in which they would have traversed later in development. In the weanling rat (4 weeks postnatal), this lesion cut the majority of CST axons. The results showed that in the neonate, left CST axons bypassed the lesion and followed an aberrent pathway in the dorsal

position in the lateral funiculus and terminated in normal CST sites. In the weanling operate, CST axons severed by the lesion did not regenerate around the site of lesion, but grew axonal sprouts. It should be noted that even in this group of animals, undamaged axons did not grow through a glial scar or across a gap. In a series of papers, Bregman & Goldberger (1982, 1983a,b,c), further examined the ILE in the immature cat CST. The initial effects of a low thoracic hemisection on hindlimb motor function are severe in the adult cat (Murray & Goldberger, 1974; Goldberger & Murray, 1978). The ipsilateral limb is passive during attempted locomotion and segmental activity is depressed. Postural reflexes are abolished ipsilaterally, but this is an expression of spinal shock, as it returns in a day or two. In these experiments, the contact paw placing reaction was used to assess the behavioral deficits. They found that contact paw placing is abolished in adult operates but not in neonates. It was concluded that since the neural circuits that mediate this behavior are not yet developed in neonates at the time of the surgery, the neonates demonstrated a sparing, rather than a recovery of function. The sparing of function is probably due to the late-developing, and therefore undamaged, CST axons that take an aberrant course around the lesion, since only part of the CST has extended throughout the cord on the day of birth. In summary, the above studies show that the CST is a late-developing tract in mammals and it is this relative immaturity that allows the tract to demonstrate remarkable

anatomical and behavioral plasticity.

The Gudden effect

The Gudden effect (GE) describes an opposite reaction to the ILE and states that younger neurons are more susceptible to injury than older, more mature neurons (Brodal, 1940). It is generally accepted that CNS injury sustained in infancy often produces fewer and less severe consequences than does identical injury sustained at maturity in some systems, as seen in the CST. However, in contrast to the plasticity of the immature CST, is the response to lesion of other systems like the rubrospinal tract (RST) to neonatal lesions. Thus, the response of the immature CNS to damage is not uniform.

An example of the GE: RST development & degeneration

The course and development of the RST was first described by von Monakow (1883, in Brown, 1972). It has been shown that the cat and monkey rubrospinal fibers terminate in precisely the same region of the spinal cord as do the fibers of the CST (Lawrence & Kuypers, 1965). In both the rat and opossum (Martin & Fisher, 1968), the RST descends in the dorsolateral funiculus, principally contralateral to its origin and projects to the base of the dorsal horn and to intermediate regions of the ventral horn.

Shieh, Leong & Wong (1983) utilized injections of HRP to describe the origin and development of this descending tract. HRP was administered to the lumbosacral region of the cord in three different age groups of rats: neonate, weanling and adult. The results showed that, unlike the relatively immature CST, the RST extends to the lumbosacral part of the cord at birth. Furthermore, labeled neurons were found bilaterally in the red nucleus with a contralateral predominance.

In studies that have examined the effects of lesioning this relatively mature tract in infant rats and cats, the Gudden effect, rather than the ILE, has been exemplified. The response of the RST to spinal cord lesion, in neonatal and adult rats and cats, was different in that the immature rubrospinal system responded to the lesion by a massive retrograde cell death not seen in the adult operates (Prendergast & Stelzner, 1976). There was little to no retrograde labeling in the red nucleus when WGA-HRP was injected into the spinal cord contralateral to the site of lesion, in either age group. It is uncertain what precipitates the retrograde cell death response seen in neonates. One hypothesis is that this phenomenon reflects a critical period where some neurons depend on an environmental factor, such as a trophic substance, or suitable relationships with postsynaptic structures that are necessary for their survival (Bregman & Reier, 1986). An obvious prerequisite for the regrowth of axons after injury is the survival of the

parent cell body. One factor that may influence the immature neuron's response to axotomy may be its degree of dependency on trophic support from its environment or targets. This need for trophic support may itself vary with the developmental stage of the neuron (Reier, Perlow & Guth, 1983).

Other early developing pathways that demonstrate the GE when lesions have been made in both the adult and neonate are the lateral vestibulospinal tract, and after high cervical lesions, the spinal component of the Edinger-Westphal nucleus (Steward, Cotman & Lynch, 1974). Unlike what the ILE and GE state, all of the above studies indicate that it is not the age or maturity of the animal in question that determines CNS plasticity and recovery of function, but some other factor(s).

Tract maturity is an issue

The question remains whether the response of the rat RST to neonatal lesion is due to characteristics not yet determined, inherent to the system, or if the retrograde cell death of the RN is due to the advanced growth of this early developing tract at birth.

Because the North American opposum is born in a highly immature state, 12 days after conception, and climbs into an external pouch in the mother

for a period of 3 months, Martin & Xu (1988) used it as a model for developmental studies to address the above questions. By injecting wheatgerm agglutinin conjugated to HRP (WGA-HRP) into the red nucleus of neonatal opossums, Cabana & Martin (1986) found that unlike the rat, RST projections do not reach the cord until postnatal day 5 and that the adult opossum pattern of rubrospinal innervation is not complete until postnatal day 35. Because the maturity of the RST occurs postnatally in this animal, as the CST does in cats and rats, the opposum serves as a good model to demonstrate plasticity of the RST. Opossums, ranging in age from postnatal day 12 to 75, were operated on while still in the mother's pouch, lesioning the RST at mid to caudal thoracic levels. Injections of WGA-HRP were made caudally and ipsilaterally to the thoracic lesion in order to label rubral neurons whose axons had grown caudal to the lesion. When RST lesions occurred between postnatal day 12 and 43, rubral neurons were labeled contralateral to the lesion. Lesions made to a mature RST in postnatal day 54 animals resulted in no retrograde labeling of the contralateral red nucleus one month later. These results show that the RST is capable of plasticity at early stages of its development and so, in the opposum, exemplifies the ILE and not the GE. This study also demonstrates that there is a sensitive period for plasticity that is dependent on the stage of development of the particular system involved (Martin & Xu, 1988). In order to determine whether this plasticity resulted from the

growth of new axons around the lesion, true regeneration of severed axons or both, Xu & Martin (conference communication, 1990) utilized 2 fluorescent retrograde markers, Fast Blue (FB) and Diamidino Yellow (DY). The long-lasting marker, FB was injected into the lumbar cord to label RST neurons in developing and adult opposums. After the appropriate survival time, the RST was lesioned several segments rostral to the injection. After approximately 30 days, injections of DY were made between the first injection and the lesion. When the red nucleus was examined, it was found that relatively few rubral neurons were labeled by FB, but many were labeled by DY, suggesting that plasticity results primarily from the growth of new axons. A few neurons were double labeled suggesting that they not only survived axotomy but that they supported regenerated axons.

