The development and recovery of motor function in spinal cats

I. The infant lesion effect

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Summary. Normal development of motor function was compared to that of cats with spinal transections at birth (newborn operates) or at approximately two weeks after birth (two week operates). Newborn operates expressed motor behavior not seen until sometime later in normal newborn cats, suggesting that this behavior is normally suppressed by descending systems in newborn cats. After reaching adulthood, the motor performance of newborn operates surpassed that of both two week operates and chronic adult operates (cats with spinal cord transection in adulthood), suggesting that the earlier transection occurs, the greater the recovery of motor function. Transection at birth may alter the course of spinal cord development, accounting for the differences in motor performance among the three age groups.

Key words: Development – Cats – Transection – Spinal cord – Paraplegia

Introduction

The infant lesion effect refers to the sparing and greater recovery of function seen when the nervous system is damaged neonatally rather than in adulthood (Bregman and Goldberger 1982). Support for this idea was found first in the monkey, where acute and chronic motor deficits are less severe in infants with cortical ablations than in adults with comparable lesions (Kennard 1936). Later, it was shown in both rats and cats, that neonatal cortical lesions also result in fewer motor deficits than in adults with similar lesions (Hicks and D'Amato 1970; Leonard et al. 1983).

In the studies cited above, a number of descending and ascending pathways between the brain and

spinal cord remain intact. It is generally assumed, and in some cases demonstrated, that modifications in these pathways occur in the developing, damaged nervous system and that these modifications mediate sparing and greater recovery of function (Hicks and D'Amato 1970; Reh and Kalil 1981; Bregman and Goldberger 1982). The possibility that the development of the spinal cord is altered by these supraspinal lesions, and therefore contributes to the infant lesion effect, has not been investigated.

The spinal cord is capable, however, of mediating considerable motor behavior. Even after the complete removal of supraspinal control (i.e., after complete spinal transection), the spinal cord functions to some extent autonomously, generating postural reflexes and locomotion. The spinal cord's production of locomotion is due to a system of motoneurons and interneurons called the spinal pattern generator for locomotion (Grillner 1975; Edgerton et al. 1976). The spinal pattern generator requires neither afferent input (Grillner and Zangger 1979) nor descending control (Grillner 1973), although it can be modified by supraspinal systems (Grillner 1976), afferent pathways (Forssberg et al. 1980) and propriospinal pathways (Miller et al. 1975).

The present studies were undertaken in order to determine: 1) the contribution of the spinal cord to the infant lesion effect after spinal transection by comparing the motor status of neonatally spinalized cats, when they matured, to the motor status of cats spinalized in adulthood. In this case, the possibility of forming aberrant ascending or descending pathways is excluded by complete transection; and 2) the extent to which motor function develops in the absence of supraspinal input by characterizing the normal motor development of kittens and comparing it to the development of kittens spinalized either at birth or at approximately two weeks of age.

Methods

Surgical

Twenty five cats were used for behavioral testing. Three groups of spinal cats were used: newborn operates with transections made within 24h of birth (n = 7), two week operates with transections made between 11 and 16 days after birth (n = 6) and adult operates with transections made in adulthood (n = 4). Two groups of normal cats were used as controls: unoperated littermates of the neonatally lesioned groups (n = 4) or unoperated adults (n = 4). Kittens were anesthetized by nitrous oxide and oxygen (1 : 1) and 1% halothane gas by volume, adults by Nembutal (35 mg/kg, i.p.). Under antiseptic conditions a low thoracic or high lumbar laminectomy was performed. After the dura was opened, the spinal cord was transected using a curved scalpel blade. The gap between the rostral and caudal spinal cord segments was filled with Gelfoam. The exposed area was then closed by layers. Newborn and two week operates were awake and nursing 30–60 min following termination of anesthesia.

After completion of behavioral testing, animals were deeply anesthetized with Nembutal and perfused with normal saline followed by 4% paraformaldehyde in sodium phosphate buffer (pH 7.4), 20 min after i.v. administration of Herparin and just after i.v. administration of 0.1% sodium nitrite. The lesion sites were embedded in paraffin and sectioned longitudinally. Serial sections were stained for axons using the Bodian method (Humason 1979) and examined in order to determine completeness of the transections.

Care of paraplegic cats

All paraplegic cats were housed in large metal cages with a bedding of shredded newspater 6" deep. The newspaper is soft, non-toxic, disposable, very absorbent and provides an excellent environment for movement. Spinal kittens were weaned approximately 9 weeks postpartum. After weaning they were housed in pairs until 5 months postpartum, when each received its own cage. Although paraplegic cats may urinate spontaneously, bladders of all spinal cats were expressed at least once a day and before behavioral testing either by cutaneous stimulation of the perigenital area or by gentle manual pressure applied to the bladder.

Behavioral testing methods

The following postural reflexes and triggered movements were examined: bipedal stepping (locomotion), monopedal stepping (hopping), placing responses and positive supporting responses. Testing procedures are described below. Placing responses, locomotion and hopping were tested in four directions (forward, backward, laterally and medially). Qualitative behavioral observations such as the presence or absence of a response and abnormalities were recorded. The development of motor behavior was evaluted for 5 months in newborn and two week operates and normals, after which cats were considered mature (Forssberg and Svartengren 1983). Data from cats transected as adults was collected for 3–17 postoperative months. Kittens were tested daily (excluding weekends) for the first 2 postoperative weeks, 3 times per week for the following 10 weeks and once per week after the third month. Adults were tested 5 times per week until treadmill locomotion could be elicited, 3 times per week for the following 10 weeks and once per week after the third month. Adults were tested 5 times per week until treadmill locomotion could be elicited, 3 times per week for the next month and twice per week thereafter.

Bipedal stepping (locomotion)

Locomotion in all animals was tested by placing both hindlimbs onto a moving rubber treadmill belt (speed range 14 cm/s to 75 cm/s) with the forelimbs either gently restrained or standing on a platform. Lateral support was maintained by gently holding the tail and the back was positioned both horizontally over and in parallel with the treadmill to approximate the position for normal quadrupedal locomotion. At birth and during the first two weeks of life the hindlimbs of a normal kitten are rotated externally at the hip. By the fourth postnatal week, this rotation is no longer apparent. For consistency, testing methods were not modified during the first two postnatal weeks to correct for the limb's rotation. No other external stimulation, e.g. pinching of the tail, was used during data collection. Responses to testing procedures at a belt speed of 14 cm/s were recorded on Super 8 film for analysis which included:

A. The number of continuous alternating hindlimb step cycles per trial. Trials representing means of more than 15 cycles were represented as > 15.

