

**ONTOGENETIC EFFECTS OF MIDTHORACIC SPINAL CORD COMPRESSION
ON HINDLIMB SENSORIMOTOR FUNCTION AND SPINAL CORD TISSUE LOSS**

By

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Traumatic spinal cord injury (SCI) in humans is most prevalent in young adult males. Although human infants are not exempt from SCI, ontogenetic changes in the behavioral and neuroanatomical sequelae following incomplete SCI have not been evaluated. Injury to the developing cord may have unique consequences for behavioral recovery, making necessary the elucidation of the effects of SCI in infancy. In the present experiments, midthoracic spinal cords of 5-, 15-, or 60-day-old rats were compressed with forceps by 0% (sham), 85%, or 95% of the uncompressed width. On postoperative days 1, 7, 14, 21, and 28, hindlimb sensorimotor function was quantified during beam- and parallel bar walking, on an inclined plane, and on a hot plate. Results from those tasks were compared to ratings of overground walking, which are typically used with adult animals following traumatic SCI. The amount of tissue at the lesion epicenter was then quantified. Despite significant spinal cord damage, few tasks distinguished between rats that received sham surgery or the lesser compression as

neonates. Hindlimb sensorimotor function was severely disrupted on most tasks following the lesser compression on postnatal day (PND) 60 or the greater compression on PND 5, 15, or 60. In contrast, ratings of overground walking suggested that hindlimb sensorimotor function was near-normal in rats that received the greater compression as neonates or the lesser compression as young-adults. The amount of tissue at the lesion epicenter decreased during ontogeny in rats that received the lesser compression, but did not differ with age in rats that received the greater compression of the cord. Results of the present experiments suggest that ratings of overground locomotion may not accurately depict sensorimotor dysfunction postinjury and that additional deficits may be revealed on tasks that challenge hindlimb function. Furthermore, hindlimb sensorimotor function postinjury is not solely dependent on the amount of tissue at the lesion epicenter, which presumably reflects the amount of communication between spinal and supraspinal circuits. Ontogenetic changes in sensorimotor performance postinjury most likely depend on the tissue reaction to injury and to the combination of neuroanatomical, neurophysiological, and behavioral compensatory mechanisms invoked during recovery.

CHAPTER 1 INTRODUCTION

Behavioral Sequelae following Injury to the Mature Spinal Cord

Traumatic injury to the human spinal cord is most prevalent in young adult males. Behavioral consequences of spinal cord injury (SCI) are dependent on the severity of the impact, as well as the level of the spinal cord that is damaged. Consequences of thoracic and lumbar injuries can range from paresis to paralysis of the legs, whereas cervical injuries can result in quadriplegia. Other consequences immediately following injury may include bladder and sexual dysfunctions. In the weeks that follow the injury, the patient may recover some motor function, but recovery is dependent on severity of injury. However, muscular spasticity and chronic pain may develop and hinder functional locomotion. Magnetic resonance imaging with clinical evaluation has enabled clinicians to better predict neurologic recovery following injury. However, resolution of the injury site is limited (Croul & Flanders, 1997). Because SCI is so prevalent, animal models of spinal cord contusion/compression have been developed to aid understanding of the behavioral changes and the underlying pathophysiological mechanisms that follow SCI. Models of spinal cord contusion/compression, rather than transection, are used because the human spinal cord is rarely transected following injury and the neuroanatomical changes that occur in the spinal cord following incomplete SCI in animals closely resemble the changes that occur in the human spinal cord. Generally, there is preferential

degeneration of spinal cord gray matter, with the amount of gray and white matter remaining at the lesion epicenter dependent on the severity of the injury.

Early attempts to study SCI in animals were crude relative to current standards (see Dohrmann, 1972). Allen (1911) was the first to attempt to produce a clinically relevant and reproducible model of human SCI by dropping a weight from various heights onto the exposed spinal cord of adult female dogs. With this model, the spinal cord lesion is due to the force of the rapid impact, the concomitant dorsoventral compression of the spinal cord, and the propagation of shock waves through the tissues and fluids. Significant correlations have been found among (1) height from which the weight is dropped and lesion length or white matter spared at the lesion epicenter; (2) behavioral score on an open field test and lesion length, gray matter at the lesion epicenter, or remaining white matter; and (3) the degree of incline that balance and position were maintained on an inclined plane and remaining gray- or white matter at the lesion epicenter (Noble & Wrathall, 1985; also see Basso, Beattie, & Bresnahan, 1996). Depending on the severity of injury, adult animals recover some hindlimb function during the 3- to 4-week postoperative period (Gale, Kerasidis, & Wrathall, 1985). Since its invention, the weight-drop model has been modified to improve reproducibility of the injury. Many current systems use electronic closed-loop feedback devices to monitor parameters of the injury (e.g. Bresnahan, Beattie, Todd, & Noyes, 1987). Thus, animals may be discarded initially if parameters of the injury do not meet criterion.

Despite these improvements, behavioral outcome following injury varies with parameters of the weight-drop method, which makes it difficult to compare treatment effectiveness across laboratories. The behavioral and neuropathological characteristics of

a given injury vary with the size of the weight used and the height from which it was dropped (Daniell, Francis, Lee, & Ducker, 1975; Dohrmann & Panjabi, 1976). Not all weight-drop devices utilize an impounder; use of an impounder requires that the height of the weight/rod be increased to produce injuries of equal severity to those in which the weight/rod impacts the spinal cord directly. Severity of injury can also vary with the size of the impounder contact area (Gerber & Corrie, 1979) or with the mass of the impounder (Koozekanani, Vise, Hashemi, & McGhee, 1976). Inherent properties of the spinal cord (see Bresnahan et al., 1987; Koozekanani et al., 1976) or differences in surgical technique (e.g. size of the laminectomy; Koozekanani et al., 1976) may also alter neurologic outcome. Furthermore, smaller spinal cords will suffer a greater degree of compression than a larger cord following a given drop-mass energy (Koozekanani et al., 1976), which makes age-related comparisons difficult.

Because of shortcomings with the weight-drop method and because a single model of SCI in animals may not represent all forms of SCI in humans, other models of SCI have been developed. Use of various models of SCI may also enable one to determine the parameters of SCI that predict behavioral outcome following injury. Rivlin and Tator (1978) studied the effects of a contusion injury by applying aneurysm clips of known forces to the spinal cord of adult female dogs for various lengths of time. Although the amount and speed of compression can not be controlled with this technique, it has the advantage that there is no confounding by movement of the spinal cord or vertebral column during impact. Those authors found that the duration of compression was important in determining recovery of function after acute compression; an 8-week postoperative recovery period was required for maximum levels of recovery in some

groups of animals. Subsequent research determined that functional recovery varies with both force and duration of compression (Dolan, Tator, & Endrenyi, 1980). Similarly, duration of compression and pressure on the spinal cord are important factors that determine hindlimb function following circumferential compression of the spinal cord by an inflatable extradural cuff (Tator, 1973), which may reproduce the symptoms and neuropathology of dislocation or fracture-dislocation of the spinal column in humans. With that model, recovery is maximal within 12 postoperative weeks. Models of slow spinal cord compression, which may reproduce the neuropathological changes in the spinal cord following tumor growth, include compression by intradural balloons (e.g. Tarlov, Klinger, & Vitale, 1953), forceps (Gruner, Yee, & Blight, 1996) or slow addition of weights to an impounder resting on the spinal cord (Eidelberg, Staten, Watkins, McGraw, & McFadden, 1976). Amount (Gruner et al., 1996), speed (Tarlov et al., 1953), and duration (Tarlov et al., 1953) of compression, affect neurologic outcome. As with contusion injuries, surviving white matter postcompression is correlated with behavioral outcome (Gruner et al., 1996) and a 3- to 4-week postoperative period is generally sufficient for maximum recovery.

Functional changes are evident with each type of SCI described, but the degree of sensorimotor impairment depends on how the spinal cord is injured. Complicating matters further, researchers of different laboratories rarely quantify these functional differences and use their own rating scale to assess hindlimb sensorimotor function postinjury. These motor performance scales include the 5- and 6-pt. modified Tarlov scales (e.g., Tator, 1973; Wrathall, Pettegrew, & Harvey, 1985), the 15-pt. Ohio State motor scale (e.g., Saruhashi & Young, 1994), the 21-pt. Basso, Beattie, and Bresnahan

(BBB) locomotor recovery scale (e.g., Basso et al., 1996), and the 100-pt. Combined Behavioral Score (CBS; e.g., Kerasidis, Wrathall, & Gale, 1987). On most scales, higher scores represent greater hindlimb function postinjury, but lower scores on the CBS scale represent greater hindlimb function. Although rating systems are adequate for rapid assessment of gross locomotor skill, rating systems are subjective and overground walking does not provide a stringent test of hindlimb function. Thus, there is a need for a standardized battery of tests that challenge hindlimb performance, for which sensorimotor function can be quantified following incomplete SCI.

Behavioral Sequelae following Injury to the Developing Spinal Cord

Although human infants are not exempt from SCI, the neuropathological and behavioral effects of spinal cord contusion/compression have not been studied in young animals. Each year, approximately 230-500 children younger than 15 years of age sustain SCIs. The most common cause of pediatric SCI is motor vehicle accidents, but an increasing number is due to domestic violence. Sporting accidents are common causes of SCI in older children and adolescents. Uncommon etiologies of pediatric SCI include injuries from automotive lap-belts, the birthing process, child abuse, and C₁-C₂ subluxation associated with tonsillitis or pharyngitis (Vogel, Mulcahy, & Betz, 1997; also see Hadley, Zabramski, Browner, Rekate, & Sonntag, 1988; Ruggieri, Smarason, & Pike, 1999; Vogel & Lubicky, 1995). Pediatric SCI is distinguished from SCI in adults in that the neuropathological and behavioral manifestations following injury in infants interact with normal processes of growth and development. Thus, the recovery process and treatments designed for adults may not generalize to infants.

Most of what is known about the effects of SCI in young animals is from studies in which the spinal cord was transected. Although most descending long fiber tracts innervate the spinal cord by birth (Leong, Shieh, & Wong, 1984), rats gradually recover hindlimb support and walking when the midthoracic spinal cord is completely transected prior to postnatal day (PND) 12. However, when the transection is made after PND 15, there is increased spinal shock and little recovery of hindlimb function, if any (Stelzner, Ershler, & Weber, 1975; Weber & Stelzner, 1977). That change in the degree of recovery from spinal cord transection corresponds to a period of rapid synaptogenesis in the lumbar spinal cord (Gilbert & Stelzner, 1979), which suggests that lumbar motor circuits become dependent on supraspinal inputs for normal function. There is no evidence for neural growth across the transection site (e.g., Cummings, Bernstein, & Stelzner, 1981; Howland, Bregman, Tessler, & Goldberger, 1995). Therefore, recovery of function after spinal cord transection in young rats is probably due to synaptic reorganization of immature circuits (Weber & Stelzner, 1977). Similar age-dependent changes in response to contusive/compressive SCI may exist. Preliminary results from our laboratory (McEwen & Stehouwer, 1998a) suggest that hindlimb dysfunction results in greater hindlimb dysfunction following midthoracic spinal cord compression on PND 30, than on PND 5 or 15. However, the hindlimb sensorimotor function of rats of all three age groups improved during the 10-day postoperative period. Although the spinal cord of 5-day-old rats was compressed by 90% of the uncompressed width, the overground locomotion of those rats was subjectively similar to that of sham controls. Because subjective ratings of overground walking may not be a sensitive test of hindlimb function, kinematic analyses were subsequently used to quantify the limb movements of

neonatal rats during L-DOPA-induced air-stepping following midthoracic spinal cord compression. On postoperative day 1 or 11, few parameters of limb movement were significantly altered from sham controls, even though the spinal cord was compressed by 90% of the uncompressed width on PND 4 (McEwen & Stehouwer, 1998b). In contrast, severe hindlimb dysfunction results when the adult spinal cord is compressed by approximately 60% of the uncompressed width (see Gruner et al., 1996). Together, these results suggest an age-related decline in sensorimotor function following spinal cord compression. However, age-related changes in properties of the spinal cord tissue (e.g., elasticity; see Horie, Ikuta, & Takenaka, 1990) or its response to injury may influence behavioral outcome.

Neuroanatomical Sequelae Postinjury

Mechanical damage to the central nervous system (CNS), not only directly causes tissue necrosis, but also triggers a cascade of neurochemical changes that ultimately results in additional, delayed tissue damage. These neurochemical changes include release of polyunsaturated fatty acids, decreased free intracellular Mg^{2+} , release of platelet-activating factor, release of free radicals, elevations in dynorphin, serotonin, and excitatory amino acids, NMDA receptor activation with increased intracellular Ca^{2+} and Na^{+} , release of inflammatory agents, decreased blood flow, and decreased glucose utilization (see Faden, 1996; Janssen & Hansebout, 1989). A primary purpose of studying SCI in animals is to understand these pathophysiological changes occurring in the spinal cord and to identify successful therapeutic treatments following injury.