The response of the immature CNS to damage is not uniform

The Gudden and infant lesion effects are two paradoxical and competing responses to neonatal lesion and illustrate the point that the anatomical and functional consequences to neonatal spinal cord lesion are quite diverse and not well understood. All of the above studies have examined specific spinal systems to characterize the anatomical and behavioral responses to neonatal spinal cord damage. The concept that has emerged from these developmental studies is that in neonatal mammals, spinal cord

systems develop and mature at different rates, and that it is this differential rate of specific tract maturity that dictates plasticity, rather than the overall maturity of the animal. If anatomical and behavioral plasticity are dependent on the individual maturity of the tract, and not on the age of the animal, it should become possible to define a type of sensitive period for recovery once the development of any tract is known.

The aim of this experiment was to investigate the anatomical consequence of lesions that are made to the spinal cord at different developmental stages of the LSTT. Both of the preceding experiments have shown that the development of the LSTT occurs prenatally in the rat. Specifically, Experiment 1 showed that some, but not all, afferents have already terminated in the VPL thalamus by FD-18 and that the LSTT has terminated in all parts of the VPL thalamus by PD-0, although maturation continues at least to PD-10. Experiment 2 found that by FD-17, LSTT afferents are present in the cervical spinal cord and so the initial growth of this tract must take place soon after neurogenesis of the spinal cord cells of origin at FD-14. For the present experiment, it was hypothesized that a sensitive period for anatomical plasticity could be demonstrated such that if lesions are made through the ventrolateral quadrant of the spinal cord when the LSTT is at a relatively immature state of development, then the system will be able to take an aberrant course around the site of lesion and

terminate in the VPL thalamus. Because it was hypothesized that the tract would be able to make appropriate contacts with the neurons of the terminal field, the integrity of the LSTT and the pain behavior that it mediates will also be maintained, thus demonstrating the infant lesion effect. In contrast, if a lesion is made through the ventrolateral quadrant of the spinal cord after the LSTT has ascended to the VPL thalamus, permanent anatomical and behavioral damage should occur. The anatomical and behavioral plasticity that is hypothesized to occur is a graded response dependent on the relative maturity of the tract. Although maximum plasticity would be expected to occur before the spinal cord cells of origin has started to send any axons through the cord, plasticity is still expected to occur at FD-17, when the LSTT is at a very early stage of its development. In the present experiment, the effects of spinal cord lesion were studied in two different age groups of rats that represent two very different developmental stages of the LSTT.

METHOD

Subjects

Twelve to fourteen day old (PD-12 to PD-14) Long-Evans hooded rat pups born in our colony were used in this experiment to study the effects of spinal cord lesion on the more mature LSTT. Fetal day 17 rats from timed-

pregnant females were used to study the effects of spinal cord lesion on the very immature LSTT. For prenatal subjects, female rats were checked daily by the vaginal smear method for the presence of sperm. Once sperm was detected, the male was seperated from the female and this day was termed FD-0. For postnatal subjects, rats were checked twice daily for the presence of pups, with the day of birth termed PD-0.

Postnatal spinal cord surgery

Pups were deeply anesthetized by inhalation of methoxyfluorane. A laminectomy was performed at the level of the mid-thoracic spinal cord, leaving the dura intact. Approximately .5 cm of the cord was exposed. Using a fine scalpel (Fine Science Tools, troutman corneal knife), and working under a binocular, stereoscopic surgical microscope (Carl Zeiss #41010, West Germany), a lateral spinal hemisection of the spinal cord was made. The area was cleansed and the incision was closed with a few drops of externally applied cyanoacrylate cement. The entire procedure took about 20 minutes. The pup was then warmed under a heat lamp and placed in an incubator until full recovery from anesthesia, about 60 minutes. Upon recovery, the pup was transported back to its homecage with its operated littermates and mother. After being weaned from its mother, all littermates were sexed and housed together with food and water available *ad libitum*.

Fetal spinal cord surgery

Following the intrauterine fetal method that was explained previously (see Fetal Retrograde Tracing Techniques), the uterus of the dam was externalized into the warm saline bath. The procedure of Gearhart, Oster-Granite & Guth (1979) in which the spinal cord was lesioned in fetal mice was followed. Care was taken to not lesion neighboring fetuses in order to put the least amount of stress on the uterine wall. Using a surgical microscope, a hemisection was made without laminectomy. Because at this age the vertebrae are very soft, cutting through the vertebral segments as well as through the cord did not pose any special problems. Once hemisections were made, the uterus was carefully placed behind the muscle wall and the female was removed from the saline bath. All incisions were closed with square knot sutures. The entire procedure lasted about 90 minutes. The dam was then brought back to her warmed homecage to recover from surgery.

Behavioral testing

In designing the battery of tests for assessing the pain mediating behavior of the LSTT both before and after hemisection, it was very important to separate those responses that are supraspinally mediated from those that are segmental reflexes. The present test battery included only those tests that were found to be supraspinally mediated in pilot work with transected

animals in which all pathways between the spinal cord and brain had been severed. If the transected animal initiated a withdrawal response to the noxious stimuli then the stimulus at that intensity was defined as segmentally mediated.

Experimental groups of rats that had spinal hemisections as fetuses underwent testing following a 30-day survival period. Postnatal experimental animals were tested 72-96 hours after surgery and following a 30 day survival period. Although all animals dragged both of their hindlimbs, care was given to insure that the limb contralateral to the lesion still had the motor capability to respond to all behavioral tests.

Noxious heat test

This test examined the supraspinal reaction of the pup to a noxious heat stimulus. A hemisection of the right side of the lumbar spinal cord causes a loss of pain sensation in the left hindlimb but spares some motor function of that limb due to the different points of decussation of both the CST and the LSTT relative to the lesion. Motor functioning of the hindlimbs is carried by the ipsilateral spinal cord and does not cross until the level of the medulla. Painful sensory information for the hindlimbs (via the LSTT), ascends in the contralateral spinal cord since the decussation of the LSTT is complete immediately after entry into the cord through the spinal gray

matter. Preliminary work with transected pups showed that water temperatures exceeding 53°C elicited a segmentally mediated reflex withdrawal response and so was not used. Limb withdrawal at lower temperatures however, requires an intact spinal cord (Paredes & Barr, unpublished data). To test the response to a noxious heat stimulus, each hindlimb was submerged in a hot water bath of 49°C and the latency to both vocalize and withdraw was recorded. In order to avoid tissue damage, a cutoff latency of 5 seconds was observed.