B. The maximum height of the hindfoot during swing phase.

C. Analysis of stepping behavior, which included:

i) The treadmill-induced passive extension of the limb required to elicit a step in the opposite direction will be referred to as the threshold for forward stepping. This was measured as the posterior angular displacement, beta (β), of a line drawn from the greater trochanter of the femur to the end of the middle toe pad, referred to as the limb line, from a true vertical axis passing through the greater trochanter of the hip (Fig. 1a).

ii) The active excursion of the hindlimb during swing, measured as the total angular displacement, phi ($\varnothing$), of the above limb line from the hindlimb's most posterior extension to its most forward position at the end of swing, rotating around the greater trochanter of the hip (Fig. 1a).

iii) The efficiency index, an indicator of locomotor performance during bipedal and monopedal stepping, was calculated for all cats within a group by subtracting the average threshold measurements to elicit stepping ($\bar{x}\beta$) from randomized total excursions ($\varnothing$) according to the formula:

$$\frac{\Sigma (\varnothing - \bar{x}\beta)}{n} \pm S.D.$$

where n = total trials per group.
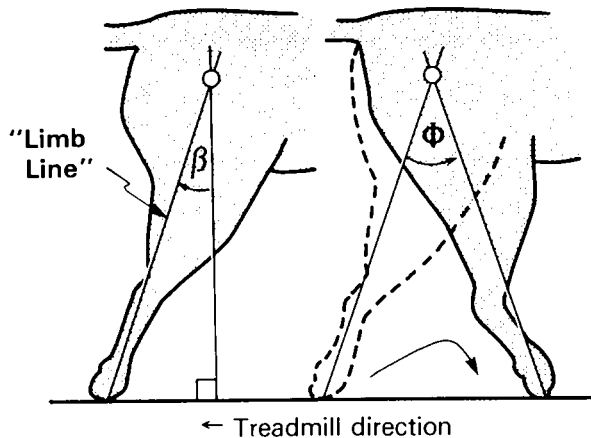
Monopedal stepping (hopping)

Monopedal stepping was elicited by placing one hindfoot onto a moving treadmill belt with the other limbs restrained. The belt displaced the limb from beneath the body's center of gravity causing the foot to be lifted and repositioned under the body to establish a new base of support. Films were analyzed as described for bipedal stepping and in addition included the angular measurements for both the passive (β) and active ($\varnothing$) displacements of the hindlimb during medial and lateral monopedal stepping (Fig. 1b) and representation of this stepping by an efficiency index.

Placing responses

The normal placing response consists of limb flexion directed to clear the edge of a surface (or other stimulus) with subsequent extension and placement of the paw onto a surface to bear weight. Placing was elicited using two methods of stimulation. The first utilized the edge of a table to deform the skin, bend the joints and

A Forward Monopedal Stepping

(Lateral View)

"Limb Line"

← Treadmill direction

B Lateral Monopedal Stepping
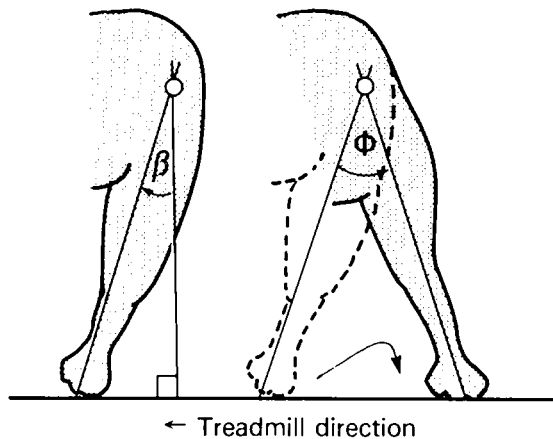
(Frontal View)

← Treadmill direction

Fig. 1A and B. Passive and active components of the step cycle: **A** During forward monopedal stepping, the tredmill moves the limb passively through the angle beta (β). In response to this movement, the cat actively moves the limb through the angle phi (∅). **B** During lateral monopedal stepping, the limb is passively moved medially by the treadmill (β). In response to this movement, the limb is moved actively in the lateral direction (∅)

stretch the muscles of the limb. This method was used to establish the existence of proprioceptive placing. The second stimulus was an inflexible plastic plate attached to a force transducer and linked to a polygraph. The plate was used to establish the threshold for placing responses by measuring the minimum force necessary to elicit them. The toes of the pendent hindlimb were brought into contact with the plate until a response was elicited. Both the behavioral response and the force required to elicit it were recorded. The force of extension during the behavioral response was not measured. Responses were scored relative to those obtained in the normal adult:

0 = no response.

1 = flexion followed by relaxation.

2 = flexion followed by extension in the direction of the stimulus without weight support.

3 = flexion followed by extension in the direction of the stimulus with partial weight support. Response may be hypermetric and/or slow.

4 = complete, efficient response in the direction of the stimulus with maintained weight support.

The force exerted by the foot on the plate was also categorized according to the type and extent of contact. To examine the relationship between force plate contact and limb stimulation, the hindlimb of an anesthetized adult cat was used to determine the force necessary for movement at any of its joints during testing. The toes of the pendent limb were brought into contact with the plate by three different examiners. The force resulting from each contact was recorded and all trials filmed. Each trial was assigned to one of six categories based on the force it generated. Films of the first five trials in each category were spliced together randomly. For orientation, naive observers were shown a training film containing known examples of either limb movement or lack of movement. They were then asked to judge whether or not the limb moved during each of the thirty test trials (field of view contained only the hindlimb and force plate, magnification 5×). Responses were scored as either yes or no. The force associated with each trial was then matched to responses.

The least force corresponding to observed passive limb movement was between 2000 and 2500 dynes. This represented the lowest force to elicit proprioceptive placing. Forces of 1000 dynes or less represented the greatest force to elicit placing by hair-bend only (hair contact placing). Forces between 1000 and 2000 dynes were assumed to deform the skin (skin contact placing).

Positive supporting responses

The hindlimb's ability to extend and bear weight in response to pressure against the toe pads was tested according to Rademaker (1931), with the limb in the standing position. Each cat was lowered onto a force plate with one hindlimb pendent and the other limbs gently restrained. The force resulting from the hindlimb's extension in response to toe pad contact was recorded.

Statistical methods

Statistical tests used are indicated in the figure legends.

Results

Normal motor development and the effect of transection at different ages

Bipedal stepping (locomotion)

Normals. Treadmill-induced hindlimb locomotion was rarely demonstrable in normal kittens at birth. The hindlimbs usually rotated externally and extended caudally in response to being lowered onto the moving belt. The hindlimbs also flexed and extended sporadically, but rarely contributed to locomotion until the end of the first week. The frequency and duration of these sporadic responses could be increased by external stimulation such as pinching the tail. By the end of the second week, the hindlimbs provided a wide base of support, but relatively few alternating cycles could be elicited at a
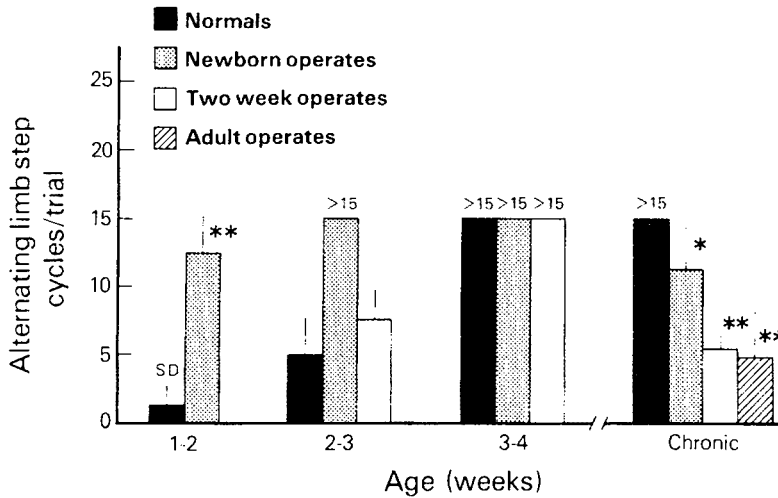
Development of Hindlimb Locomotion