Although the biochemical changes in the infant spinal cord following incomplete SCI have not been elucidated, the biochemical changes and neural tissue reactions following hypoxic-ischemic injury to the neonatal brain (Berger & Garnier, 1999; Giacoia, 1993) resemble the changes that occur in the mature spinal cord following traumatic SCI. However, behavioral consequences of such injury-induced changes are not necessarily equivalent in infant and adult animals. Insulin-induced hypoglycemia is neuroprotective to hypoxic-ischemic damage to the adult brain (Nedergaard & Diemer, 1987), whereas hypoglycemic conditions exacerbate the lesion in the infant brain (Yager, Heitjan, Towfighi, & Vannucci, 1992). Cell death is greater following transection of the developing spinal cord than following transection of the mature spinal cord (e.g., Prendergast & Stelzner, 1976). However, the spinal cord of young rats has greater functional autonomy following complete transection than does the mature spinal cord (Stelzner et al., 1975; Weber & Stelzner, 1977). Recovery processes following injury to the neonatal central nervous system occur in conjunction with normal processes of growth and development, which may afford the neonate greater recovery of locomotor skill following SCI. Therefore, treatments designed for adults may not have the same effects in infants. Elucidation of the neonatal reaction to traumatic SCI is needed.

Locomotor Development

Development of coordinated locomotor behavior is dependent on the maturation and integration of numerous neural and nonneural subsystems, each of which has its own developmental time-course. Human infants gradually develop independent walking over the first 15 postnatal months (Forssberg, 1985; McGraw, 1940; Shirley, 1973). Human

infants generally progress through stages of head-raising, raising their upper body with their forearms, and crawling before independent walking (Shirley, 1973), but perform stepping movements from birth if held erect (Forssberg, 1985; McGraw, 1940).

However, the capacity for infant stepping disappears after the first two months, which has been described as the "inactive period" (Forssberg, 1985; McGraw, 1940). After the inactive period, infants initiate locomotor movements by crawling and then as supported locomotion when held under the arms (Forssberg, 1985). Several months later children begin to walk without support (Forssberg, 1985; McGraw, 1940; Shirley, 1973).

Gradually, the walking pattern is transformed, late in the second postnatal year, to the adult plantigrade pattern, which is characterized by a heel-strike in front of the body (Statham & Murray, 1971; Sutherland, Olshen, Cooper, & Woo, 1980).

Rats are also born relatively immature, but rapidly develop the raised, quadrupedal posture for walking during the first 15 days. By PND 4 or 5, forelimb movements become frequent. At this stage, the forelimbs act as paddles, but provide minimal forward propulsion because the hindlimbs do not support the body. Since the hindlimbs are anchored, the forelimbs pivot the animal around the pelvis. "Pivoting" is the predominant form of locomotion by the end of the first postnatal week. By PND 8, head raising is observed and the hindlimbs begin to provide some propulsive force, although the hindlimbs are often unable to keep up with the forelimbs and are dragged behind. Trunk elevation emerges around PND 10 or 11 and is predominant by day 12 or 13. True quadrupedal walking emerges when rats open their eyes on PND 14 or 15. Locomotor speed subsequently increases and fast running appears by PND 16. However, immaturity of the locomotor skills of the rats are evident beyond PND 21 when rats are required to

ambulate over a slippery terrain (Altman & Sudarshan, 1975). In fact, the mature pattern of paw contact with the surface continues to develop beyond PND 30 (Clarke & Williams, 1994).

Although rats do not show the raised, quadrupedal posture and walking prior to about PND 15, that does not mean that the neural circuits that mediate coordinated locomotion are not in place and functional. In fact, coordinated locomotor behavior is observed during the first postnatal week under conditions in which the postural demands are reduced, such as during swimming (e.g., Bekoff & Trainer, 1979), during air-stepping (e.g., Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991), following administration of catecholaminergic agents *in vivo* (e.g., Kellogg & Lundborg, 1972), or following administration of drugs to a brainstem-spinal cord preparation *in vitro* (e.g., Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991). Similarly, human infants show rhythmical, coordinated movements shortly after birth when held erect (McGraw, 1940) or placed in a prone position in water (McGraw, 1939). Human infants also continue to step during the "inactive period" (Forssberg, 1985; McGraw, 1940) when the weight of the limbs is reduced by submerging them in water (Thelen, Fisher, & Ridley-Johnson, 1984).

Because the neural and nonneural mechanisms required for adultlike locomotion in rats mature postnatally, investigation into the development of locomotion in rats may provide insight into how the motor system is constructed. In fact, some behavioral traits of the damaged, mature system are analogous to the behavioral traits of the immature system. Infant reflexes return in aged individuals (Paulson & Gottlieb, 1968) and patients with Parkinson's Disease no longer walk with a heel strike, but walk on their toes

like young children (cf. Forssberg, Johnels, & Steg, 1984; Statham & Murray, 1971).

The nervous system utilizes similar mechanisms to reinnervate denervated tissue postinjury as are used by the developing system for initial outgrowth and innervation of target tissues. Because recovery from injury to the developing nervous system occurs on a background of normal processes important for growth and development, the developing nervous system may compensate for lost inputs postinjury, resulting in reconstruction of the developing nervous system. Determination of the behavioral effects of damage to the immature nervous system is important because prolonged activation of those compensatory mechanisms may have numerous behavioral effects, ranging from complete sensorimotor recovery to additional sensorimotor impairments.

Significance of Current Research

SCI in humans occurs in a wide variety of ways, so the tissue (see Bunge, Puckett, & Hiester, 1997; Kraus, 1996) and behavioral responses to injury may vary with injury type. Thus, all forms of SCI in humans may not be simulated by a single model of SCI and justifies the development of different animal models. However, with such different techniques to produce SCI, accurate determination of the extent of the lesion and residual hindlimb function is necessary. Knowledge of age-related differences in the effects of SCI will aid determination of the generalizability of postinjury treatments. The purpose of the present experiments was to develop a set of behavioral tasks that challenge hindlimb sensorimotor function postinjury and to examine the effects of age on recovery of function. Although the size of the spinal cord increases with age, proposed procedures enabled compression of the spinal cord by the same relative amount during ontogeny.

Descending, ascending, and segmental systems that survive SCI and guide recovery of hindlimb sensorimotor function may vary with the age of the animal, as well as severity of injury. Therefore, age-related differences in the amount of tissue at the lesion epicenter following midthoracic spinal cord compression will suggest age-related changes in the relative importance of descending inputs or compensation by spinal systems in recovery of hindlimb sensorimotor function. Because human infants and rats progress through similar stages of locomotor development, a greater understanding of the effects of injury to the spinal cord of human infants may be achieved by studying the effects of injury to the spinal cord of developing rats.

CHAPTER 2 MATERIALS AND METHODS

Subjects

Subjects were 5-, 15-, and 60-day-old Sprague-Dawley rats born in the vivarium at the University of Florida Department of Psychology. Adult breeder Sprague-Dawley rats (Zivic-Miller) were housed and bred in a temperature-controlled colony room on a 12:12 hr light/dark cycle with food and water available *ad libitum*. Breeding cages were checked twice daily for litters and the day of birth was considered PND 0. Litters were culled to 10 pups (5 males and 5 females, when possible) within 24 hr of birth and remained with their dams until surgery and behavioral testing. On PND 25, rats were weaned and housed in gang-cages by sex. On PND 45, rats were housed in pairs and were then handled daily prior to surgery.

Surgery

A split-litter design was used so surgery was performed under aseptic conditions on two litters on the same day; half of the male and female rats from each litter received one of the three surgical treatments below. On PND 5, 15, or 60, rats were anesthetized (hypothermia on PND 5; Metofane on PND 15 or 60) and dorsal incisions were made in the skin and underlying muscles. A midthoracic laminectomy was performed and the width of the spinal cord was measured using the coordinate system of a

micromanipulator. Tips of a pair of forceps (Dumont #5) were bent so the arms closed in parallel to each other and were inserted into the vertebral column, between the vertebral walls and the lateral margins of the spinal cord. Sham surgery consisted of no further manipulation. To produce the SCI, forceps were slowly closed until contact was made with an automotive ignition gauge inserted between the arms of the forceps (see Gruner et al., 1996). Spinal cords were compressed for 15 s by 0% (sham), 85%, or 95% of the initial, uncompressed width (also see McEwen & Stehouwer, 1998a). At most ages, muscle and skin were closed in layers using silk sutures and the wound was covered with *New Skin* liquid bandage (Medtech Laboratories, Jackson) to prevent wicking; the skin of 60-day-old rats was closed with wound clips. Rats were placed on a heating pad for 2-3 hr to recover and were placed in the colony room until behavioral testing and sacrifice. Bladders of 60-day-old rats were manually expressed three times daily until self-expressing and the hindquarters were washed as needed. Bladders of the 5- and 15-day-old rats were not expressed manually because the dam performed this function and micturition was not severely compromised following injury. Weight gain of all rats was monitored daily. Seven to 8 subjects comprised each of the 9 experimental groups.

Behavioral Testing

Froot Loops[®] cereal was the food reward during behavioral testing and was placed into the home cage, beginning the week prior to the testing phase to reduce the effects of neophobia. On postoperative days (PODs) 1, 7, 14, 21, and 28, rats were tested for hindlimb sensorimotor function on a battery of tasks. On PODs 1 and 7 for rats of the youngest age group, the battery of tasks included ratings of olfactory-induced walking

(from Jamon & Clarac, 1998), followed by righting, and then determination of latencies to withdraw one hindpaw from the surface of a hotplate. For all other experimental groups and PODs, the sensorimotor battery included ratings of voluntary overground walking, followed by walking across beams of various widths, walking across parallel bars of various distances apart, maintenance of position and balance on an inclined plane, and finally latencies to lick one hindpaw on a hot plate. Except during testing on the parallel bars where rats received multiple successive trials with the bars various distances apart, rats of all experimental groups were tested on one particular task before proceeding to the next behavioral task. Because rats were allowed to rest in the home cage between tasks, sensorimotor performance was probably not significantly affected by fatigue of the rat. A lateral view of each rat was videotaped during testing to aid determination of forelimb and hindlimb performance. A mirror was placed at a 45-degree angle beneath each elevated apparatus to enable simultaneous recording of the lateral and ventral views of each rat. A 5-cm-thick foam pad was placed below these apparatuses to prevent injury if an animal fell.

Righting

Rats younger than 15 days of age were placed supine on a flat surface and allowed to right. Approximately 5 righting episodes were initiated on PODs 1 and 7 for each rat of the youngest age group. Righting matures rapidly during the first week of postnatal life in rats (Tilney, 1934). Because righting by infant rats is dependent on hindbrain and spinal cord structures (Bignall, 1974), observations of righting by infant rats of the present experiments enabled an assessment of the functional efficacy of descending systems following spinal cord compression early in postnatal life.

Overground Walking

Rats, 15 days of age or older, were placed on a flat surface and allowed to walk freely. Prior to PND 15, a plastic tube filled with soiled shavings was placed over the nose of the rat for olfactory-induced locomotion (Jamon & Clarac, 1998). The 21-point BBB locomotor recovery scale was used to assess hindlimb locomotor function (see Appendix A). Because rating scales are subjective, hindlimb motor function was rated on two separate occasions by the same individual for subsequent determination of intrarater reliability. Spontaneous locomotion occurs following a precollicular-premamillary transection through the brain, suggesting that descending systems of the midbrain activate spinal motor circuits. Coordinated walking of all four limbs then requires the integrated activity of spinal and supraspinal systems (see Grillner, 1975; Whelan, 1996). Therefore, ratings of overground locomotion during the recovery period were included to suggest the degree of spared function and plasticity of (1) supraspinal systems, which are required for activation of spinal motor circuits during spontaneous locomotion, (2) propriospinal systems, which are important for coordinated movements between limb girdles, and (3) intraspinal systems, which are important for hindlimb joint movement and weight support following spinal cord compression.

Beam Walking

Rats, 15 days of age or older, were placed on one end of 112 cm long, inclined beams (7 degrees) of various widths (3.0, 2.2, or 0.5 cm) and allowed to walk the length of the beam to reach the goal box with the food reward. The beams were covered in smooth contact paper to prevent soiling of the wood by the rats. The corticospinal tract is important for limb positioning during walking (Hicks & D'Amato, 1975). Therefore, rats

were required to walk across beams of various widths to provide a measure of the functional efficacy of the corticospinal system.