Noxious mechanical test

In following the rationale of the above test, this test examined the pup's supraspinally mediated withdrawal response to a noxious mechanical stimulus. Preliminary work with PD-1 to PD-10 transected pups has shown that at high forces (above 64 g), the response to withdraw is segmental but that at more conservative weights of 57 g, pups reliably emitted an audible vocalization and a withdrawal response. In younger animals, a blunt metal probe fashioned from a dulled 23 gauge needle, was slowly lowered to the dorsal surface of each hindpaw at a force of 57 g. The probe was bevelled to 30° and had a surface area diameter of .2 cm. The latency to initiate a withdrawal and vocalization response was recorded. A cutoff latency of 5 seconds was observed. After careful preliminary observations with the older age group, it became necessary to change the testing stimulus that was used in order to obtain reliable, supraspinal responses to

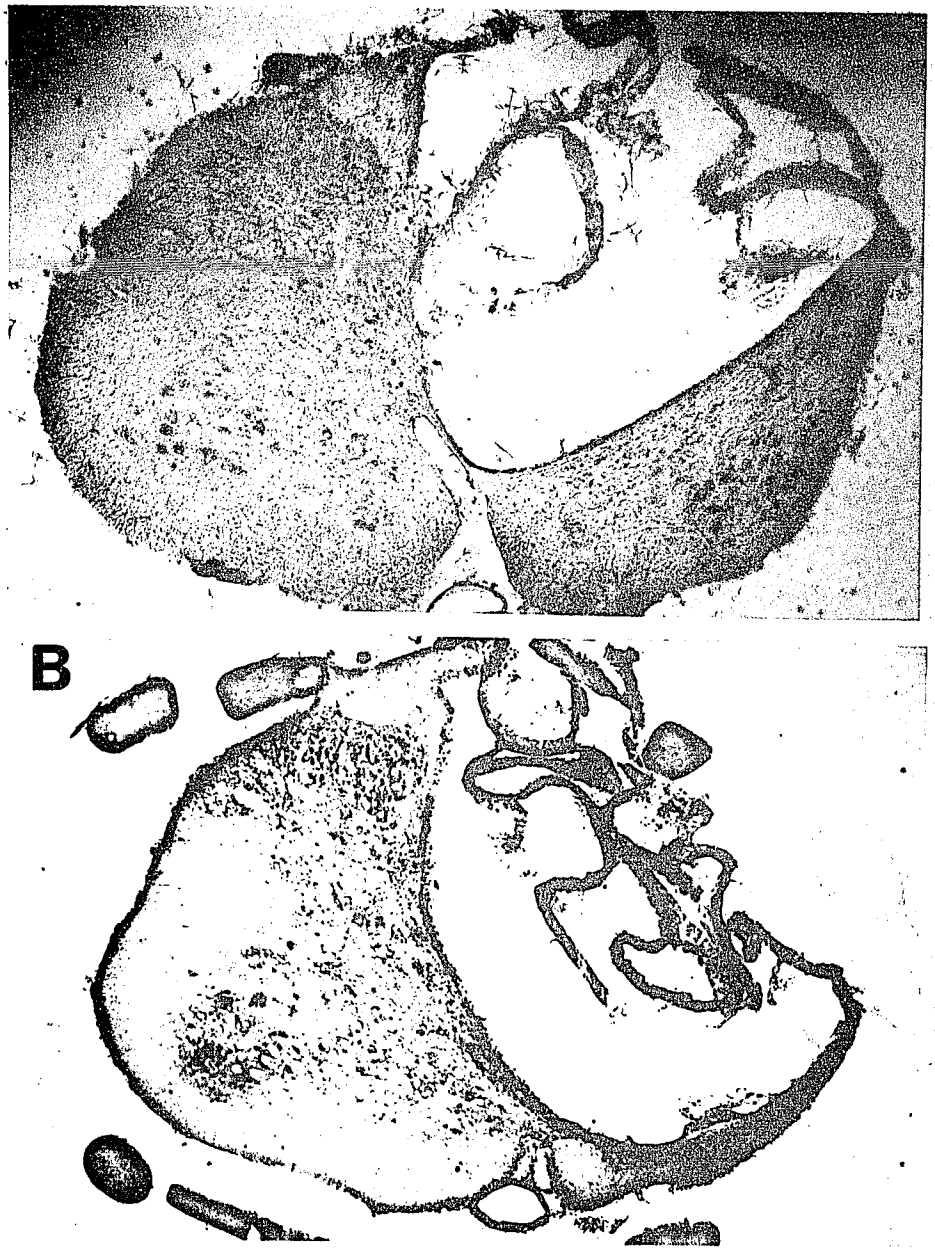
a noxious mechanical stimulus. In this age group, use of the blunt metal probe would require an excessive amount of weight to elicit a response and would make the separation between a spinal and supraspinal response difficult to make. The strength of this age group of animals (the average weight is 200 g) also made it difficult to accurately determine a withdrawal. In order to alleviate the above problems, a small alligator clip was used. The metal clip has a mouth that measures approximately 10 mm in length and when the mouth is maximally opened, it measures 6 mm wide. The serrated edges of the teeth were filed down until blunt. The alligator clip was gently put on each hindpaw only when the animal was not visually orienting towards it. The biting response was a clear, reliable, supraspinally mediated behavior. The latency to initiate this response was recorded, and the cutoff latency of 5 seconds was again observed.

Experimental design

The anatomical plasticity that was hypothesized to occur was expected to be a graded response dependent on the relative maturity of the tract. Therefore, maximum plasticity would be demonstrated to occur before the spinal cord cells of origin had started to send any axons to the VPL thalamus. But, plasticity would still be expected if the lesion was made at any early developmental stage. Because the youngest age at which spinal cord lesions could reliably take place was FD-17, and the results of

Experiment 1 and 2 demonstrated this to be a very early stage of LSTT development, this age group was chosen as the optimal group to reliably demonstrate anatomical plasticity in the present experiment. Experiment 1 also found that the LSTT had already terminated in all parts of the VPL thalamus and appeared in its mature form by PD-10, and so animals aged between PD-12 and PD-14 was also investigated to demonstrate the response of the mature LSTT to axotomy. Experimental groups of 3-9 animals were used for both ages studied. Both groups underwent a lateral spinal cord hemisection of the mid-thoracic cord and was allowed to survive for a period of either 35 or 52 days. The older group of animals underwent behavioral testing to noxious stimuli 48-72 hours after surgery and again at the end of the 35-day survival period. The group of animals hemisected *in utero* at FD-17 were tested in an identical manner as the PD-10 group with the exception that testing began 48-72 hours after birth and then at the end of a 52-day survival period. The different survival times for the two groups was done so that both groups were examined at the same age (surgery done at PD-12 + 35 day survival period = PD-47 at the time of testing and sacrifice; surgery done at FD-17 + 52 day survival period = PD-47 at the time of testing and sacrifice). A control group of PD-12 animals was also behaviorally tested at the same time periods as experimental animals after receiving only a spinal laminectomy. The fetal control group was made up of unlesioned littermates of the experimental group. At the end of the

Figure 15. Photomicrographs showing coronal sections of the site of thoracic spinal cord lesion in PD-12 animals. *A.*The smallest lesion destroyed most of the lateral half of the cord except for a portion of the ventral horn. *B.*The most extensive lesion included the contralateral dorsal funiculus as well as the lateral half of the cord.


B

survival period, after behavioral testing was completed, animals of each age group were stereotaxically injected with WGA-HRP into the contralateral VPL thalamus and processed as described in a previous section (see Postnatal retrograde tract tracing methods). Alternate sections from all animals used in this study were stained with cresyl violet in order to verify spinal cord hemisections.

RESULTS

The results are based on rats in which qualitative and quantitative behavioral data and morphological information indicated with certainty, that thoracic spinal cord lateral hemisections were successfully performed. In PD-12 operates, the smallest lesion destroyed the entire lateral half of the spinal cord except a small portion of the ventral horn (Fig.15a), and the largest lesion included the contralateral dorsal funiculus (Fig.15b). In fetally lesioned animals, the extent to which the lesions destroyed the spinal cord was difficult to verify anatomically as no open cavity or lesion was ever seen in cresyl violet sections and so was assessed using behavioral data and other visual means such as disrupted or missing laminar organization in the lower segments of the spinal cord or shrunken appearance of the caudal spinal cord as compared to controls.