Fig. 2. Development of hindlimb locomotion. The number of complete, alternating cycles per trial expressed by newborn operates, two week operates and normals at different ages. Chronic data for all groups except adult operates were collected when cats were at least 5 months old. Adult operate data were collected when cats in this group were at least 3 months post-operative. During the first postnatal weeks, newborn operates completed significantly more cycles than normals (**, $p < 0.01$). Chronically, newborn operates (*, $p < 0.05$), two week operates (**, $p < 0.01$) and adult operates (**, $p < 0.01$) completed fewer cycles than normal. Two week and adult operates also showed significantly fewer cycles compared to newborn operates ($p < 0.05$). All statistical comparisons were made with the Mann-Whitney U Test
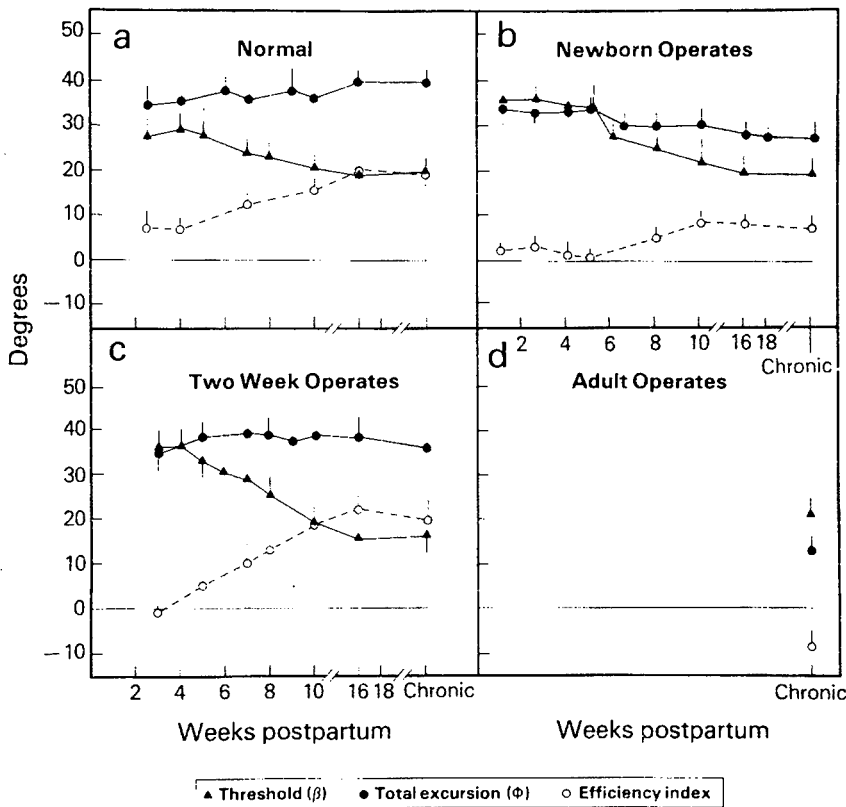
BIPEDAL STEPPING

Fig. 3a–d. Development of passive (β) and active ($\emptyset$) components of bipedal stepping. The efficiency index is derived from these two components (see *Methods*). All spinal groups except adult operates show efficiency indices above zero. In this analysis, two week operates are more similar to normal than newborn operates. No developmental data are shown for adult operates, because they were already mature at the time the lesion was made

time (Fig. 2). The toes dragged during swing phase and the limbs were still externally rotated. During the next two weeks, development of locomotion was dramatic; the hindlimbs were positioned under the torso with little external rotation and the hindfeet no longer dragged during swing. After the fifth week,

normals showed consistent continuous alternating steps, a narrow base of support and the height during swing reached adult levels of 2.5 cm. The posterior angular displacement i.e., the threshold (beta), necessary to elicit a step, was greatest and most variable early in development, dropping to adult

levels by the end of the second month (Fig. 3a). The active angular excursion (phi) of the limb was also most variable early in development, but did not change significantly throughout development. In response to increasing the speed of the treadmill from 14 cm/s to 30 cm/s, normals responded with bilateral flexion followed by relaxation until the fourth week when short bursts of galloping could be elicited. These galloping responses were gradually replaced by responses of increasingly faster walking. After five months normals used fast walking up to 75 cm/s and would rarely gallop.

Newborn operates. In contrast to normal newborns, most newborn operates began hindlimb locomotion as early as 24 h after surgery (i.e., 2 days postpartum) and increased the number of treadmill-induced alternating locomotor cycles dramatically compared to normal littermates (Fig. 2). This superior performance persisted until the middle of the third week, when normals reached the same level. Throughout the first weeks, the hindlimbs of spinal kittens rarely exhibited the external rotation or caudal extension and dragging seen in normals during elicited locomotion. Compared to normal littermates during the first five weeks, the passive displacement (i.e., the threshold) to elicit stepping was greater in newborn operates, but the excursions during stepping were the same as normals. In chronic newborn operates, the threshold (β) for stepping was the same as normals (Fig. 3), but the total excursion decreased. After the fourth month, the number of alternating step cycles decreased in newborn operates relative to normal (Fig. 2). The height of the paw during swing was greater than normal throughout development (Fig. 4). Doubling the treadmill speed to 30 cm/s often elicited galloping only during the first few weeks post surgery, but after that, an alternating gait was seen when treadmill speed changed.

Two week operates. Two week operates began to show bipedal locomotion between 3 and 5 days after surgery. By the end of the first month, two week operates displayed the same number of alternating cycles as normals and newborn operates (Fig. 2). Unlike newborn operates, however, two week operates showed a decrease in the number of elicited locomotor cycles by the beginning of the third month (Fig. 2). The hindfoot height during swing was similar to newborn operates during the first two months of development, but it later decreased to approximately normal values (Fig. 4). Early in development the displacement required to elicit forward stepping was the same as for newborn operates. By the end of the third month, and
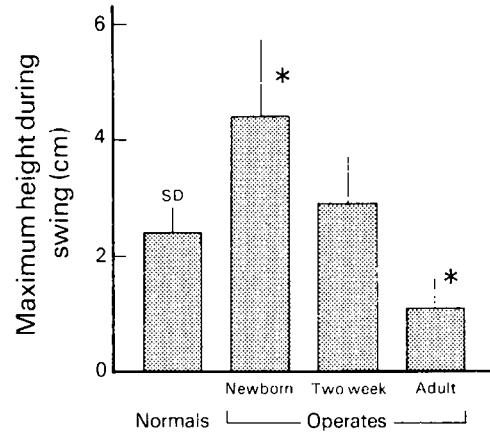

Fig. 4. Maximum height of the hindfoot during forward monopedal stepping in chronic spinal cats. The stepping height of newborn operates was significantly greater than normals ($*$, $p <$ 0.05), indicating hypermetria. In contrast, adult operate heights were also significantly lower than normal ($*$, $p < 0.05$). Adult operate heights were also significantly lower compared to newborn operates ($p < 0.01$). All statistical comparisons were made using the Mann-Whitney U Test

thereafter, two week operates' threshold decreased for bipedal stepping without any change in the total excursion (Fig. 3c), yielding an efficiency index closer to normal than the hypermetric responses of newborn operates. Until the end of the third month two week operates also accomodated to changes in treadmill speed to the same degree as newborn operates and normals, but later decreased due to the use of a non-alternating gait (galloping) at treadmill speeds above 40 cm/s.