Object Avoidance

This test utilized the same procedures as described for beam walking, except that 4 obstacles of various heights (0.5, 1.2, 1.7, and 2.7 cm above the surface of the runway) were equally spaced along each of the three types of beams. Obstacles were centered along the width of each beam and were 1.1, 0.7, or 0.2 cm in diameter for the 3.0, 2.2, or 0.5 cm wide beams, respectively. The coordinated walking of decerebrate animals (precollicular-premamillary transection) is described as purposeless because the animals walk aimlessly around the room and do not respond properly to obstacles in the environment (see Grillner, 1975; Hinsey, Ranson, & McNattin, 1930). Therefore, the obstacle avoidance task, which requires the rats to step over obstacles during beam walking, was included as a further test of the functional efficacy of forebrain/cortical descending (corticospinal) systems. Because stimulation to the dorsum of the foot of spinal animals initiates limb flexion (see Grillner, 1975), performance on this task will also provide evidence for the functional efficacy of spinal systems if stepping over the obstacles requires activation of spinal reflexes.

Parallel Bar Walking

Rats, 15 days of age or older, were placed on one end of parallel, inclined (7 degrees) wooden rods (112 cm long, 9 mm diameter) that were initially 2.7 cm apart. Rats were allowed to traverse the parallel bars to reach the goal box with the food reward. The distance between the bars was increased in 0.5 cm increments until the rat failed to

traverse the two bars using completely weight-supported steps or the rats walked along a single bar to reach the goal box. The parallel bars were covered with smooth contact paper to prevent soiling of the wood by the rats. Because walking across parallel bars requires greater accuracy of limb placement and coordination between limb girdles than does walking overground or across flat beams, this task was included as a more stringent test of the functional efficacy of the corticospinal system, as well as efficacy of propriospinal systems of the spinal cord.

Inclined Plane

Rats, 15 days of age or older, were tested for the maximum degree of incline on which they maintained position and balance without falling for at least 5 s. Modified from Rivlin and Tator (1977), the inclined plane was a flat board, covered in smooth contact paper, that was initially set 15-degrees above horizontal. Rats were placed onto the inclined plane at a 45-degree angle from horizontal (head up) and tested for maintenance of position and balance for at least 5 s. The incline of the plane was increased in 10-degree increments until the rat failed to maintain position and balance for the duration of the time limit. To determine the threshold value within that 10-degree window, the range was repeatedly narrowed by half the distance until threshold was determined. Although hindlimb weight-support is accomplished by spinal motor circuits in animals with complete spinal cord transections, the hindquarters generally fall to one side or the other (see Grillner, 1975), suggesting that the pathways important for balance were disrupted. Therefore, the inclined plane task was included in the sensorimotor battery of the present experiments to suggest alterations following spinal cord compression in the functional efficacy of ascending and descending systems, such as the

spinocerebellar and vestibulospinal tracts, that would be important maintenance of balance on the inclined plane.

Hot Plate

Rats, 15 days of age or older, were placed on a hot plate preheated to 51° C and monitored for the time elapsed before the rat licked one of its hindpaws. Rats younger than 15 days of age were held around the trunk with the plantar surface of the foot touching the hot plate and observed for the latency to withdrawal one hindpaw from the hot plate surface (on PODs 1 and 7 following surgery on PND 5). A time limit of 30 s was used to ensure that there was no tissue damage to the feet of an unresponsive rat. Based on previous research with adult (see Irwin, Houde, Bennett, Hendershot, & Seevers, 1951) and infant (see Stelzner et al., 1975) animals, withdrawal responses to sensory stimulation are primarily controlled by spinal sensorimotor circuits. In contrast, forebrain circuits are required for the hindpaw lick response on the hotplate (Woolf, 1984). Therefore, the hotplate task was included to provide an assessment of the functional efficacy of spinal sensorimotor circuits during the first postoperative week in rats of the youngest age group and of ascending and descending sensorimotor circuits in rats of all other ages and on all other PODs.

Histology

On POD 29, rats were deeply anesthetized with sodium pentobarbital and perfused with heparinized-saline and 1.0% paraformaldehyde-1.25% glutaraldehyde in 0.1M phosphate buffered saline (PBS; pH 7.2-7.4). Spinal cords were removed and stored at 4° C in 30% sucrose-formalin for at least 3 days and a 2 cm section of spinal cord

containing the injury site was embedded in 30% albumin-3% gelatin. Once hardened in formaldehyde, the blocks were trimmed and embedded in 3% agar to improve adherence of the block to the microtome stage. Spinal cords were then cut into 40 μm transverse (coronal) sections on a freezing-stage sliding microtome, mounted on chrom-alum gelatin-coated slides, and air-dried. Sections were stained using a modification of the Kluver-Barrera method: (1) dehydrate in 95% ethanol (30 min); (2) immerse in 0.1% Luxol Fast Blue MBSN, preheated to 60° C (16-24 hr); (3) rinse in 95% ethanol; (4) rinse in distilled water; (5) differentiate in 0.1% lithium carbonate (2-5 min); (6) differentiate in 70% ethanol (5-30 min); (7) wash in distilled water; (8) immerse in 0.1% cresyl violet (5 min); (9) rinse in distilled water; (10) immerse in 70% ethanol, then 95% ethanol (2 min each); (11) differentiate in 95% ethanol + glacial acetic acid; (12) dehydrate in 95% ethanol (2 min), then 100% ethanol (2 x 1 min); (13) clear in xylene, coverslip with Permount.

Data Analyses

Righting

Videotapes were played in slow-motion ($1/26^{\text{th}}$ normal speed) for qualitative assessment of surgery-related behavioral changes in righting. Videotapes were then played frame-by-frame (30 frames/s) and the positions of the head, trunk, and limbs were drawn for one rat that represented the righting of all rats within that experimental group. Successive images were usually separated by less than 0.23 s (7 frames). In a few cases, righting was interrupted by a brief period of quiescence so as much as 1.03 s (31 frames)

elapsed between drawings. For this and all subsequent analyses, videotapes were scored under experimentally blind conditions with respect to the surgical condition of the rat.

Overground Walking

From videotapes, hindlimb locomotor function was assessed with the BBB locomotor recovery scale as described above.

Beam Walking

Episodes from rats that crossed the 3.0- and 2.2 cm wide beams to reach the goal box were analyzed from videotapes for the number of hindlimb slips. Because the number of steps required to cross the beams decreased with age, the number of hindlimb slips was converted to a percentage of the number of hindlimb steps used to cross the beam. Any step during which the abdomen of the rat touched the beam (non-weight-supported) or any step during which the abdomen remained elevated above the beam (weight-supported), but the foot fell below the beam, was counted as a slip. For rats in which the majority of steps during beam walking were weight-supported, videotapes were also played frame-by-frame (60 frames/s) for determination of footfall patterns and the time interval between stance and lift off of successive limbs. Two limbs were considered to be in stance together if 67 msec or more (4 frames or more) elapsed between surface contact by one foot and lift off of the successive limb. The instances in which 2, 3, or 4 feet were simultaneously in stance were counted and converted to percentages of the total number of footfall patterns used to traverse the beam. Except for rats that received sham surgery on PND 60, most rats did not traverse the 0.5 cm wide beam using weight-supported steps, so the results of that task are not considered further.

Obstacle Avoidance

On each POD, only those rats that successfully traversed the 3.0 and 2.2 cm wide beams were tested for obstacle avoidance. Videotapes were used to determine the tallest obstacle that rats stepped over, without falling or dragging the abdomen. Most rats did not walk across the 0.5 cm wide beam with obstacles using weight-supported steps, so the results of that task are not considered further.

Parallel Bars

All rats, 15 days of age or older, were tested for walking across the parallel bars. From videotapes, behavioral analyses were as described above.

Inclined Plane and Hot Plate

Measures were as described above.

Histologic Evaluation

The section through the lesion that depicted the greatest amount of tissue loss was designated as the lesion epicenter and was magnified and projected onto a digitizing tablet. Outlines of all patches containing clear CNS tissue were traced using a digitizing pen and a computer was used to calculate the area of the spinal cord tissue at the lesion epicenter. Tissue that was characterized by reactive astrocytes, fibrous scarring, or Wallerian degeneration (see Reier, Eng, & Jakeman, 1989) were excluded. Because the cross-sectional area of the spinal cord increases during ontogeny, the absolute amount of spinal cord tissue at the lesion epicenter of rats of the present experiments that received a spinal cord compression was determined and converted to a percentage of control tissue size. Because the myelin was not evenly stained with luxol fast blue in all tissue sections

following the differentiation process, no distinctions were made between gray and white matter during quantification of the cross-sectional area of tissue at the lesion epicenter. Due to problems with tissue adherence to the slides, several tissue sections were lost for each subject and prevented quantification of lesion length or lesion volume.

Statistics

A descriptive analysis of surgery-related alterations to righting was intended and, therefore, no statistical analyses were performed on data from that behavioral task. One-factor Kruskal-Wallis nonparametric statistics were used to test for significant effects of age, surgery, or POD on data that were characterized by mutually exclusive categories with an inherent order among them. Those data included ratings of overground walking, stepping over obstacles the four obstacles of different heights, and walking across parallel bars that were separated in 0.5 cm increments. Post-hoc comparisons were performed using Mann-Whitney U tests ($\alpha = 0.05$). Intrarater reliability on ratings of overground locomotion was determined with Kendall's coefficient of concordance for nonparametric statistics ($W = 0.9932, p < 0.001$). In cases where the two locomotor ratings were not in agreement, the mean of those values was assigned to that subject for subsequent statistical analyses. All other data were measured at the cardinal level and were tested for significant main effects of age, surgery, and POD, as well as their interactions, using analyses of variance (ANOVAs) as specified. For ease of data interpretation, 3-factor ANOVAs were not conducted. Data from the hotplate and inclined plane tasks were analyzed with 2-factor ANOVAs with 1 repeated measure (surgery x POD) for each age group and with 2-factor ANOVAs with 1 repeated measure (age x POD) for each surgery

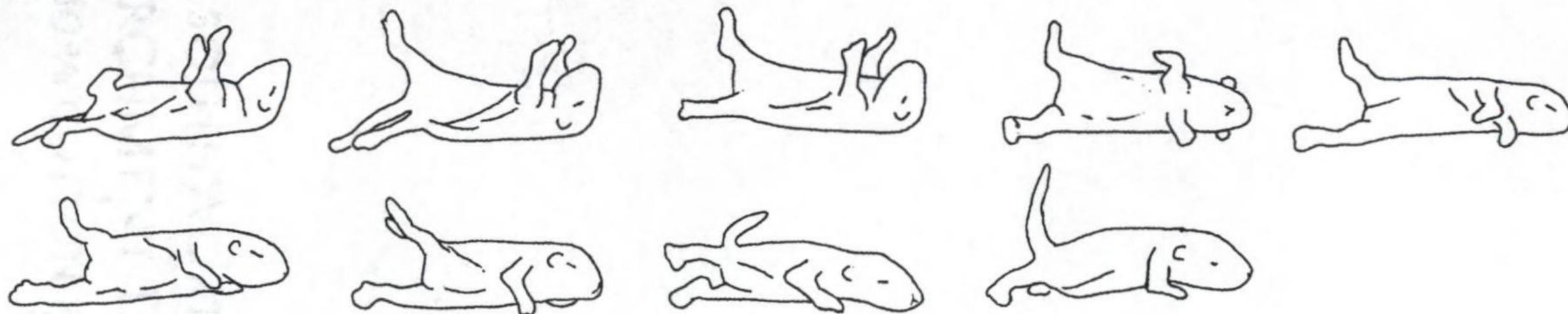
group. The percentage of hindlimb slips and the percentage of footfall patterns characterized by 2, 3, or 4 feet in stance during beam walking were analyzed using 2-factor ANOVAs with 1 repeated measure (surgery x POD) for each age group, as well as 2-factor ANOVAs with 1 repeated measure (age x POD) for each surgery group. Following surgery on PND 60, only rats that received sham surgery walked across the beams using weight-supported steps so data on percentages of footfall patterns was analyzed with 1-factor repeated measures ANOVAs (POD). Some rats failed to traverse the beams on a particular day (generally on POD 1), which resulted in missing values for the repeated measure. In those cases, the missing values were estimated and the degrees of freedom were reduced accordingly (GB-STAT). Absolute amounts of tissue at the lesion epicenter and percentages of control tissue size were analyzed with 2-factor ANOVAs (age x surgery). Post-hoc multiple comparisons were performed using Duncan's new multiple range test ($\alpha = 0.05$). For each age and injury group, ratings of overground walking on POD 28 were plotted as a function of injury level and absolute tissue size at the lesion epicenter was plotted as a function of injury level. Data were tested for the strength of the correlation using Spearman's Rank Correlation Coefficient ($\alpha = 0.05$). Because the number of subjects that successfully completed some of the tasks varied by POD, the number of subjects comprising each statistical analysis is included as Appendix B.