In-utero surgery and behavior at PD-4

Two litters of fetuses were operated on *in utero*. In the first dam, #245, 5 of the 9 fetuses were given spinal lesions. In dam #231, 6 of the 12 fetuses were also given lateral hemisections. Because at this age, all membranes and vertebrae surrounding the spinal cord are transparent, use was made of the posterior spinal vein that runs down the center of the spinal cord as a landmark that divided the right and left halves of the cord when lesioning. Both dams gave birth without incident on FD-22. Surgically lesioned animals were verified at birth by an open wound to the back that was immediately closed by cyanoacrylate cement. Lesioned animals were then immediately given a small tattoo in the forepaw. At birth, there was a very low survival rate among both lesioned and unlesioned animals. Dam #245 gave birth to 2 living (one was lesioned, #L245-1) and 7 dead animals. Dam #231 gave birth to 4 living (two were lesioned #L231-1, #L231-2) and 8 dead animals. Because surgery was done without laminectomy, and through all membranes encasing the fetus, it was difficult to determine at birth which side of the cord was hemisected. All lesioned animals had a definite and obvious paralysis of at least one hindlimb; in all cases one hindlimb was more impaired than the other and this was designated as the side ipsilateral to the lesion. At PD-4, all lesioned animals were tested and showed behavioral deficits. In the limb contralateral to the lesion, the mean latency to withdraw from a noxious thermal stimulus was 3.68 sec. (in

Table 4: Latency means (seconds) of fetally and postnatally lesioned rats.

	Noxious Mechanical		Noxious Thermal	
	After Lesion	**After Survival Period**	**After Lesion**	**After Survival Period**
Fetal Lesions n=3	*3.59 sec. ± 2.43 (sd)*	*3.51 ± 2.48*	*3.68 ± 2.29*	*3.85 ± 1.99*
Fetal Controls n=3	.76 ± .24	.78 ± .19	.71 ± .18	.84 ± .25
Postnatal Lesions n=9	*3.49 ± 1.85*	*4.26 ± 1.10*	*2.48 ± 1.71*	*3.68 ± 1.45*
Postnatal Controls n=9	.91 ± .47	1.16 ± .48	.58 ± .14	.85 ± .21

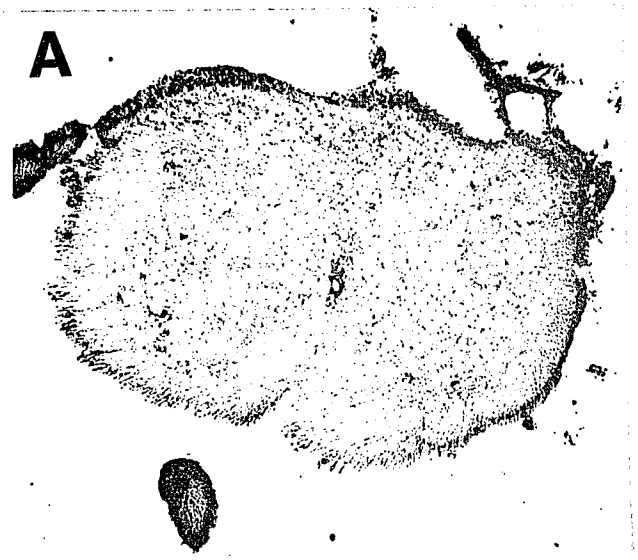
controls, x=.71 sec.). To withdraw from a mechanical stimulus, x =3.59 sec. as compared to controls, x = .66 sec. (See Table 4)

Behavior in fetal lesioned animals after survival period at PD-47

After the 52 day survival period, #L231-2 maintained normal grooming and elimination behavior, but the other two lesioned animals needed to be washed and groomed by the experimenter. All three animals had bodyweights comparable to that of controls, about 185 g.

All 3 lesioned animals had serious behavioral deficits ranging from dragging both hindlimbs and no movement (#L245-1), to both hindlimbs dragging and some movement (#L231-1 and #L231-2). In two lesioned animals (#L245-1, #L231-1), the plantar surface of both dragging hindlimbs was facing dorsally and in #L245-1, the hindlimb ipsilateral to the lesion was atrophied at a 30° angle to the ground. In animal #L231-2, both hindlimbs were oriented normallly to the ground, although dragging. This animal appeared to have adopted a different behavior to adapt to the impairment by pushing off of the hindlimbs, similar to a hop, in order to escape or to obtain food, indicating that some motor ability was spared. All animals did have voluntary movement in the contralateral hindpaw as they did at PD-4 and the means of both thermal and mechanical tests were unchanged. In lesioned animals after the survival period, the mean latency to respond to a noxious thermal stimulus was 3.85 sec. (in controls, *x =.92 sec.). In*

Figure 16. Coronal sections of the spinal cord from an adult animal lesioned at FD-17. *A.* Caudal to the site of lesion, the spinal cord appeared smaller in diameter and lacked clear laminar organization, often displaying much fewer cells than in a normal, unlesioned control animal. *B.* In coronal sections of the sacral spinal cord, several millimeters caudal to the lesion, sections appeared relatively normal.(Magnification is approximately 60X)


A

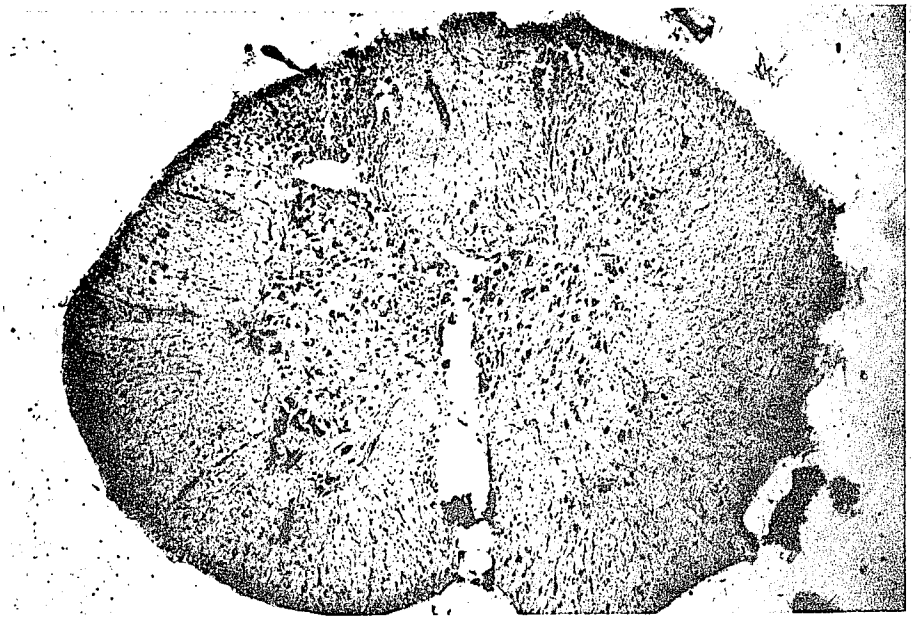

B

responding to a noxious mechanical stimulus, x= 3.51 sec. as compared to unlesioned controls, x= .91 sec.