Adult operates. Data from three adult operates are presented here. One additional adult operate's performance (see Robinson and Goldberger 1986b) differed from the rest of the group and, since its spinal cord immunocytochemistry also differed, it is presented seperately. Adult operates began to display locomotion on the treadmill 5 to 7 weeks after surgery and was characterized by few continuous alternating steps (Fig. 2) and stepping onto the dorsum of the foot. The feet dragged during swing and as in monopedal stepping (Fig. 4), very little height during swing was attained. The hindlimbs tended to be adducted during locomotion and a thin plate had to be placed between the limbs to prevent them from impeding each other's locomotion. This was not necessary for newborn operates or two week operates. The mean angular displacement necessary to elicit the swing phase of locomotion in chronic adult operates was the same as for normals, but mean excursions were reduced to 30% of normal values
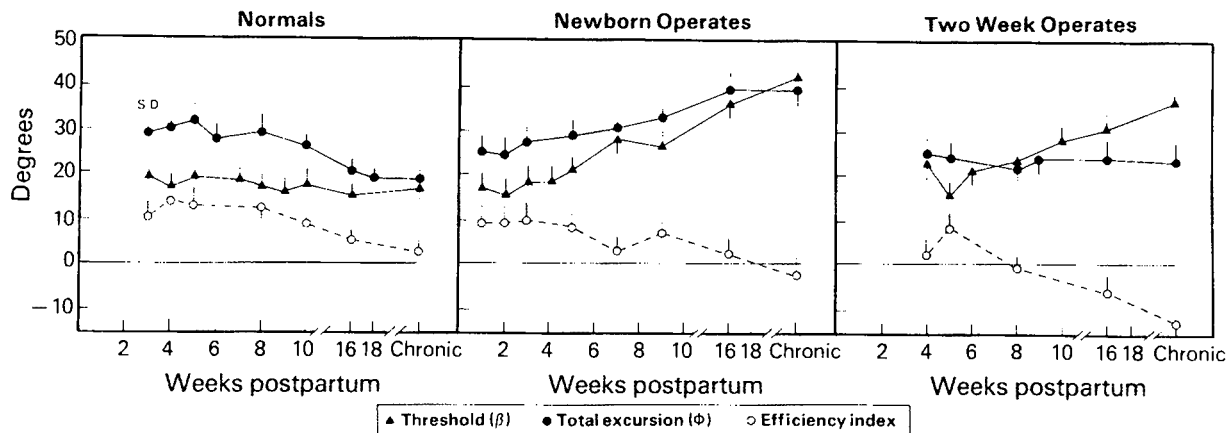
LATERAL MONOPEDAL STEPPING

Fig. 5. Development of the passive (β) and active (∅) components of lateral monopedal stepping. The efficiency index is derived from these two components (see *Methods*). The hopping reflex repositions the foot for weight support in newborn operates and normals, but not in two week operates. Adult operate data are not shown, because lateral hopping did not recover

(Figs. 3a,d). Adult operates were able to accomodate to changing speeds only up to 28 cm/s.

Side-stepping (i.e., lateral and medial) and backward treadmill-induced locomotion were also examined in all groups. Normals developed side- and backward stepping by the end of the third postnatal week. No spinal group developed consistent backward treadmill-induced locomotion. Newborn operates developed and retained the ability to side-step by the end of the first week, two weeks ahead of normal. Although two week operates developed side-stepping by the end of the first postoperative week, their performance deteriorated during the chronic period. Their hindlimbs failed to be repositioned under the torso for adequate weight support. Adult operates failed to recover side-stepping.

Monopedal stepping (hopping)

Normals. Lateral hopping could be elicited, although infrequently, in half of the normal kittens tested at birth and in all normals by the end of the first week. Forward treadmill hopping was not elicited at birth. Backward and medial hopping could be elicited at the end of the first week, followed shortly by forward, with all directions consistently demonstrable by the beginning of the third week. The threshold (β) for forward treadmill hopping remained constant once hopping developed. Initially the active excursion (∅) was small and increased to adult levels by the end of the second month. Thresholds for medial and lateral hopping also
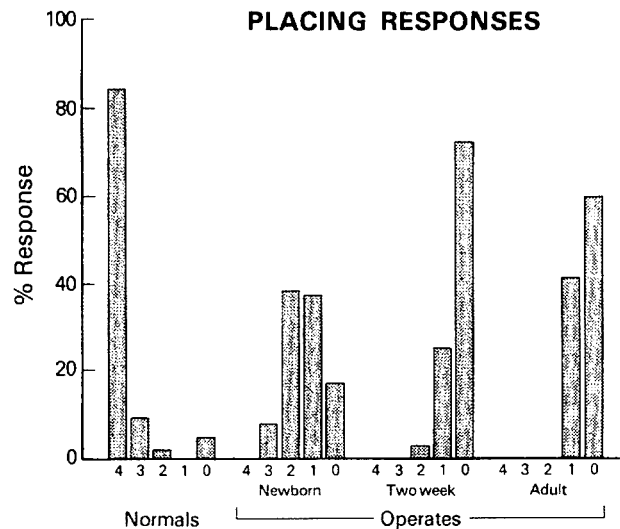

Fig. 6. Sparing and recovery of medial placing responses in chronic spinal cats. 4 = fast, complete weight-supporting response, 0 = no response (see *Methods*). Newborn operates are the only spinal group to demonstrate directed, weight-supported responses (3s). In contrast, adult operates responded with only the least sophisticated movements (1s) or with no movement at all (0s)

remained constant throughout development (Fig. 5a), but the excursions were hypermetric until the end of the third month.