CHAPTER 3 RESULTS

Righting

When placed in a supine position on POD 1, rats that received sham surgery or the lesser compression rocked their outstretched fore- and hindlimbs in opposite directions, until rostrocaudal axial rotation of the body and righting was achieved (Fig. 1A). When rats that received the greater compression were placed in a supine position on POD 1, the hindlimbs remained flexed and the hindquarters immediately fell to one side, which was followed by righting of the head and shoulders. Sometimes righting was aided in those rats by ventroflexion of the upper body, which lifted the head and shoulders off of the ground. Some rats that received the greater compression never pulled the hindlimbs up under the body to complete the righting sequence. Instead, the hindlimbs continued to lie to one side of the body (Fig. 1B). On POD 7, limb extension and axial rocking of the fore- and hindquarters of rats that received sham surgery or the lesser compression was exaggerated relative to the limb and body movements on POD 1. Eventually, righting began at the head and shoulders and proceeded caudally until axial rotation of the entire body was achieved (Fig. 2A). On POD 7, the amount of rocking of the fore- and hindquarters during attempts to right was diminished and the hindlimbs were slightly flexed in rats that received the greater compression, relative to sham controls. Righting was then achieved by axial rotation of the head and shoulders, followed by the hindlimbs.

A



B

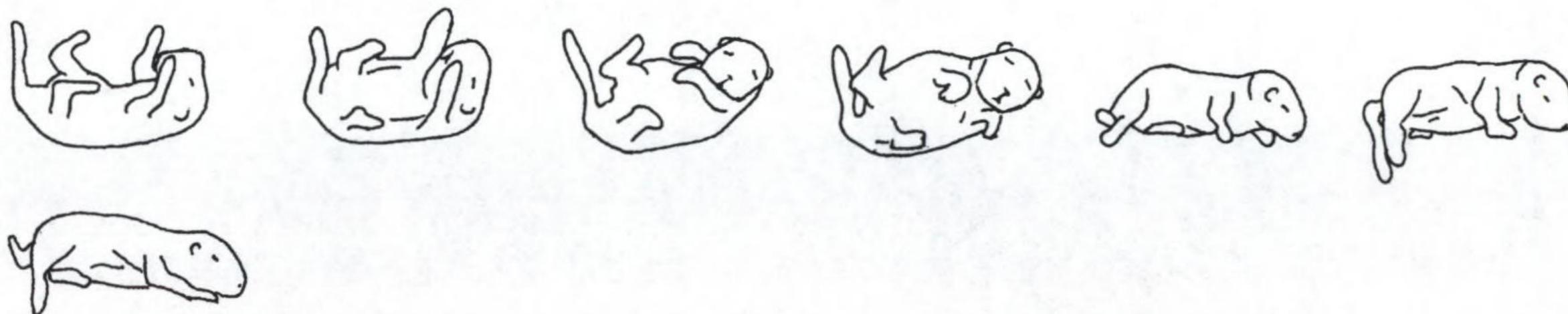


Figure 1. Righting sequence on postoperative day 1 day following compression of the spinal cord of 5-day-old rats.

Depicted from left to right are: A) a series of postures during righting of a representative rat that received sham surgery on postnatal day 5 and B) a series of righting postures of a rat that received 95% compression of the spinal cord on postnatal day 5. Righting of age-matched rats that received 85% compression were indistinguishable from sham controls and, therefore, not included.

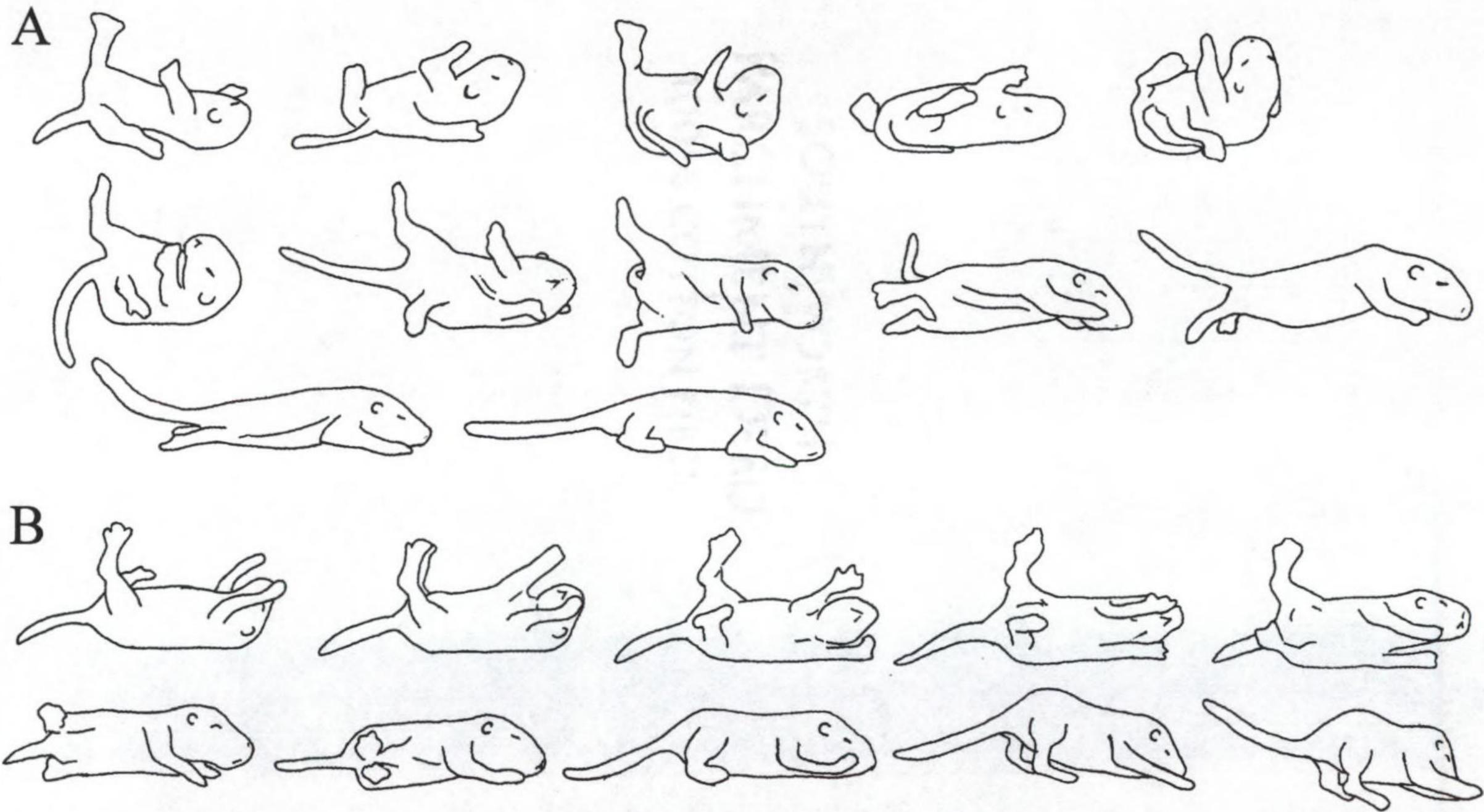


Figure 2. Righting on postoperative day 7 following compression of the spinal cord of 5-day-old rats. Depicted from left to right are: A) a series of postures during righting of a representative rat that received sham surgery on postnatal day 5 and B) a series of righting postures of a rat that received 95% compression of the spinal cord on postnatal day 5. Righting of age-matched rats that received 85% compression were indistinguishable from sham controls and, therefore, not included.

Often the hindlimbs were pulled under the body, as found for sham controls. However, the hindlimb joints were unusually extended during weight support, which lifted the hindquarters above the table surface (Fig. 2B).

Overground Walking

There was an age-related decline in the quality of overground walking in rats that received compression of the spinal cord (Fig. 3). In rats of the youngest age group, surgery-related differences in overground walking were apparent on PODs 1, 14, 21, and 28 [$H_s(2) > 8.72$, $p_s < 0.05$]. By the end of the postoperative period, rats of the youngest age group that received the greater compression retained deficiencies in toe clearance, paw position at lift off, tail position, and trunk stability during walking ($p_s < 0.05$). Overground walking of age-matched rats that received the lesser compression or sham surgery did not differ significantly ($p_s > 0.05$). Compression of the 15-day-old spinal cord also had surgery-dependent effects on overground walking throughout the postoperative period [$H_s(2) > 15.0$, $p_s < 0.005$]. Specifically, overground walking was indistinguishable throughout the postoperative period between rats that received the lesser compression or sham surgery on PND 15 ($p_s > 0.05$). Rats that received the greater compression on PND 15 dragged the hindlimbs on POD 1 and only slightly moved one or two of the hindlimb joints, if any, during locomotion. By POD 7, those rats recovered hindlimb weight-supported stepping, but remained deficient throughout the postoperative period in either toe clearance or paw position, tail position, and trunk stability, relative to rats of the other two groups ($p_s < 0.05$). Surgery on PND 60 also significantly altered overground walking throughout the postoperative period [$H_s(2) >$

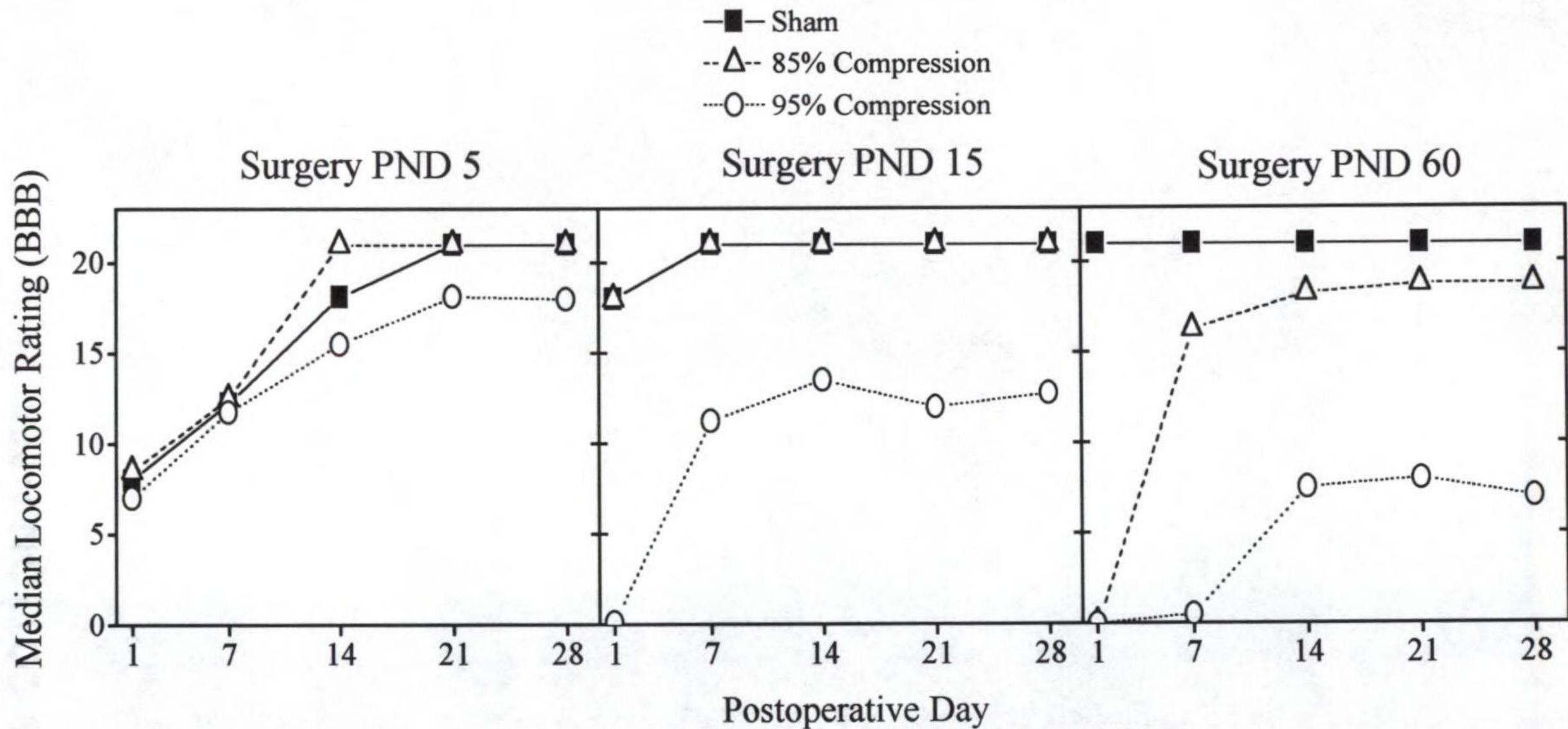


Figure 3. Median ratings of overground walking throughout the 4-week postoperative period for rats that received sham surgery, 85% compression, or 95% compression on postnatal day 5, 15, or 60. On the Basso, Beattie, and Bresnahan (BBB) 21-point scale, higher numbers reflect greater motor function, with a score of 21 representing overground walking of uninjured, adult rats. Ratings less than 21 in rats that received sham surgery on postnatal day 5 or 15 reflect immaturity of the motor system, rather than surgery-related deficits in motor function.