Anatomical results of FD-17 operates

In all rats lesioned as fetuses, gross observation of the whole cord showed no open cavity in the spinal cord corresponding to the site of lesion. In all cases, there was damage in the longitudinal axis marked by an obvious tapering of the cord that continued to the caudal end. In cresyl violet and neutral red sections, the gray matter appeared cytoarchitectually normal and of normal size to within a few millimeters rostral to the lesion. At that point, and through the lesion site, the gray matter progressively disappeared giving the spinal cord the tapered appearance. Many times, the spinal cord would break on dissection at this point due to the weakened state of the tissue. Disorganization could be seen unilaterally in the gray matter of transverse spinal cord sections caudal to the site of lesion. In #L245-1, one dorsal horn appeared to be missing. In the other animals, there was an apparent reduction in the size of the dorsal horn. In both #L231-1 and #L245-1, the ventral horn was also greatly reduced with an obvious lack of large motor neurons, and the entire section appeared to lack laminar organization (Fig.16a). However, several millimeters caudal to the lesion, cresyl violet sections revealed sacral sections that appeared normal in terms of both size and organization (Fig.16b). Corresponding to both the relative

Figure 17. Different degrees of spinal cord disorganization was observed in the adult sections of animals lesioned at PD-12. In one animal, transverse sections revealed a minimum of damage in the dorsal horn. (Magnification is approximately 60X)



lack of motor impairment and behavioral data from pain tests, the spinal cord sections rostral and caudal to the site of lesion from #L231-2 were intact with little damage.

Due to reasons unknown, the WGA-HRP reaction failed in these animals. In this important group of animals, care was taken to insure that WGA-HRP injections were made carefully by testing that the needle was not clogged both prior to and after each injection. All tissue samples from this group of animals were handled identically to each other and to previous groups of animals in earlier experiments. It can only be speculated that the sample of WGA-HRP used on these animals was the nature of the problem as it was mixed fresh with each new experiment.

Surgery at PD-12 and behavior at PD-14

Eleven animals were given lateral spinal cord hemisections in the thoracic cord with laminectomy. Autophagia occurred in two of these animals and they were immediately euthanized. All other animals (n=9; control animals n=5) survived surgery and the survival period without incident and were included in the study.

After the 48 hour recovery period from surgery, all lesioned pups dragged the ipsilateral hindlimb. Most times, the plantar surface of the paralyzed

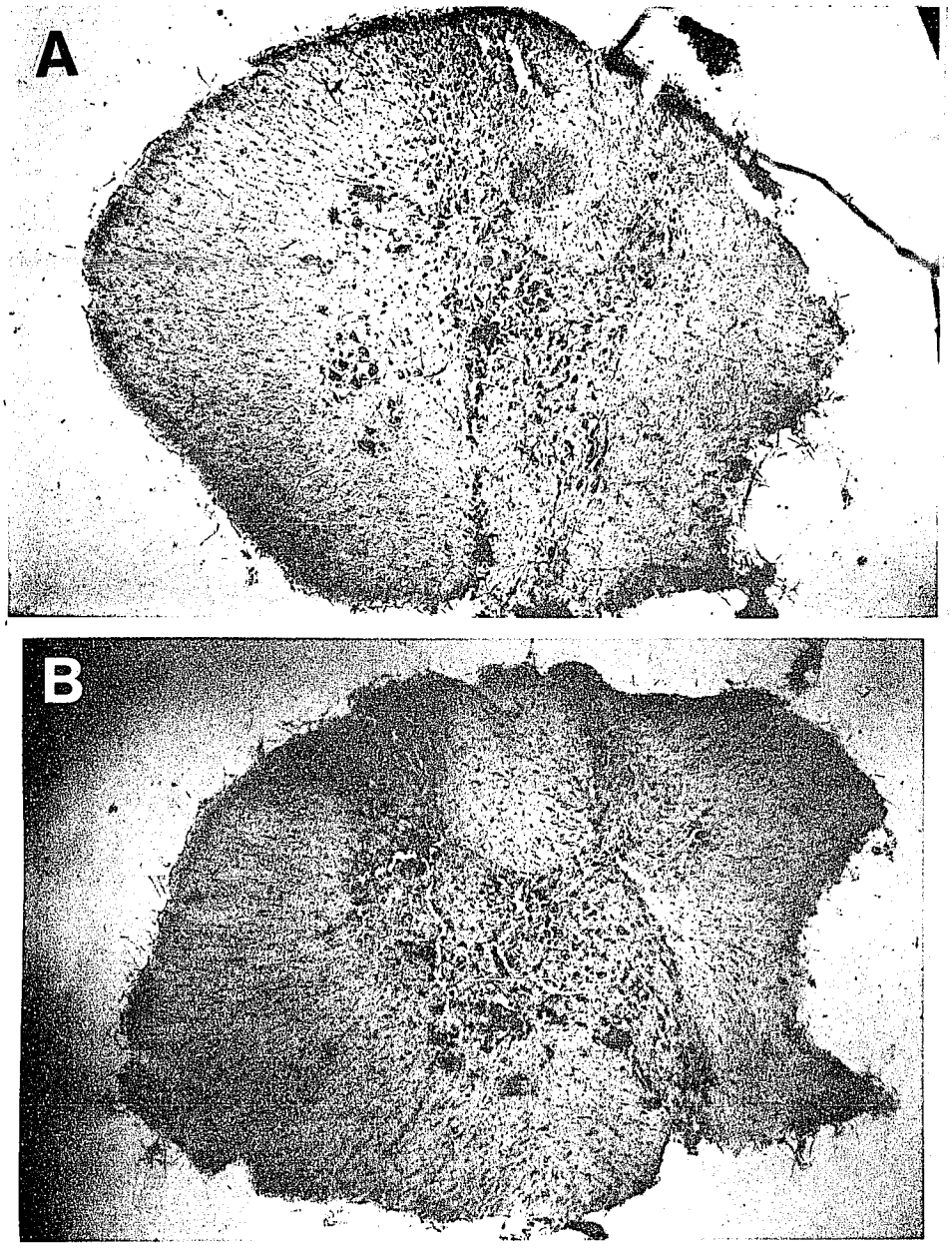
hindlimb faced dorsally. At PD-14, all animals were behaviorally tested. In the limb contralateral to the lesion, the mean latency to withdraw from a noxious thermal stimulus was 2.48 sec. as compared to 0.67 sec. for control animals. To withdraw from a noxious mechanical stimulus, x=3.49 sec., in control animals x=0.80 sec.

Behavior in postnatally lesioned animals after survival period at PD-47

After the 35 day survival period, all lesioned animals remained paralyzed in the ipsilateral hindlimb dragging it as they moved, with the plantar surface of the atrophied limb facing dorsal, in only 2 cases (#L507-1, #L507-5) was the paralyzed limb oriented normally to the ground. All animals maintained grooming and elimination behavior. In all cases, the contralateral hindlimb appeared normal and although food and water were easily accessible to the animals, the average weight for these animals was about 140 g as compared to 191 g for laminectomized control animals.