Newborn operates. Forward hopping was consistently elicited in newborn operates within 24h after surgery, approximately two weeks before normal littermates. Medial and lateral hopping were consistently elicited by the end of the first week. Backward hopping was
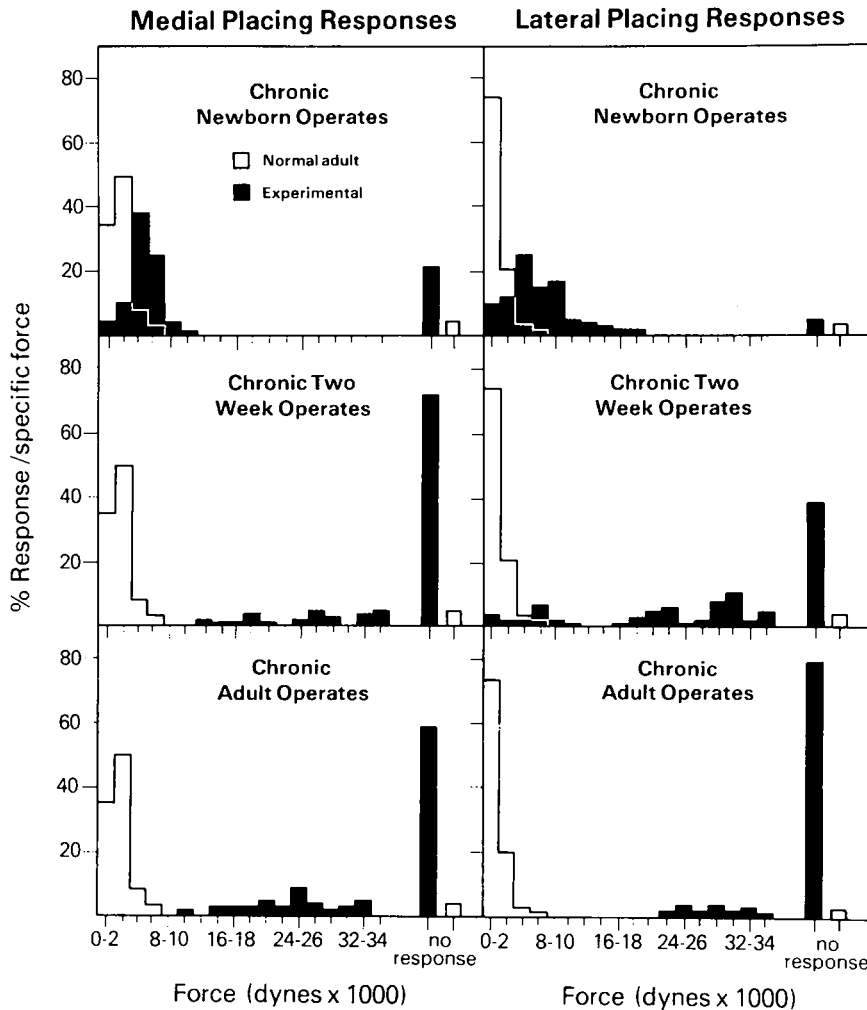
Medial Placing Responses   Lateral Placing Responses

Fig. 7. Effects of chronic spinal transection on thresholds for placing. Thresholds to elicit medial and lateral responses in chronic newborn, two week and adult operates. Normal data are included in each graph. Force is expressed in thousands of dynes. Percent of responses is out of n = > 100 trials. Normal forces required to elicit placing are clustered below 8000 dynes. In newborn operates, these forces are also clustered at the low end of the force scale. In contrast, both two week and adult operates require greater forces to elicit responses and also demonstrate an increased percentage of "no responses" at any force of stimulation

only variably present during the first week and failed to develop into a consistently elicited response. The thresholds for lateral and medial hopping were the same as for normals until the sixth postnatal week, when they increased (Fig. 5b). The excursions also increased, bringing the hindpaw under the pelvis and establishing support during stance (Fig. 5b). During the first two months the threshold for forward hopping was the same as in normals, but the excursion was hypermetric. The thresholds and excursions for forward hopping in chronic newborn operates were the same as for normals, although the height of the foot during swing was greater than normal.

Two week operates. Two week operates never developed backward hopping. Forward hopping recovered first; approximately 3 days after surgery with medial and lateral emerging between 5 and 10 days later. Until the middle of the second month, two week operates showed the same thresholds and excursions during medial and lateral hopping as

normals (Fig. 5c). Whereas newborn operates then developed larger than normal excursions in response to larger than normal displacements medially and laterally, two week operates, in the chronic period, only responded to large angular displacements (similar to newborn operates) with excursions too small to reposition the foot under the hip's center of gravity for weight support.

Adult operates. Adult operates failed to recover backward and lateral hopping. Medial and forward responses recovered by two weeks in two cats and in all by one month. Hopping in adult operates lacked adequate extensor force during the stance phase to support the pelvis and often showed flexion of the toes after swing phase as well as the tendency to drag the toes during swing. The thresholds for forward and medial hopping were the same as in normals, but the excursions displayed great variability. In two cats, these were half as large as in normal adults, the other had excursions within the range of normals.

Placing responses

Normals. Proprioceptive placing responses, although elicited in normal newborns in all directions, were slow and characterized by maintained flexion followed by relaxation. During the next two weeks, responses matured to include extension in the direction of the stimulus and partial weight support, finally reaching the maturity of fast, accurate and weight-supporting responses by the tenth week.

Low threshold placing responses (forces < 1000 dynes) in all directions tested were also observed in 10% of the trials, resulting in partially weight-supporting responses during the first 10 postnatal days only. After that, only proprioceptive placing was seen until the fourth week, when low threshold, partially weight-bearing placing returned. It matured by the eight week. As adults, normals responded with fast, fully weight-supporting placements (Fig. 6) in response to low threshold stimuli (Fig. 7a). Normal adult cats failed to respond in 5% of all trials, regardless of stimulus strength.

Newborn operates. Newborn operates showed low threshold backward responses early in development, which never matured past the stage of limb-flexion. In 30% of the trials newborn operates demonstrated low threshold lateral and forward responses with weight support during the second postoperative week. In chronic newborn operates, the force required to elicit placing responses increased (Fig. 7) and performance deteriorated, but still included weight-bearing responses (Fig. 6). Newborn operates failed to respond, at any force, during approximately 26% of the medial and backward trials, and during 5% of forward and lateral trials.

Two week operates. Backward placing was abolished in two week operates. Lateral, forward and medial placing could be elicited by the third week postpartum (one week postoperative), mostly by strong proprioceptive stimuli. A small percentage of forward and medial responses with weight support were elicited during the first month, but thereafter only flexion followed by extension in the direction of the stimulus (Fig. 6) could be elicited by strong proprioceptive stimulation (Fig. 7c,d). Two week operates failed to respond, at any force, during 37 % of forward and lateral and 72% of medial trials.

Adult operates. Adult operates failed to recover any weight-supporting placing responses. Only simple limb flexion could be elicited (Fig. 6) after strong proprioceptive stimulation medially and laterally (Fig. 7e,f). Backward responses could not be elicited.
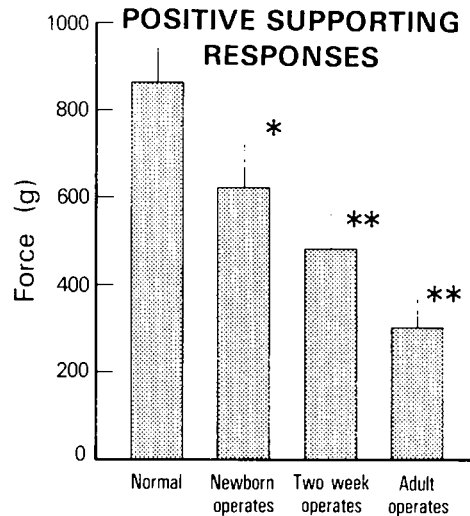

Fig. 8. Force of extension during positive supporting responses. Force expressed in grams. Forces in newborn operates (*, $p < 0.05$), two week operates (**, $p < 0.01$) and adult operates (**, $p < 0.01$) were significantly less than normal. Newborn operates also showed more force than adult operates ($p < 0.01$) and two week operates, although the differences with the latter were not statistically significant ($p < 0.09$). All statistical comparisons were made using the Mann-Whitney U Test

Forward responses varied among animals. One cat showed consistent medium threshold (10–17 thousand dynes) flexion followed by extension. Another showed a small percentage of low threshold (2–6 thousand dynes) responses of limb flexion followed by relaxation. The third adult operate showed high threshold (> 20 thousand dynes) flexion, also without active extension or any supporting response. Adult operates failed to respond to maximal stimulation during 15% of forward trials and during 70% of medial and lateral trials.