15.0, $ps < 0.005$]. Rats that received midthoracic spinal cord compression on PND 60 dragged the hindlimbs on POD 1 ($ps < 0.05$), which did not differ significantly between rats of the two compression groups ($ps > 0.05$). Young-adult rats that received the lesser compression recovered hindlimb weight-supported stepping and coordination across limb girdles within the first postoperative week, but deficiencies in toe clearance, paw placement, tail position, and trunk stability remained throughout the remainder of the postoperative period. Young-adult rats that received the greater compression recovered extensive movement of the hindlimb joints by POD 14, but never recovered weight-supported stepping or coordination across limb girdles ($ps < 0.05$). Except for rats that received sham surgery on PND 60 and were rated as 'normal' throughout the postoperative period [$H(4) = 0.00$, $p > 0.05$], overground walking of rats of all other age and surgery groups improved during the postoperative period [$Hs(4) > 18.73$, $ps < 0.005$].

Between PODs 1 and 14, locomotor function of sham controls differed between rats of the three age groups [$Hs(3) > 11.76$, $ps < 0.005$]. Following sham surgery, rats of the youngest age group had lower locomotor scores on PODs 1, 7, and 14 and rats of the intermediate age group had lower scores on POD 1, relative to rats of the oldest age group ($ps < 0.05$). Those findings reflect the locomotor immaturity of neonatal rats on those PODs. On PODs 21 and 28, overground walking was mature and was indistinguishable among rats of the three age groups that received sham surgery [$Hs(2) = 0.00$, $ps > 0.05$]. Overground walking of rats that received either amount of spinal cord compression also resulted in significant effects of age throughout the postoperative period [$Hs(2) > 12.13$, $ps < 0.005$]. Once overground walking was mature on PODs 21 and 28, rats of the youngest two age groups that received the lesser compression were

indistinguishable from each other ($p > 0.05$), whereas rats that received a similar compression on PND 60 maintained deficiencies in paw position at lift off, tail elevation, and trunk stability ($p < 0.05$). In contrast, there was an age-dependent decrease on PODs 21 and 28 in hindlimb motor function during overground walking of rats that received the greater compression ($p < 0.05$).

Beam Walking

3.0 cm Beam

Percentage of hindlimb slips

Rats that received sham surgery on PND 5 traversed the 3.0 cm beam by POD 14, when a raised, quadrupedal posture was achieved. Rats that received sham surgery on PND 15 or 60 traversed the beam throughout the postoperative period. Not all rats of each age and compression group crossed the beam on all PODs (see Appendix B). Following surgery on PND 5, the percentage of slips during beam walking resulted in a significant effect of surgery \times POD [$F(4, 42) = 21.23, p < 0.001$; Fig. 4]. The percentage of hindlimb slips during beam walking was indistinguishable on PODs 14 to 28 between rats that received the lesser compression or sham surgery ($p > 0.05$). In contrast, the percentages of hindlimb slips were significantly elevated in rats that received the greater compression ($p < 0.05$), except on POD 21 when the percentage of hindlimb slips was not different from rats that received the lesser compression ($p > 0.05$). Although the percentage of hindlimb slips of rats of the youngest age group was relatively constant throughout the postoperative period following the lesser compression or sham surgery ($p > 0.05$), the percentage of hindlimb slips of the 3 rats that crossed the beam following

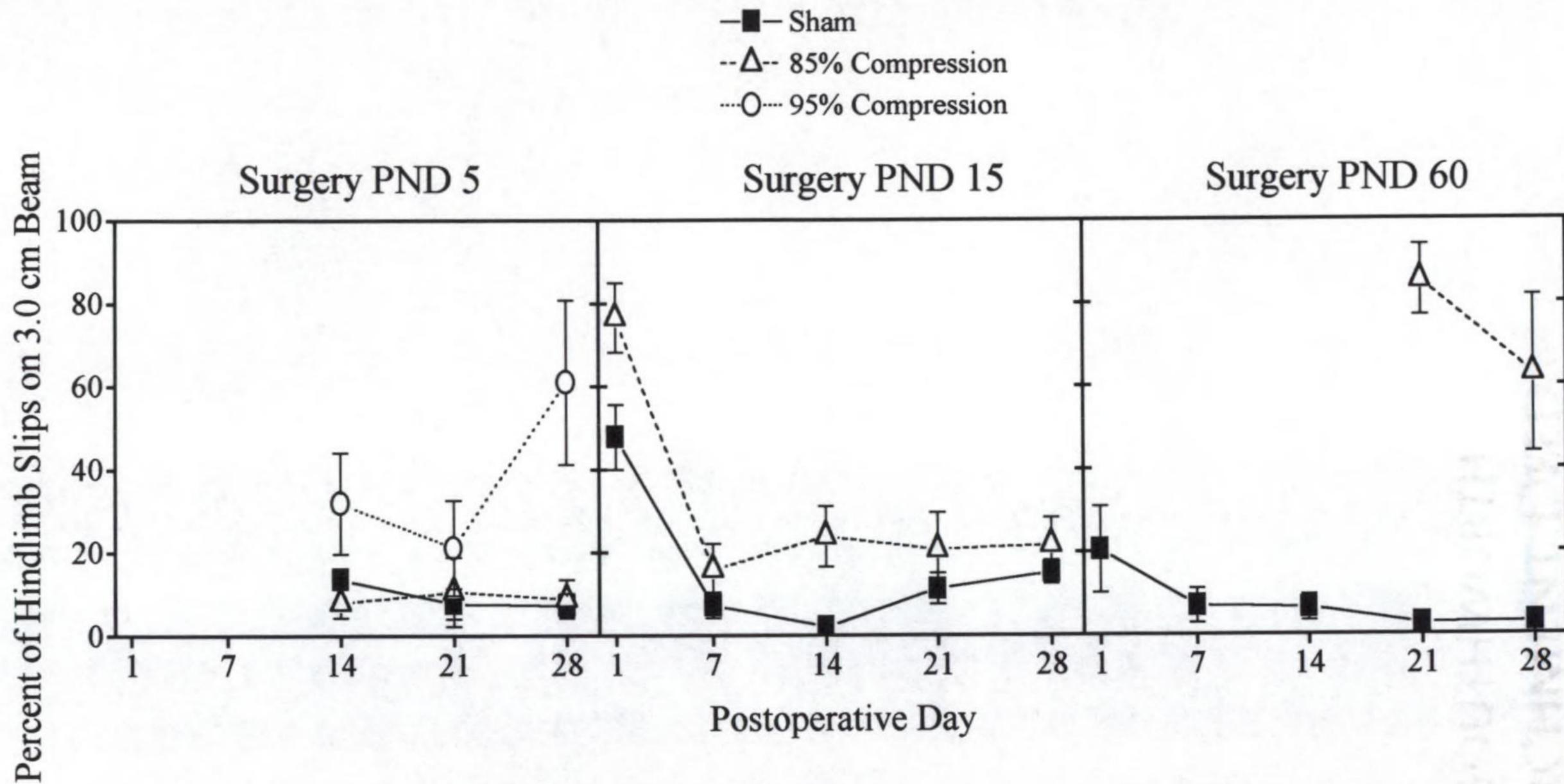


Figure 4. Numbers of hindlimb steps that were characterized by slips are represented as percentages of the total number of hindlimb steps required to cross the 3.0 cm wide beam. Percentages of hindlimb slips (\pm SEM) are shown for each postoperative day of interest for rats that received sham surgery, the 85% compression, or the 95% compression on postnatal day 5, 15, or 60. Only 3 rats that received the 95% compression on PND 5 and 3 rats that received the 85% compression on PND 60 traversed the beam.

greater compression of the cord significantly increased between PODs 21 and 28 ($p < 0.05$). Rats that received the lesser compression on PND 15 had a higher percentage of hindlimb slips than age-matched rats that received sham surgery [$F(1, 14) = 8.15, p < 0.05$]. The percentage of hindlimb slips in those groups of rats decreased during the postoperative period [$F(4, 56) = 47.83, p < 0.001$], mainly between PODs 1 and 7 ($p < 0.05$), with little change thereafter ($ps > 0.05$). Following surgery on PND 60, the percentage of hindlimb slips resulted in a significant surgery x POD interaction [$F(1, 14) = 27.48, p = 0.001$]. When rats that received the lesser compression on PND 60 began traversing the beam on POD 21, the percentage of hindlimb slips was elevated over sham controls ($ps < 0.05$), but the percentage of hindlimb slips decreased between PODs 21 and 28 ($p < 0.05$). The percentage of hindlimb slips did not change significantly during the postoperative period in rats that received sham surgery on PND 60 [$F(4, 28) = 2.39, p > 0.05$].

Following sham surgery, the percentage of hindlimb slips of rats of the three age groups resulted in a significant age x POD interaction for PODs 14 to 28 [$F(4, 42) = 3.93, p < 0.01$]. Specifically, rats that received sham surgery on PND 5 had a higher percentage of hindlimb slips on POD 14 than rats that received sham surgery on PND 15 ($p < 0.05$) and rats that received sham surgery on PND 15 had more hindlimb slips on POD 28 than rats that received sham surgery on PND 60 ($p < 0.05$). The percentage of hindlimb slips of rats that received surgery on PND 15 or 60 also resulted in a significant age x POD interaction between PODs 1 and 28 [$F(4, 56) = 3.59, p < 0.05$]. Specifically, the percentage of hindlimb slips was elevated on POD 1 in rats that received sham surgery on PND 15, relative to rats that received sham surgery on PND 60 ($p < 0.05$).

The percentage of hindlimb slips during beam walking of rats that received the lesser compression resulted in a significant effect of age x POD when data were compared across rats of all three age groups [$F(2, 21) = 8.30, p < 0.01$]. Among rats that received the lesser compression, there was an age-dependent increase in the percentage of hindlimb slips on PODs 21 and 28 ($ps < 0.05$). Most rats that received the greater compression of the cord did not walk across the beam, which precluded an examination of age-dependent effects of the greater compression on beam walking.

Footfall patterns

Footfall patterns could not be determined for rats that dragged their abdomens across the beam, rather than walking with weight-supported steps (see Appendix B). While traversing the 3.0 cm wide beam, rats primarily used a lateral sequence walking gait in which 3 limbs remained in contact with the beam and stepping of the left hindlimb, was followed by the left forelimb, then the right hindlimb and finally by the right forelimb. Among all rats that received the greater compression of the spinal cord, only 2 rats that received the compression on PND 5 traversed the beam using weight-supported steps on PODs 14 and 21 and only 1 rat of that age group traversed the beam on POD 28. Therefore, footfall patterns were statistically analyzed for rats that received either the lesser compression or sham surgery, but data for rats that received the greater compression on PND 5 were included in the figures for comparison. Between PODs 14 and 28, the percentage of footfall patterns in which rats of the youngest age group maintained 2 (Fig. 5), 3 (Fig. 6), or 4 (Fig. 7) limbs on the beam was not dependent on surgery [$F_s(1, 14) < 0.06, ps > 0.05$] or on POD [$F_s(2, 28) < 1.97, ps > 0.05$]. In contrast, the percentage of footfall patterns in which rats that received surgery on PND 15