In all experimental animals, the contralateral hindlimb remained mobile and normal in all appearances. In the noxious mechanical test, the subject bit at the clip on the contralateral hindlimb only after the animal visually oriented to it. No vocalizations or apparent urgency to remove the clip was ever seen, unlike the control animals. In lesioned animals after the 35 day survival period, the mean latency to respond to a noxious mechanical

Figure 18. In typical transverse spinal cord sections caudal to the site of lesion in animals lesioned at PD-12, the dorsal and ventral horn showed disorganization not seen in fetal operates. *A.*The dorsal horn of this animal appeared shrunken due to the lack of cells and the entire gray matter was shifted relative to the white matter. *B.*Many times the ventral horn was completely missing, replaced by glial cells or was of an unusual shape due to the lack of neurons. (Magnification is approximately 60X)


A
B

stimulus was 4.64 sec. (in controls, x=1.16 sec.). In responding to a noxious thermal stimulus the mean latency to remove the hindpaw was 2.48 sec. versus a mean latency of .85 sec. in control animals.

Anatomical results of PD-12 operates

In rats lesioned at PD-12, an area of damage at the site of the lesion was noticeable on visual inspection of the dorsal side of the spinal cord. As in fetally lesioned animals, there was a tapering of the spinal cord caudal to the lesion. In cresyl violet and neutral red transverse sections, the spinal cord appeared normal rostral to the lesion. Immediately caudal to the lesion, different degrees of disorganization of the lateral half of the spinal cord was seen. In one animal, the dorsal horn was faint and lacked cells. However, from a majority of animals, the dorsal horn was either missing or shrunken exhibiting greater distortion in general than fetal operates (Fig.18a). Unidentified large neurons were also seen in the area of the dorsal horn. The ventral horn displayed disorganization not seen in fetal operates in that it was either missing and\or elongated, tapering to an end in the ventrolateral quadrant (Fig.18b). Sacral sections, several millimeters caudal to the site of lesion, were unavailable for examination as the spinal cord disintegrated at this point.

Cresyl violet sections of the thalamus showed that WGA-HRP injections

Figure 19. In either control or in animals lesioned at PD-12, adult animals showed heavy labeling of the contralateral PrV nucleus following injections of WGA-HRP into the VPL thalamus. (Magnification is approximately 150X)

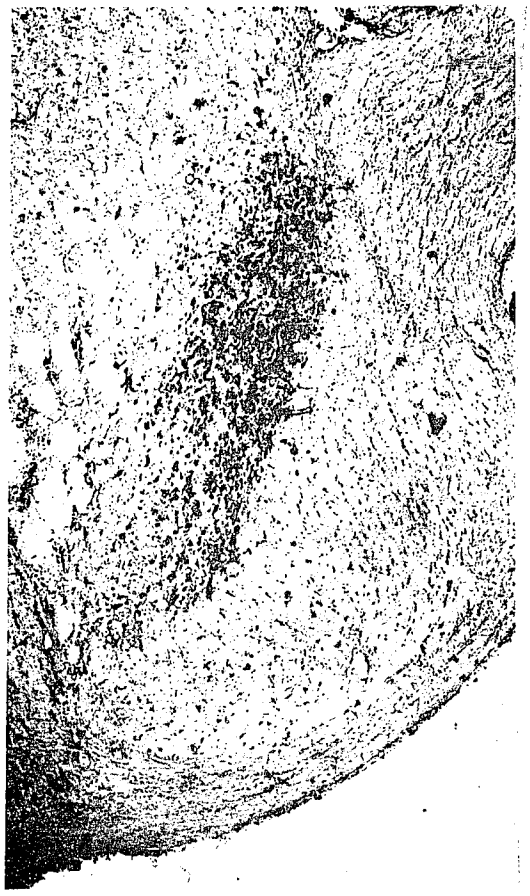
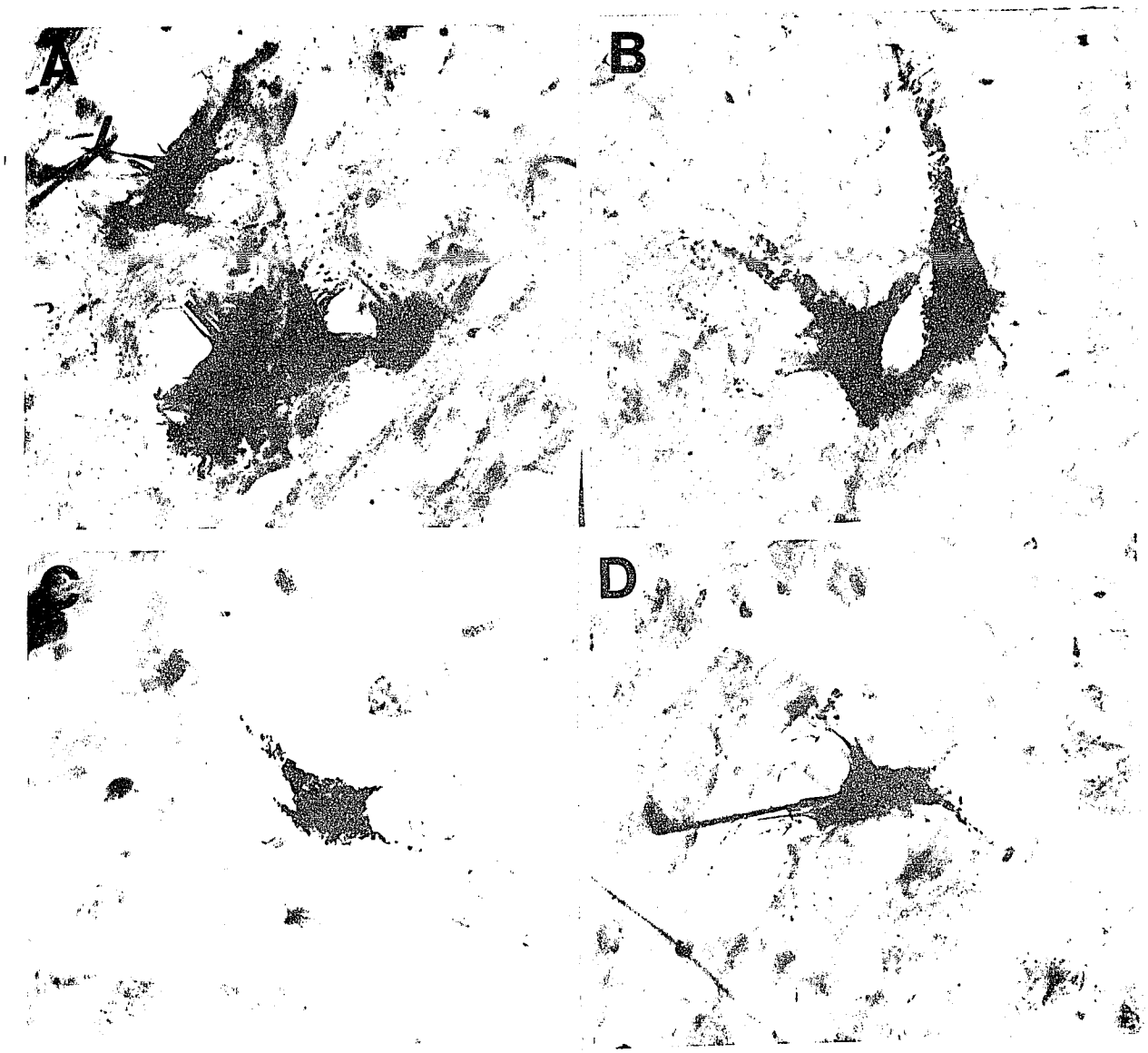