Positive supporting responses

Normals. During the first week, normal kittens lacked a positive supporting response in the hindlimbs. By the end of the second week, kittens showed partial support on a flexed limb which matured to full support on an extended limb by the middle of the third week. Mature normals extended and maintained weight support following contact with the force plate.

Newborn operates. Newborn operates developed strong supporting responses on partially extended hindlimbs by the end of the second postnatal week. Spontaneous standing in their cages was not uncommon. Mature newborn operates developed approxi-
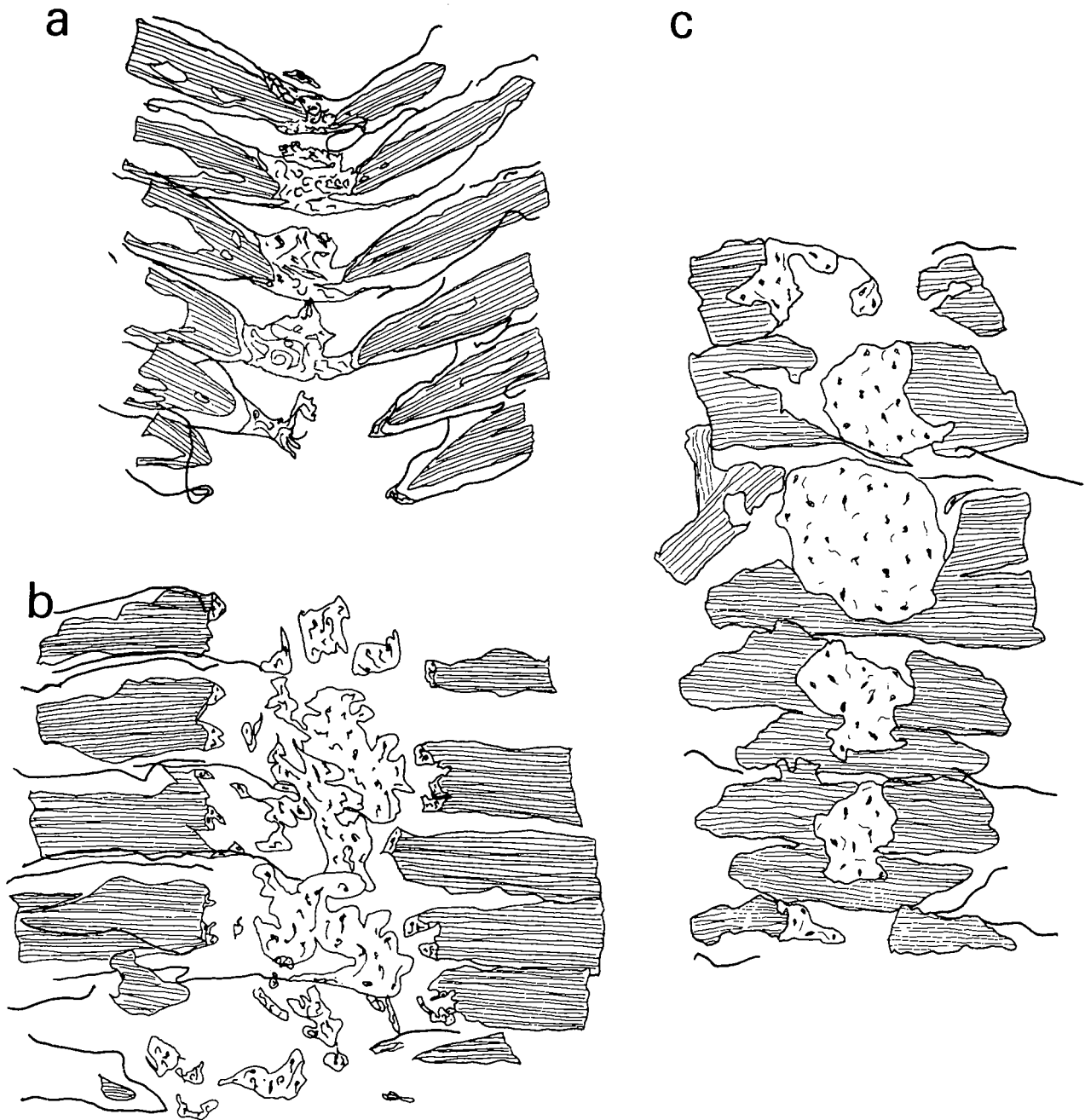
Fig. 9a–c. Serial reconstruction of lesion sites stained by the Bodian method for axons (see *Methods*). Sections were examined for any remaining fibers crossing the lesion site. **a** chronic newborn operate lesion site (scale bar = 2 mm). **b** chronic adult operate lesion site (scale bar = 2.5 mm). **c** incomplete adult operate lesion site (scale bar = 2 mm). The difference between incomplete and complete lesions could not be discerned at the time of surgery

mately 70% of the force generated by normals and would maintain this support for minutes during testing (Fig. 8).

Two week operates. Two week operates developed strong supporting responses from the fourth week until the fourth month. Afterwards, the hindlimbs no

longer provided weight support and the force generated diminished to 50% of normal values (Fig. 8).