Percent Support Patterns with 2 Feet on 3.0 cm Beam

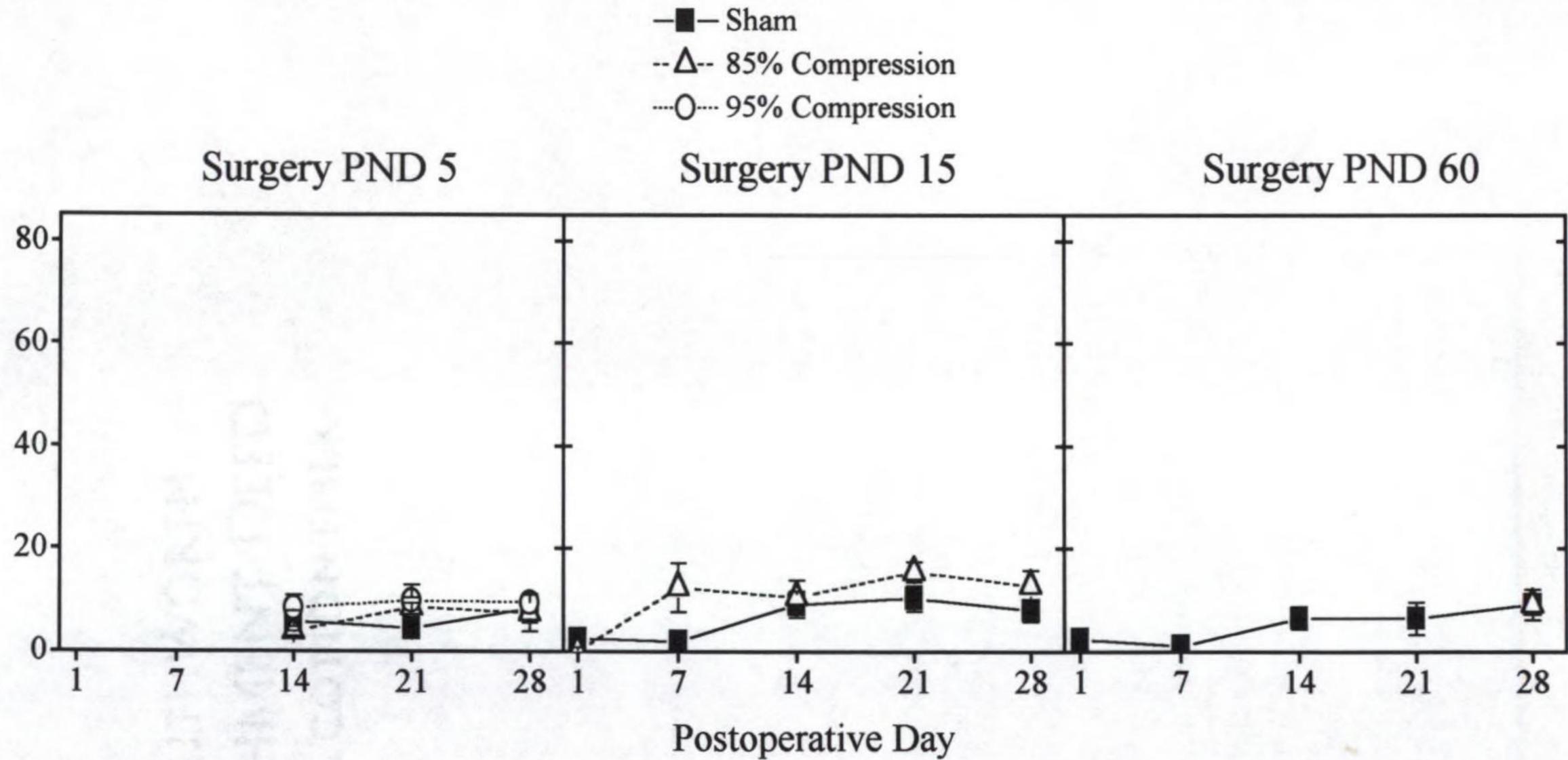


Figure 5. Rats crossed the 3.0 cm beam with either 2, 3, or 4 limbs in stance simultaneously. This figure depicts the percentage of support patterns (\pm SEM) used throughout the postoperative period in which the body weight was supported by 2 feet on the beam for rats that received surgery on postnatal day 5, 15, or 60. Most rats that received the 95% compression at any age or the 85% compression on postnatal day 60 did not cross the beam using weight-supported steps and were not included in this analysis.

Percent Support Patterns with 3 Feet on 2.2 cm Beam

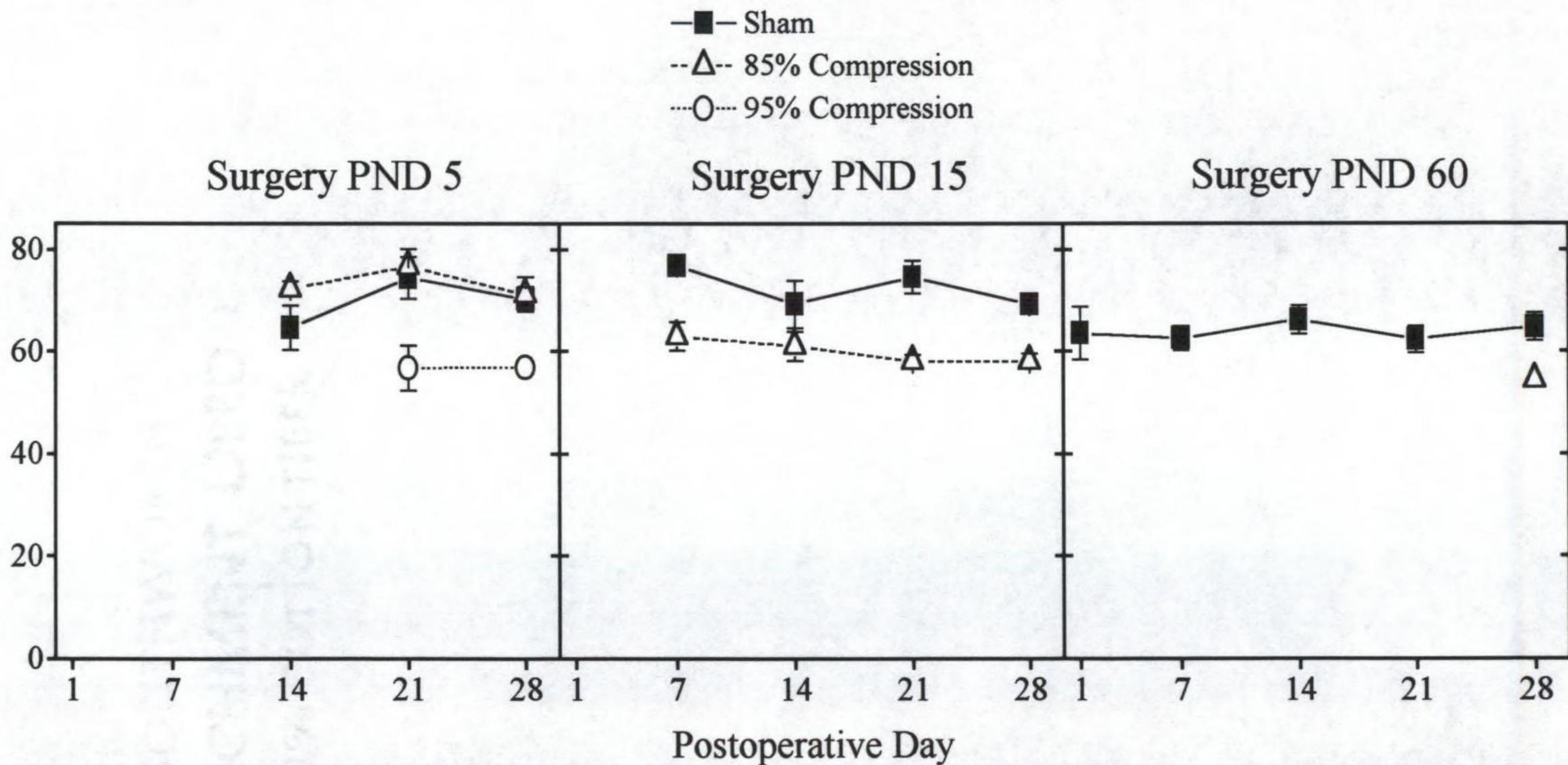


Figure 6. Rats traversed the 3.0 cm beam with either 2, 3, or 4 limbs in stance simultaneously. This figure depicts the percentage of support patterns (\pm SEM) used throughout the postoperative period in which the body weight was supported by 3 feet on the beam for rats that received surgery on postnatal day 5, 15, or 60. Most rats that received the 95% compression at any age or the 85% compression on postnatal day 60 did not cross the beam using weight-supported steps and were not included in this analysis.

Percent Support Patterns with 4 Feet on 3.0 cm Beam

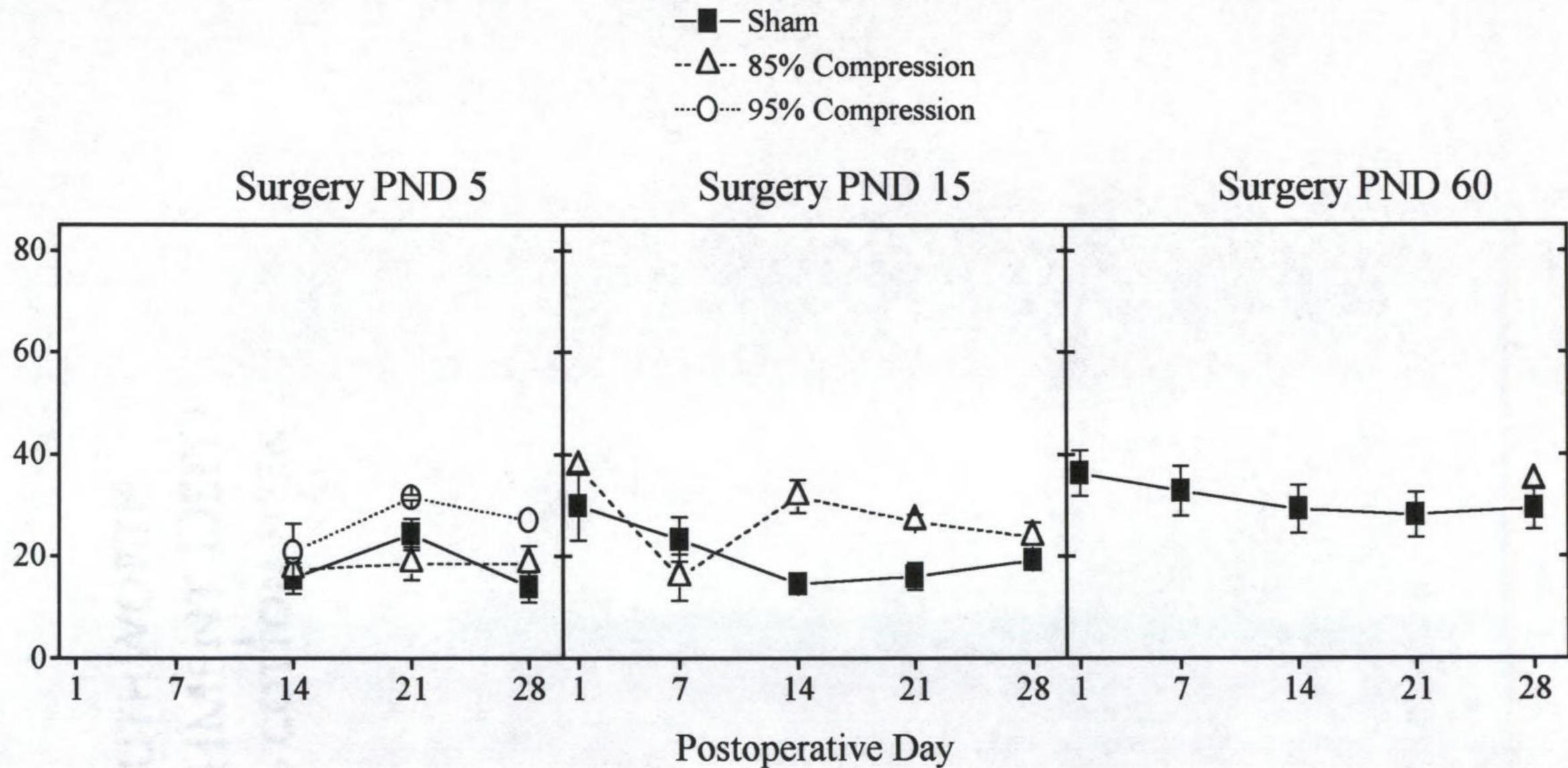


Figure 7. Rats traversed the 3.0 cm beam with either 2, 3, or 4 limbs in stance simultaneously. This figure depicts the percentage of support patterns (\pm SEM) used throughout the postoperative period in which the body weight was supported by 4 feet on the beam for rats that received surgery on postnatal day 5, 15, or 60. Most rats that received the 95% compression at any age or the 85% compression on postnatal day 60 did not cross the beam using weight-supported steps and were not included in this analysis.

maintained 2, 3, or 4 feet on the 3.0 cm wide beam resulted in significant effects of surgery x POD [$F_s(4, 56) > 2.97$, $ps < 0.05$]. Rats that received sham surgery had a higher percentage of footfall patterns with 3 limbs in stance between PODs 14 and 28 ($ps < 0.05$), but a lower percentage of footfall patterns with 4 limbs in stance on PODs 14 and 21 ($ps < 0.05$), relative to rats that received the lesser compression. In addition, the percentage of footfall patterns with 2 limbs in stance was lower on POD 7 in rats that received sham surgery on PND 15 than in age-matched rats that received the greater compression ($p < 0.05$). Although footfall patterns were analyzed statistically only for rats that received sham surgery on PND 60, data from the 1 rat that received the lesser compression on PND 60 and traversed the beam using weight-supported steps on POD 28 was included in the figure for comparison. Although the percentage of footfall patterns characterized by 3 or 4 feet in stance did not change significantly during the postoperative period [$F_s(4, 28) < 2.31$, $ps > 0.05$], there was a significant change in the percentage of footfall patterns characterized by 2 feet in stance in rats that received sham surgery on PND 60 [$F(4, 28) = 3.44$, $p < 0.05$]. Specifically, the percentage of footfall patterns with 2 feet in stance was higher on PODs 21 and 28 than on other days of the postoperative period ($ps < 0.05$).