Figure 20. Cells labeled with WGA-HRP were found in the lumbar spinal cord of adult control rats but not in adult animals lesioned at PD-12. *A,B.*The majority of cells were found in groups or in pairs in the ventromedial dorsal horn. *C.*Cells were also seen in the neck of the dorsal horn. *D.*Smaller bodied, angular cells were sometimes encountered in the superficial layers of the dorsal horn. (Magnification is approximately 1500X)


A
B
C
D

were accurately placed in the VPL nucleus and were verified by the results that demonstrated heavy labeling of the contralateral principle sensory trigeminal nucleus (PrV) both in lesioned animals (Fig.19) and unlesioned controls. As was found in postnatal rats in Experiment 1, densely labeled cells were found in groups or in pairs (Fig.20a,b) in the lumbar cord of unlesioned control animals. Also, as was found in Experiment 1, most cell bodies were spindle shaped with extensive processes in the VMDH. These neurons were the most numerous, as well as the largest. Neurons with round somata and multiple processes were encountered in the neck of the dorsal horn (Fig.20c). Small multipolar neurons were located in the superficial lamina of the dorsal horn (Fig.20d). Labeled spinal cord cells were never seen in experimental groups although the contralateral PrV nucleus and ipsilateral medial lemniscus was labeled.

DISCUSSION

It was hypothesized that lesions made to the spinal cord during the development of the LSTT in rats may result in a differential amount of anatomical and behavioral plasticity dependent on the relative maturity of the tract. Lesions made to the spinal cord at FD-17 were predicted to result in greater amount of anatomical plasticity and behavioral recovery than in animals lesioned at PD-12.

Behaviorally, both groups were similar in tests using noxious stimuli, when tested immediately after surgery and at the end of the survival period. Morphologically, the disorganization of the spinal cord was also approximately the same for both groups and so demonstrated neither the ILE or the Gudden effect. However, the complete lack of HRP results from the fetally lesioned animals makes it impossible to make firm conclusions and the possibility that the LSTT was able to reroute through the cord to the thalamus cannot be eliminated. Also, because no metabolic assays or staining was done to verify the neurochemical integrity of the spinal cord sections, firm conclusions about differences either between or within the two experimental groups cannot be made. Although the amount of damage was approximately the same in both groups, the fetally-lesioned group adapted to the injury more successfully than the PD-12 group in maintaining normal bodyweight. It is also noted that despite the paralysis, one animal of the fetal group adopted the "hop" behavior, not seen in any animal of the PD-12 group whereas 2 animals of the PD-12 group had to be euthanized due to autophagia of both hindlimbs, indicating a severe loss of function.

Although in the present experiment, the VPL thalamus of experimental groups appeared normal, in agreement with other investigators that have lesioned the spinal cords of neonatal animals (Prendergast & Stelzner;

1976a, 1976b) the reduced area of the dorsal, ventral and lateral funiculi and the lack of dorsal horn organization following midthoracic lesion indicates a massive retrograde cell and fiber loss of supraspinal systems. In all animals of the fetal group a type of reorganization was seen that was not evident in the older group; the large bodied neurons of the ventral horn appeared to be displaced into the area of the dorsal horn and laterally at the neck of the dorsal horn.

Although Experiment 3 needs to be replicated before conclusions can be made, we attempted to show a type of critical period or a specific amount of time that exists during which the nervous system is reacts differently to damage, namely early in the development of the LSTT before it has projected to and made terminations in the thalamus. However, in the true sense of a critical period, the nervous system is reactive to manipulations presumbably because they are made at a sensitive point in the development of the system. For example, within the somatosensory system, studies have indicated that reorganization of the terminal thalamic field is dependent on age at the time of damage to the mystacial vibrissae of rodents, indicating a type of critical period (Killackey, Belford, Ryugo & Ryugo, 1976). The mechanism behind this period of malleability is probably due to a trophic substance that is crucial to the organization of this system during a short, specific critical period of development. Once the system is established, it is

no longer under the influence of the trophic substance. Saporta (1986) examined the LSTT cells of origin in adult rats after they had undergone neonatal capsaicin treatment that specifically destroys the small-diameter primary afferent fibers. Neonatal rats were injected with capsaicin at either PD-1, PD-2, PD-7 or PD-15. A critical period was found for the cells of the LSTT whereby rats injected with capsaicin before PD-7 demonstrated a significant reduction of dorsal horn cells. No differences were found in the amount of labeled spinal cord cells between control adult rats and in rats injected with capsaicin on PD-7 or PD-15, indicating that for the spinal cord cells of the LSTT, a critical period of development exists early in the neonatal period, before postnatal day 7. This is in clear agreement with the results of the present study that showed the development of the LSTT to continue into the first postnatal week of life.

The design and methods of this study did not address the issue of regeneration or collateral sprouting but was interested in examining the question of whether LSTT neurons would be present in the dorsal horn caudal to the lesion indicating the growth of the tract around the lesion to the proper site of termination in the thalamus. It is not known why the WGA-HRP reaction failed in the most important group of animals to this study and so the replication of this experiment in more animals is necessary before conclusions can be drawn. However, from the HRP results of the

PD-12 group of animals, the Gudden effect does not appear to be demonstrated in the LSTT since no spinal cord labeling occurred following VPL thalamus injection.

GENERAL DISCUSSION

These experiments were designed to examine the development of a behaviorally important system of fibers, the LSTT, and then to study the effects of lesioning this system in relation to its state of development in order to understand better some of the laws of anatomical and behavioral plasticity.

From Experiments 1 and 2, it can be concluded that the development of the LSTT, like other sensory systems, is an early event taking place in the rat fetus as early as FD-17. At this time, LSTT axons are already present in the cervical spinal cord and maturity continues into the early postnatal period. This is evidenced by the addition of labeled neurons with extensive projections at PD-10. Although there have been no studies that have examined directly the development of the LSTT, these results are in agreement with the neurochemical (Otsuka et al., 1974; Randic et al., 1977; Schoenen, 1978; Semba et al., 1982; Fitzgerald et al., 1984; Pignatelli et al., 1989) and physiological (Fitzgerald et al., 1984; Fitzgerald, 1985; Fitzgerald,

1988) developmental studies that indicate the LSTT to be a system that undergoes much of its maturation in the late fetal period soon after the neurogenesis of the dorsal horn cells, with major developmental changes probably occurring at about the same time that nociceptors are becoming functional. Lesioning the spinal cord *in utero,* rather than during maturity at PD-12, did not result in greater behavioral recovery or anatomical reorganization, although additional results and replication are necessary before accurate conclusions can be made as to whether the infant lesion effect is illustrated by this system during the fetal period.