Adult operates. Weight support during testing of positive supporting responses never recovered in adult operates. Forces generated were approximately 16% of normal.
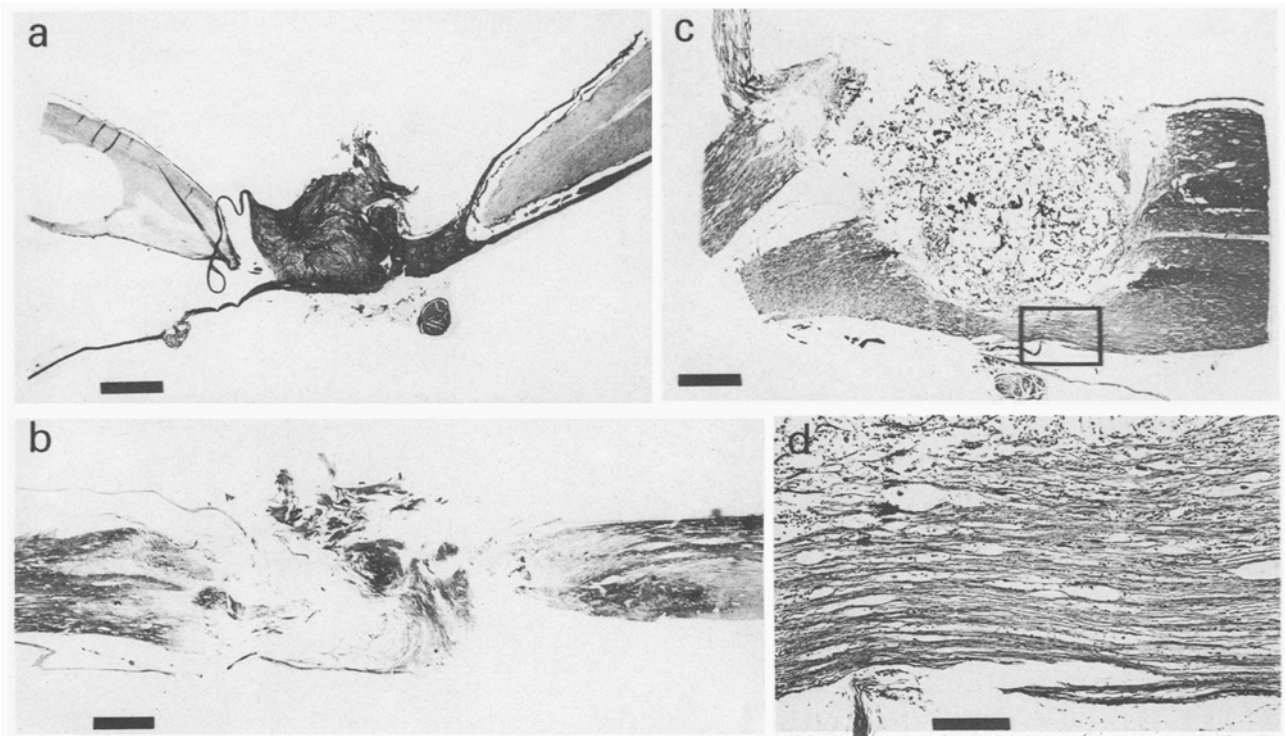
Fig. 10a–d. Lesion sites stained for axons by the Bodian method. a chronic newborn operate (scale bar = 1 mm). b chronic adult operate (scale bar = 1.5 mm). c adult operate with incomplete lesion (scale bar = 1 mm). d magnified view of crossing axons in c) (scale bar = 200 μm). All photomicrographs were taken midway through the lesion block

One adult operate differed both in motor performance and spinal cord anatomy (see *Results* in Robinson and Goldberger 1986b) and is therefore discussed separately. This cat showed more alternating step cycles than other adult operates, approaching the performance of chronic newborn operates. The thresholds and angular excursion during locomotion were within the range of normals. The foot height during swing phase was also within the range of normals, but the legs required separation by a thin plate so that they would not hit each other. This cat was also able to adjust, although inconsistenty, to changes of treadmill speed up to 60 cm/s with fast walking. During monopedal stepping trials, the cat showed adequate extensor tone during stance to support its weight. Rarely did the toes flex at the end of swing phase, but they often dragged during initiation of swing. As with other adult operates, placing never recovered in this cat. Positive supporting trials generated extensor forces which were comparable to normal, but maintained only briefly.

Lesion analysis

Serial reconstructions of longitudinal sections through the lesion sites stained by the Bodian method for axons (Figs. 9a, 10a) revealed no crossing or connecting axons in any experimental animal. The site of an incomplete lesion from an adult cat (not used in the present series for behavioral evaluation) is included for comparison (Figs. 9c, 10c and d). The most common feature encountered at lesion sites was either cyst formation surrounded by scar tissue or a dense scar mixed with bone (Fig. 10a and b).

Summary

Normals. Consistent forward treadmill-induced locomotion and hopping were observed approximately 3 weeks postpartum. By the end of the third week, normals accomodated to changing treadmill speeds. Proprioceptive placing reponses were present at, or shortly after, birth and low threshold, mature responses developed by the end of the eighth week.

Newborn operates. Treadmill locomotion and hopping were observed 24h after spinal transection, 2 weeks before normal littermates. Chronic newborn operates generated the most alternating step cycles of the 3 spinal groups, but fewer than normals with consistent accomodation to changing treadmill

speeds. Low threshold, weight-supporting placing responses developed by the second week. Chronic newborn operates generated the strongest and most consistent positive supporting responses of the 3 spinal groups.

Adult operates. Treadmill locomotion and hopping were observed 5–7 weeks post-transection. Weight-supported locomotion never recovered in 3 cats, while one cat showed locomotion approaching that of newborn operates. Only high threshold placing responses without weight support could be elicited from this group. Very small positive supporting forces were generated in 3 cats, but one adult operate generated brief forces within the range of normals.

Two week operates. Treadmill locomotion, including accomodation to changing treadmill speeds, and hopping were observed 3–5 days after transection, but deteriorated later. Placing responses developed similarly, with the best performance seen early in development. Positive supporting responses developed by the fourth postnatal week, but then deteriorated.

Discussion

These behavioral results indicate that: i) the earlier in life that spinal transection is made, the greater the recovery and sparing of motor function when animals are examined as adults; and ii) motor centers in the neonatal lumbosacral spinal cord are suppressed by descending systems in newborns since kittens whose spinal cords are transected at birth express, almost immediately, motor function that is not yet seen in their normal littermates.

Demonstration of the infant lesion effect

Greater recovery (and sparing) of motor function was seen in newborn operates than in two week operates or adult operates. An example is seen in the low threshold placing response. Low threshold placing in adults is known to be dependent on supraspinal control (Bard 1933; Amassian and Ross 1978). The presence of low threshold placing in cats with spinal transection at birth and at 6 days of age (Forssberg et al. 1974), suggests that the circuitry responsible for the response is located within the spinal cord of the kitten, but becomes dependent on supraspinal input during development. Placing was essentially abolished in adult operates; forward and medial proprioceptive placing recovered minimally, with fragmentary, relatively high threshold responses

remaining that were poorly oriented. Placing thresholds for two week operates were similar to those of adult operates, with a small percentage of fragmentary, relatively high threshold responses remaining in the forward and medial directions, but these proprioceptively elicited responses in two week operates were directed toward the stimulus. Newborn operates demonstrated the lowest thresholds, most closely approaching normal values (Fig. 7), and their placing responses were the most mature (Fig. 6) of the 3 spinal groups. They were severly impaired, however, compared to normals, in that only a small percentage of responses were of low threshold.