2.2 cm Beam

Percentage of hindlimb slips

Rats that received sham surgery on PND 5 traversed the 2.2 cm wide beam, beginning on POD 14 when a raised, quadrupedal posture was achieved. Most rats that received sham surgery on PND 15 or 60 traversed the beam throughout the postoperative period. Not all rats of each age and injury group traversed the beam on all PODs (see

Appendix B). Following surgery on PND 5, the percentage of hindlimb slips during beam walking resulted in significant effects of surgery [$F(2, 21) = 5.24, p < 0.05$] and POD [$F(2, 42) = 23.27, p < 0.001$; Fig. 8]. Specifically, the percentage of hindlimb slips was higher in rats that received the greater compression than in rats that received the mild compression ($p < 0.05$) and the percentage of hindlimb slips decreased between PODs 14 and 21 ($p < 0.05$), with no significant change thereafter ($p > 0.05$). The percentage of hindlimb slips of rats that received the lesser compression on PND 15 was elevated, relative to age-matched sham controls [$F(1, 14) = 7.91, p < 0.05$]. Furthermore, the percentage of hindlimb slips of those rats during beam walking resulted in a significant effect of POD [$F(4, 56) = 14.99, p < 0.001$]. Specifically, the percentage of hindlimb slips decreased between PODs 1 and 7 ($p < 0.05$), with little change thereafter ($ps > 0.05$). When rats that received the lesser compression on PND 60 began traversing the beam on POD 21, the percentage of hindlimb slips resulted in a significant effect of surgery x POD [$F(1, 14) = 13.12, p < 0.01$]. Specifically, the percentage of hindlimb slips of rats that received the lesser compression on PND 60 was elevated over sham controls on PODs 21 and 28 ($ps < 0.05$), but decreased significantly in those rats between the two PODs ($p < 0.05$). The percentage of hindlimb slips of rats that received surgery on PND 60 also resulted in a significant effect of POD [$F(4, 28) = 2.72, p < 0.05$]. Specifically, the percentage of hindlimb slips was higher on POD 1 than on PODs 14, 21, or 28 ($ps < 0.05$).

Between PODs 14 and 28, the percentage of hindlimb slips by rats that received sham surgery resulted in a significant age x POD interaction [$F(4, 42) = 3.45, p < 0.05$]. On POD 14, the percentage of hindlimb slips decreased with increased age of the rat

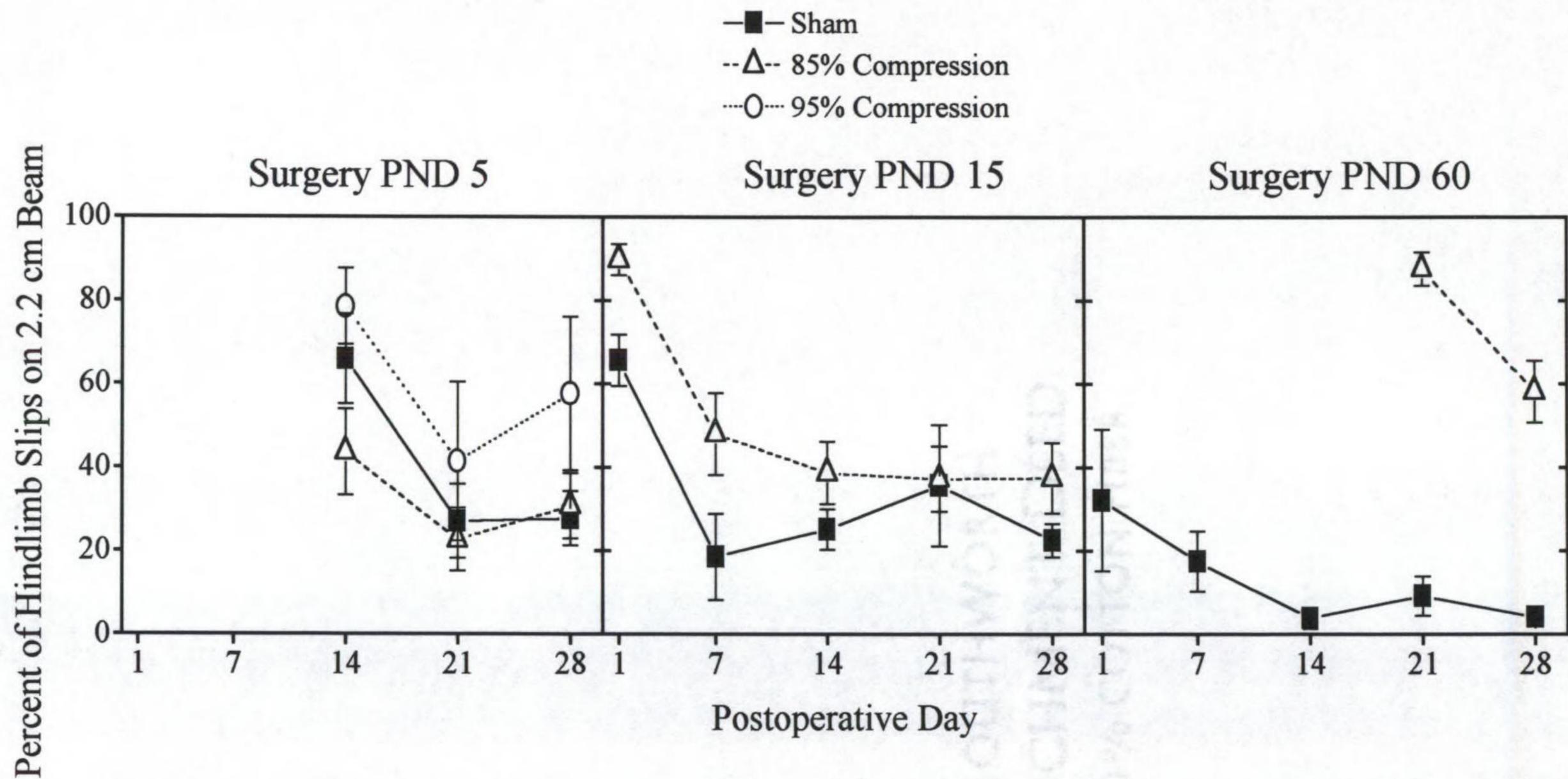


Figure 8. Numbers of hindlimb steps characterized by slips are represented as percentages of the total number of hindlimb steps required to cross the 2.2 cm wide beam. Percentages of hindlimb slips (\pm SEM) are shown for each postoperative day of interest for rats that received sham surgery, the 85% compression, or the 95% compression on postnatal day 5, 15, or 60. Only 3 rats that received the 95% compression on PND 5 and 3 rats that received the 85% compression on PND 60 traversed the beam.

($p < 0.05$). On POD 21, rats that received sham surgery on PND 15 had higher percentages of hindlimb slips than the young-adult rats ($p < 0.05$), but rats of the two youngest age groups did not differ from each other ($p > 0.05$). The percentages of hindlimb slips of rats of the three age groups that received sham surgery did not significantly differ on POD 28 ($p > 0.05$). When the entire postoperative period was examined in rats that received surgery on PND 15 or 60, the percentage of hindlimb slips was higher in rats that received surgery on PND 15 than on PND 60 [$F(1, 14) = 10.76, p < 0.01$]. Furthermore, the percentage of hindlimb slips throughout the postoperative period for rats of those two age groups resulted in a significant effect of POD [$F(4, 56) = 6.77, p < 0.001$]. The percentage of hindlimb slips decreased between PODs 1 and 7 ($p < 0.05$), with little change thereafter ($p > 0.05$). When rats received the lesser compression on PND 5 or 15, there was no significant effect of surgery [$F(1, 14) = 0.32, p > 0.05$] or POD [$F(2, 28) = 2.94, p > 0.05$] on the percentage of hindlimb slips between PODs 14 and 28. When the effects of the lesser compression were examined on PODs 21 and 28 among rats of the three age groups, the percentage of hindlimb slips resulted in a significant effect of age \times POD [$F(2, 21) = 24.72, p < 0.001$]. Specifically, the percentage of hindlimb slips was elevated on PODs 21 and 28 in rats that received the lesser compression on PND 60, than in rats of the youngest two age groups ($p < 0.05$). Rats that received the lesser compression on PND 15 had a higher percentage of hindlimb slips on POD 21 than rats that received a similar compression on PND 5 ($p < 0.05$).

Footfall patterns

Footfall patterns could not be determined for rats that dragged their abdomens across the beam, rather than walked with weight-supported steps (see Appendix B).

While crossing the 2.2 cm beam, rats primarily used a lateral sequence walking gait in which 3 limbs remained in contact with the 2.2 cm wide beam and stepping of the left hindlimb was followed by the left forelimb, then the right hindlimb and finally by the right forelimb. Following surgery on PND 5, only 2 rats that received the greater compression traversed the beam using weight-supported steps on POD 21 and 1 rat traversed the beam on POD 28. Therefore, footfall patterns were statistically analyzed for rats that received either the lesser compression or sham surgery, but data for rats that received the greater compression were included in the figures for comparison. Following surgery on PND 5, the percentage of footfall patterns in which 2 (Fig. 9), 3 (Fig. 10), or 4 (Fig. 11) limbs were in stance during beam walking did not result in significant effects of surgery [$F_s(1, 14) < 3.01$, $p_s > 0.05$]. The percentage of footfall patterns characterized by 3 limbs in stance changed during the postoperative period [$F(2, 28) = 5.56$, $p < 0.01$]. Specifically, the percentage of footfall patterns with 3 feet in stance was higher on POD 21 than on any other POD in rats of the youngest age group ($p_s < 0.05$). Following surgery on PND 15, the percentages of footfall patterns in which 2 or 4 limbs were simultaneously in stance during walking across the 2.2 cm wide beam were higher in rats that received the lesser compression than in age-matched rats that received sham surgery [$F_s(1, 14) < 9.65$, $p_s < 0.01$], whereas the percentage of footfall patterns with 3 feet in stance was lower in rats that received the lesser compression than in rats that received sham surgery [$F(1, 14) = 52.37$, $p < 0.001$]. The percentage of footfall patterns characterized by 3 feet in stance changed significantly between PODs 7 and 28 [$F(3, 42) = 3.36$, $p < 0.05$] and was characterized by a higher percentage on POD 7 than on POD 28 ($p < 0.05$). Although footfall patterns were only analyzed for rats that received sham