The definitions and issues of CNS plasticity for future investigation

The present experiments were interested in developing a model that could be used in the investigation of some of the basic laws that govern behavioral recovery following spinal cord injury. It represented only the first step of many that are needed before we can begin to understand more fully what the important questions in CNS plasticity are.

It has long been known that following axonal lesion to the peripheral nervous sytem of the adult mammal, regrowth of lesioned axons, sprouting of the axonal collaterals of either the injured system (regenerative sprouting) or of a neighboring, intact system (collateral sprouting) may occur, resulting in recovery of function. All of these phenomena have

resulted in at least partial reinnervation of the denervated regions, and in most cases restoration of lost function (Edds, 1953; Guth, 1956; Guth & Bernstein, 1961; Williams, Jew & Palay, 1973). However, the axons of the CNS appear to be more complex in that generalizations about their recovery and regenerative capacity cannot be made and a number of isssues need to be addressed such as regeneration, collateral sprouting and regenerative sprouting. 1) Regeneration, or the actual regrowth of severed axons may occur, but it remains a challenge to show unequivocally that this has taken place. An NINCDS Advisory Task Force outlined 5 criteria, that if met, would guarantee that regeneration had been achieved. These criteria were devised with specific reference to spinal cord injury. The 5 criteria are: a) the experimental lesions must disconnect the nerve processes; b) the processes of CNS neurons must bridge the level of injury; c) the regenerated fibers must make junctional contacts; d) the regenerated fibers must guarantee postjunctional contacts; e) changes in function must derive from regenerated connections (in Cohen, Mackler & Selzer, 1988). Even with this set of criteria, problems remain in demonstrating that the recovery is based on the actual regrowth of severed axons. Most commonly, the problem has been to rule out collateral sprouting. 2) Collateral sprouting occurs when intact fibers of a different system that share the same terminal field as the injured fibers, increase the density of their projections into the shared terminal field. These collaterals are from axons that were not

injured or severed by the lesion. Collateral sprouting in the adult CNS was first described by Liu & Chambers (1958). In what has become the classic preparation for the examination of collateral sprouting, the spared root preparation, in which all dorsal roots but one are unilaterally cut from the spinal cord. One year later, when all stainable degeneration disappears, the projections of the spared root are compared to that of the same root on the normal side of the spinal cord. In the absence of the adjacent roots, the projections of the spared root increased in density, with the area of increased density being in the region of maximum overlap among adjacent roots (Goldberger & Murray, 1978). Unit recordings show these sprouts to be functional (Pubols & Goldberger, 1980) as do behavioral tests (Murray & Goldberger, 1974). This represents an example of specific collateral sprouting since there is an increase in the existing projection by the spared root and it is to a region of convergence of the spared root with adjacent roots that were destroyed. Many questions remain as to whether sprouting in the adult CNS is a nonspecific phenomenon, elicited from all nearby axons such as in the developing, immature CNS (Gall, McWilliams & Lynch, 1979), or whether sprouting becomes limited to particular systems during the course of development. The stimulus for sprouting also remains unclear although it has been suggested that sprouting may be due to the presence of a partially denervated field and/or the lack of competition for a specific trophic factor. 3) Regenerative sprouting is another phenomenon

that may underlie anatomical plasticity. It is defined as new growth of the lesioned axons themselves.

In examining the two types of sprouting, collateral and regenerative, Prendergast & Stelzner (1976) hemisected the spinal cords of newborn rats and of weanling age rats (21 days of age) and studied the resulting pattern of degeneration after both groups of rats received a second hemisection, ipsilateral to the first, in the high cervical region, 5-7 months after the original thoracic hemisection. Several responses to the lesions were found. First, no regeneration or growth of lesioned axons was observed caudal to the thoracic hemisection in either group. In the neonate, this may be due to a dramatic loss of cells from supraspinal systems that were already present at the time of lesion. However, other factors are also likely to be involved since some supraspinal tracts, not yet present at the time of lesion, were not cut by the procedure, but still did not grow caudal to the lesion. The lack of regeneration in the weanling operates may have been due to the presence of a heavy glial scar that was not present in the neonate group. Although there was no axonal regeneration in either the neonatal or weanling operates, more degeneration was found in the gray matter ipsilateral and rostral to the thoracic hemisection of both groups than was observed in the cervically lesioned control animals. The increase in degeneration probably represents resultant sprouting following the thoracic

lesion and could be due to either collateral or regenerative sprouting. There was more degeneration of the ipsilateral white matter, rostral to the thoracic lesion in the neonates when compared to either the contralateral side of the cord or to the weanling operates. This indicates collateral sprouting since any growth in this group would be from unlesioned axons whereas, in the weanling operates the mechanism is unclear since sprouting may be due to both lesioned and unlesioned axons. Addressing this question, Bryz-Gornia & Stelzner (1986) examined the early maturing ascending tracts of the spinal cord in young rats. Because lesions did not result in retrograde cell death and many of these axons remained present near the site of axotomy, they hypothesized that ascending projections are kept viable due to collaterals terminating below the site of the lesion, maintaining these neurons or that the immature neurons are able to grow regenerative sprouts and form synaptic connections with these neurons.

Regenerative sprouting of severed axons has been postulated by Bernstein & Bernstein (1971, 1973a,b) after hemisection of the spinal cord of the rat and monkey and by others when studying the regenerative capacity of the monoamine neurons (Nygren, Olsen & Seiger, 1971; Bjerre, Bjorklund & Stenevi, 1973; Baumgarten, Bjorklund, Lachenmayer, Rensch & Rosengren, 1974). Bernstein proposed that these regenerative sprouts may make the premature inappropriate synapses in denervated zones near the lesion thus

creating an important factor limiting axonal regeneration.

The LSTT model that I have presented to investigate issues of development and plasticity is an exciting one that lends itself to future experimentation and investigation of the above issues. For example, by using a reliable anterograde tracer, comparison can be made of the VPL thalamus after injection of the PrV nucleus in the two different age groups in order to investigate collateral sprouting by the unlesioned axons of the trigeminothalamic tract. An anterograde tracer would also make it possible to investigate other fundamental issues of development such as, initial axonal outgrowth and extraneous projections by the LSTT. Experiment 1 also discussed the issue of axon-target interaction that is probably necessary in order to form a normal pattern of connectivity. The replication of Experiment 3 along with the results of Experiments 1 and 2 will have important consequences for our understanding of the development of spinal pathways and to some of the basic laws that govern behavioral recovery after spinal cord lesion. Future experimentation with this model should also include the influence that neonatal and prenatal VPL thalamic lesions have on normal LSTT development.

In summary, all of the above studies along with the replication of Experiment 3 and the results of Experiments 1 and 2 will have important

consequences for our understanding of the development of spinal pathways and to some of the basic laws that govern behavioral recovery after spinal cord lesion.

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