Locomotion, hopping and positive supporting responses also followed the same pattern of superior performance in neonatally lesioned animals compared to adult operates which, in general, showed weaker support and also the greatest intragroup variability. Though it is possible for 33% (Giuliani et al. 1983) of adult operates to perform well during forward treadmill-induced locomotion, we have shown that as a group, adult operates showed the most variability and the poorest performance of placing and treadmill-induced hopping and locomotion. In contrast, newborn operates tested as adults showed the least variability within the group. Backward hopping was abolished in all groups, but only adult operates failed to recover lateral hopping. The lateral hopping which recovered in two week operates and developed in newborn operates differed from each other and from normal. In response to a small medial displacement of the limb by the treadmill, the normal adult response is to lift the leg quickly and reposition it under the body's center of gravity for support. Mean thresholds (i.e. amount of medial limb displacement) for lateral hopping in newborn operates were higher than for normals, but newborn operates compensated with larger excursions to establish support. Responses by two week operates were also high threshold, but failed to re-establish support. It is interesting that backward hindlimb movements are rarely used spontaneously by cats in natural or laboratory environments (personal observations) and that these responses fail to recover consistently after transection at any age. In contrast, forward responses are the most extensively used during normal behavior and are best preserved after transection, suggesting that the circuitry responsible for forward placing, hopping and locomotion may be more strongly represented in the spinal cord than for other directions. Our results, taken together, suggest that since all lesions were studied histologically and showed no axons crossing the gap, an infant lesion effect can be mediated by the spinal cord without the requirement of spared or

aberrant descending pathways (see Hicks and D'Amato 1970; Reh and Kalil 1981; Bregman and Goldberger 1982). Previous studies using spinal cats (Shurrager and Dykman 1951; Grillner 1973; Forssberg et al. 1974; Smith et al. 1982), rats (Stelzner et al. 1975) and rabbits (Viala et al. 1985) support this idea.

The mechanisms underlying this infant lesion effect may be understood better by considering the normal development of the spinal cord. During the first month of life several anatomical and physiological changes occur in the normal kitten spinal cord which are thought to be largely independent of supraspinal control (Skoglund 1969). At birth, some excitatory reflex pathways, including those which mediate the excitatory input to flexor reflexes, are present (Malcomb 1955; Wilson 1962; Skoglund 1969). However, the pathways mediating intraspinal inhibitory control of reflexes are either not present or not expressed (Malcomb 1955), and limb responses are biased toward flexion. During the first month, there is an elaboration of dorsal root collaterals (Wilson 1962) and gamma efferent control of muscle spindle feedback develops (Skoglund 1960; Skoglund 1969). During the same time, not only do motoneurons increase in size, but the synapses upon them increase in number (Conradi and Skoglund 1969; Mellstrom and Skoglund 1969) and undergo reorganization (Ronnevi 1977). Only at the end of the first month do the descending pathways establish the normal balance of excitation and inhibition (as revealed by the appearance of decerebrate rigidity after brainstem transection) (Skoglund 1969). Thus, although many descending tracts appear to be present at birth (Bregman and Goldberger 1982; Leonard et al. 1984), considerable development of segmental and propriospinal systems takes place postnatally and may therefore be affected differently by lesions made either neonatally or in adulthood.

Suppression of motor function in normal newborns

The earlier onset of treadmill-induced locomotion and monopedal hopping in newborn operates compared to normals indicates that the local circuitry responsible for these motor functions, although suppressed, is present in the spinal cord at birth. The removal of descending input neonatally may therefore be seen as the removal of an influence, the net effect of which is to suppress the function of intraspinal motor networks. The release of hindlimb motor function by transection in the newborn suggests a paradox in the developmental relationship between descending pathways and local, segmental pathways.

Motor development has traditionally been viewed as a reflection of increased input due to maturation of descending motor pathways to spinal cord neurons (Amassian and Ross 1978; Martin et al. 1982). In contrast, our results suggest that the contribution of descending pathways in the newborn prevents the expression of hindlimb segmental motor programs. A later increase in the development of facilitatory input may occur at a time closer to weaning, when quadrupedal locomotion is more consistent with survival.

Our results also indicate that the spinal cord, transected early in development, reveals greater autonomous motor function than when transected in adulthood. Thus, there is an apparent ontogenetic loss of autonomous spinal motor function as supraspinal dominance becomes apparent, i.e., encephalization of function occurs. As the nervous system matures, descending control over the spinal cord's independent motor capacity becomes increasingly dominant. Comparative anatomical studies (Kuypers and Huisman 1982) suggest that a similar process occurs in phylogenetic development, with supraspinal control being greatest in primates. Thus, the capacitiy for spinal motor function present in the neonatal cat spinal cord declines as intraspinal motor networks submit to developing supraspinal domination. How this occurs is not known. One possibility is that the greater extent of autonomous spinal motor function after neonatal transection may result from an increased afferent input to intraspinal motor systems. This would occur if, for example, sprouting of dorsal root afferents resulted from the denervation caused by transection. The extent of sprouting has not been established in cats with neonatal lesions, but increased dorsal root input has been shown in the adult cat after spinal transection (Thor et al. 1982) or hemisection (McCouch et al. 1958; Murray and Goldberger 1974) and ipsilateral to a neonatal, but not an adult, hemisection in the rat (Stelzner et al. 1979). An increased afferent input could also occur if an exuberant dorsol root projection, normally present at birth (see Thor et al. 1982), failed to undergo retraction during development. If the maturation of descending pathways contributed to the normal retraction of segmental collaterals, then transection might result in retention of an immature exuberant pattern during development. Either mechanism by which afferent input increased might account for the observed sparing of function. The possibility that, after transection, the immature spinal cord is under greater afferent control than after transection in the adult is also suggested by the work of Viala et al. (1985), who found that the spinal motor systems could be altered in spinal rabbits by the use of different training programs if the spinal cord was

transected before 12 days postpartum. Thus, it appears that the processing of information in the spinal cord is altered by transection and this alteration depends on the age at which the spinal cord is damaged. The diminished plasticity characteristic of the adult operate in general and of the afferent systems in the present case may account for the poorer recovery of most adult operates.

The decline in the capacity for autonomous spinal motor function during ontogeny implies that motor systems present in the newborn cat spinal cord are altered by the normal postnatal development of supraspinal systems and that the alterations mediating this decline are fully expressed only in the adult. Spinal motor systems in chronic newborn operates, having developed without supraspinal influence, mediate the best motor performance. The two week operate's spinal cord has matured normally prior to transection and may therefore have begun to lose its autonomy. The intraspinal motor network of adult operates has been dominated by supraspinal control throughout development and has apparently suffered the greatest loss of autonomy since, on average, it expresses the poorest motor performance. Since excitatory input to the adult spinal pattern generator is provided by supraspinal systems which initiate and modify locomotion (Grillner 1976), a normal relationship of net supraspinal excitation balanced by intraspinal inhibition may be hypothesized. When the normally developed excitatory input is removed in adult spinal animals, intraspinal motor networks may be dominated by remaining local inhibition. If local inhibition normally develops in proportion to the amount of descending control, then it would be anticipated not only that this inhibition would be more developed in adults than in neonates, but also that in would be more developed in primates than in carnivores and rodents. This is consistent with the observation that primates exhibit far greater permanent motor impairment after transection than cats (Eidelberg et al. 1981), rats (Stelzner et al. 1975), or rabbits (Viala et al. 1985). Thus, the development of local inhibition may be altered or arrested after neonatal transection due to the absence of descending systems. An alteration in the development of spinal inhibitory pathways may therefore permit greater recovery and sparing of function after neonatal transection whereas the normal development of inhibition may prevent greater recovery in adults.

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