Percent Support Patterns with 2 Feet on 2.2 cm Beam

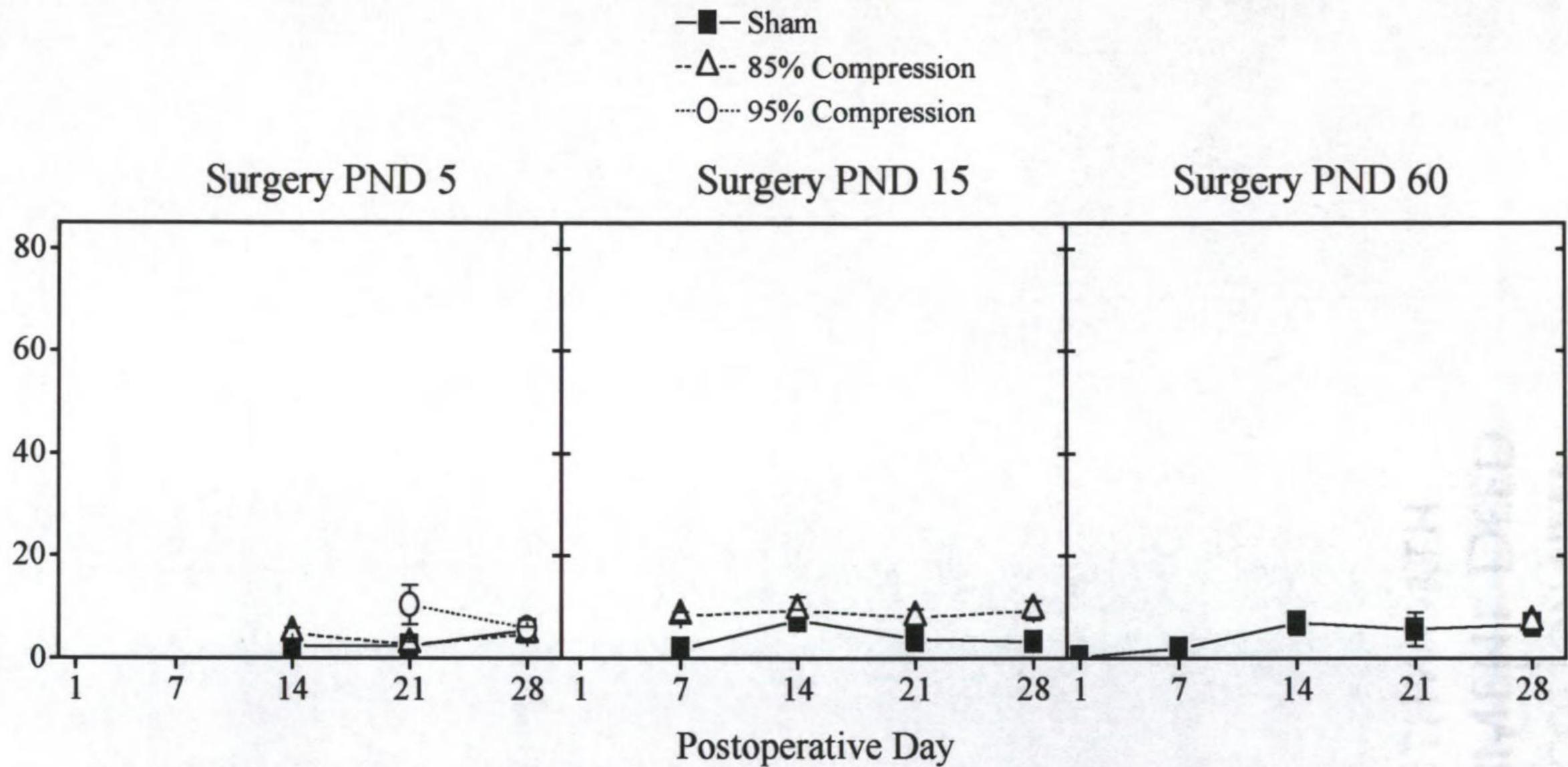


Figure 9. Rats crossed the 2.2 cm beam with either 2, 3, or 4 limbs in stance simultaneously. This figure depicts the percentage of support patterns (\pm SEM) used throughout the postoperative period in which the body weight was supported by 2 feet on the beam for rats that received surgery on postnatal day 5, 15, or 60. Most rats that received the 95% compression at any age or the 85% compression on postnatal day 60 did not cross the beam using weight-supported steps and were not included in this analysis.

Percent Support Patterns with 3 Feet on 2.2 cm Beam

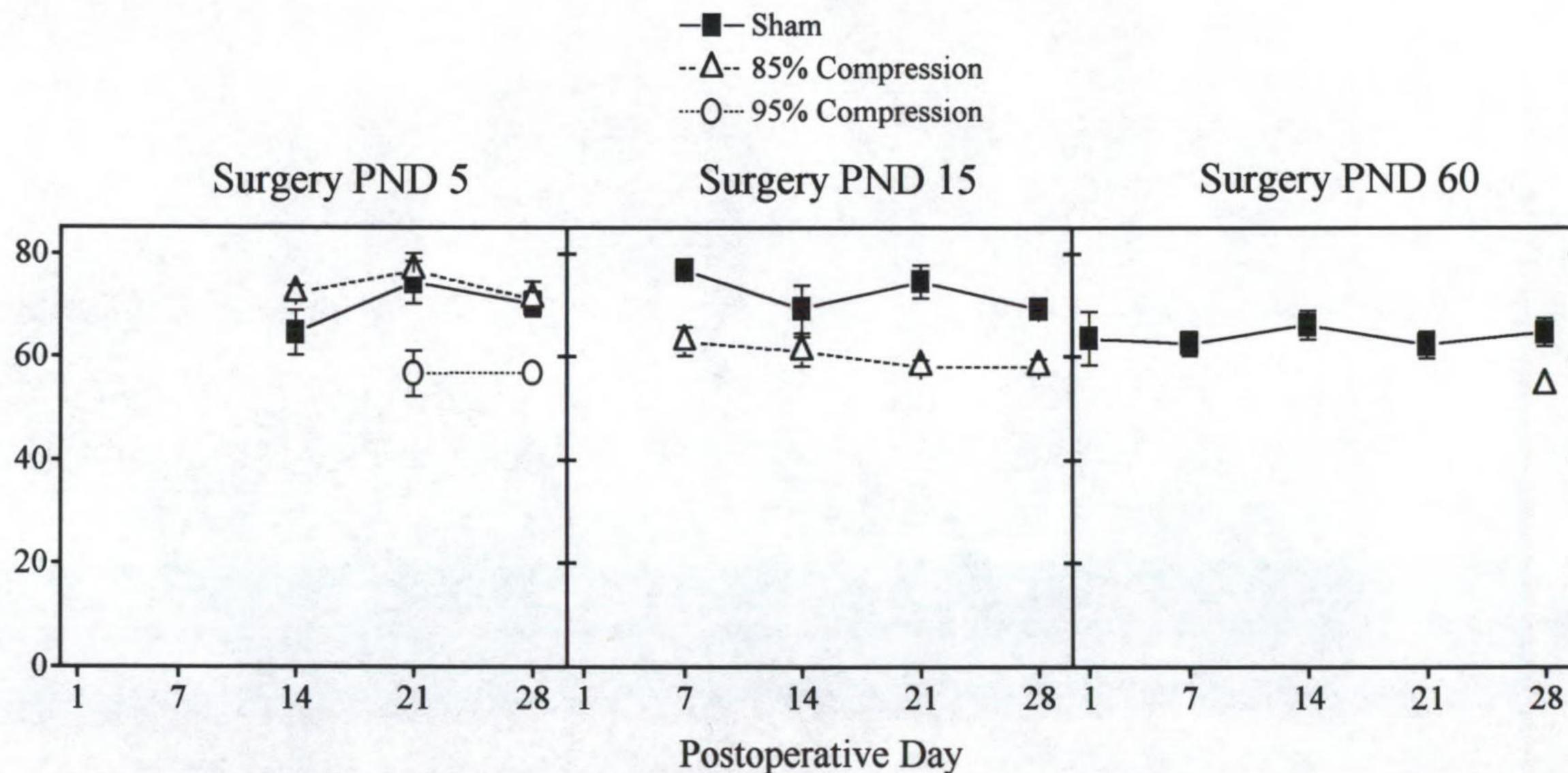


Figure 10. Rats crossed the 2.2 cm beam with either 2, 3, or 4 limbs in stance simultaneously. This figure depicts the percentage of support patterns (\pm SEM) used throughout the postoperative period in which the body weight was supported by 3 feet on the beam for rats that received surgery on postnatal day 5, 15, or 60. Most rats that received the 95% compression at any age or the 85% compression on postnatal day 60 did not cross the beam using weight-supported steps and were not included in this analysis.

Percent Support Patterns with 4 Feet on 2.2 cm Beam

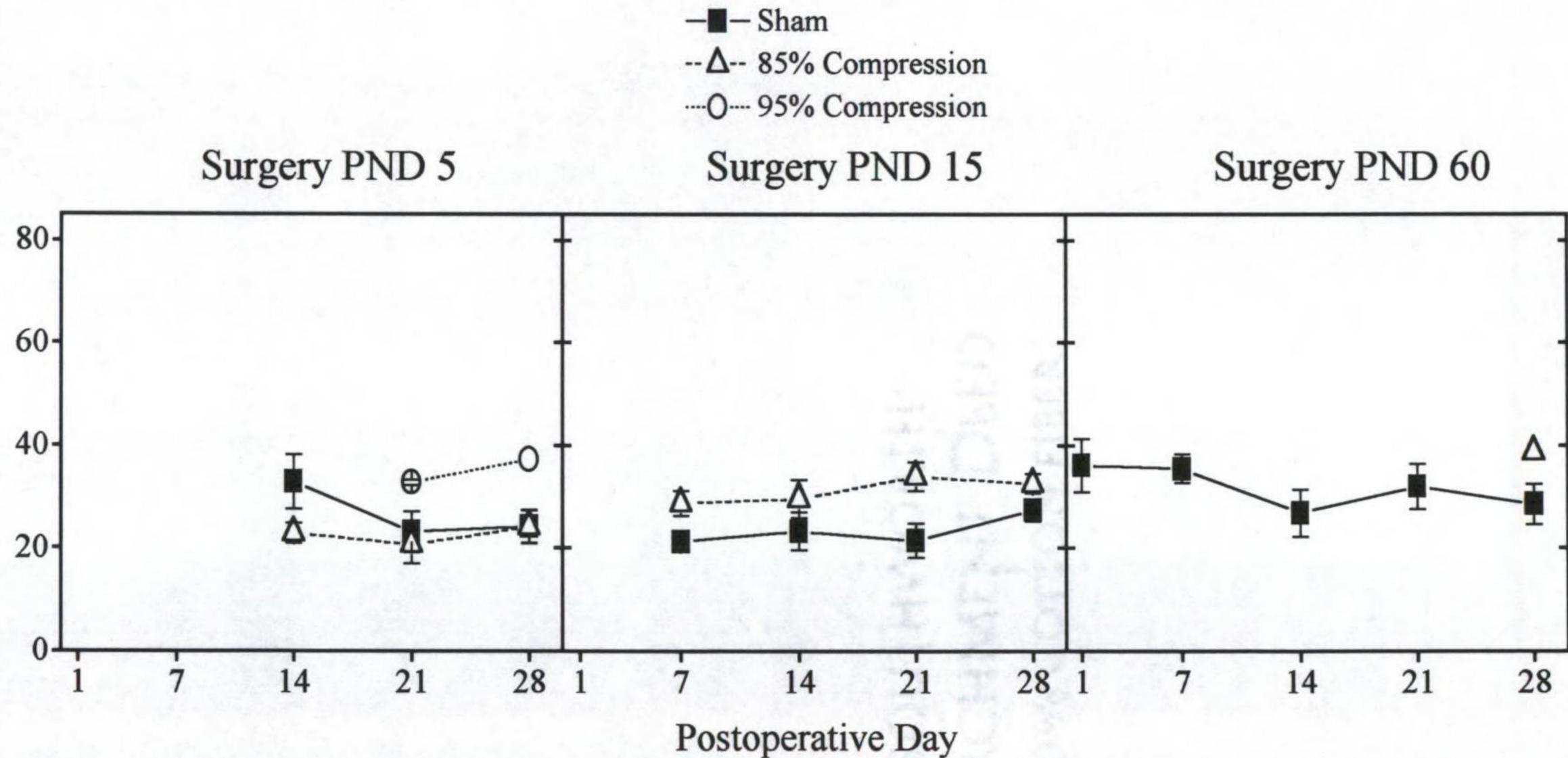


Figure 11. Rats traversed the 2.2 cm beam with either 2, 3, or 4 limbs in stance simultaneously. This figure depicts the percentage of support patterns (\pm SEM) used throughout the postoperative period in which the body weight was supported by 4 feet on the beam for rats that received surgery on postnatal day 5, 15, or 60. Most rats that received the 95% compression at any age or the 85% compression on postnatal day 60 did not cross the beam using weight-supported steps and were not included in this analysis.

surgery on PND 60, data from the 1 rat that traversed the beam using weight-supported steps following the lesser compression were included in the figure for comparison. As found for rats of the other age groups, rats that received sham surgery on PND 60 primarily used a footfall pattern in which 3 limbs were simultaneously in stance during beam walking. However, the percentage of footfall patterns characterized by 2 or 4 limbs in stance changed significantly during the postoperative period [$F_s(4, 28) > 3.23$, $p_s < 0.05$]. The percentage of footfall patterns with 2 limbs in stance was higher on PODs 14 and 28 than on POD 1 ($p_s < 0.05$). The percentage of footfall patterns with 4 limbs in stance was higher on PODs 1 and 7 than on PODs 14 and 28 ($p_s < 0.05$).

Obstacle Avoidance

3.0 cm Beam

Only rats that successfully crossed the 3.0 and 2.2 cm wide beams were tested for obstacle avoidance (see Appendix B). Throughout the postoperative period, the height of the obstacles that rats successfully stepped over while traversing the 3.0 cm beam was not significantly altered by spinal cord compression on PND 5 [$H_s(2) < 5.11$, $p_s > 0.05$], PND 15 [$H_s(1) < 2.26$, $p_s > 0.05$], or PND 60 [$H_s(1) = 2.67$, $p_s > 0.05$; Fig. 12]. Only rats that received sham surgery on PND 5 stepped over significantly taller obstacles on the 3.0 cm beam during the postoperative period [$H(2) = 10.64$, $p < 0.005$]. On POD 7 when rats that received sham surgery on PND 15 or 60 both traversed beams, there was an age-related increase in the height of obstacles that rats successfully stepped over [$H(1) = 6.62$, $p < 0.01$]. Among rats of all three age groups that received sham surgery, there were significant age-related increases in the height of the obstacles that rats successfully

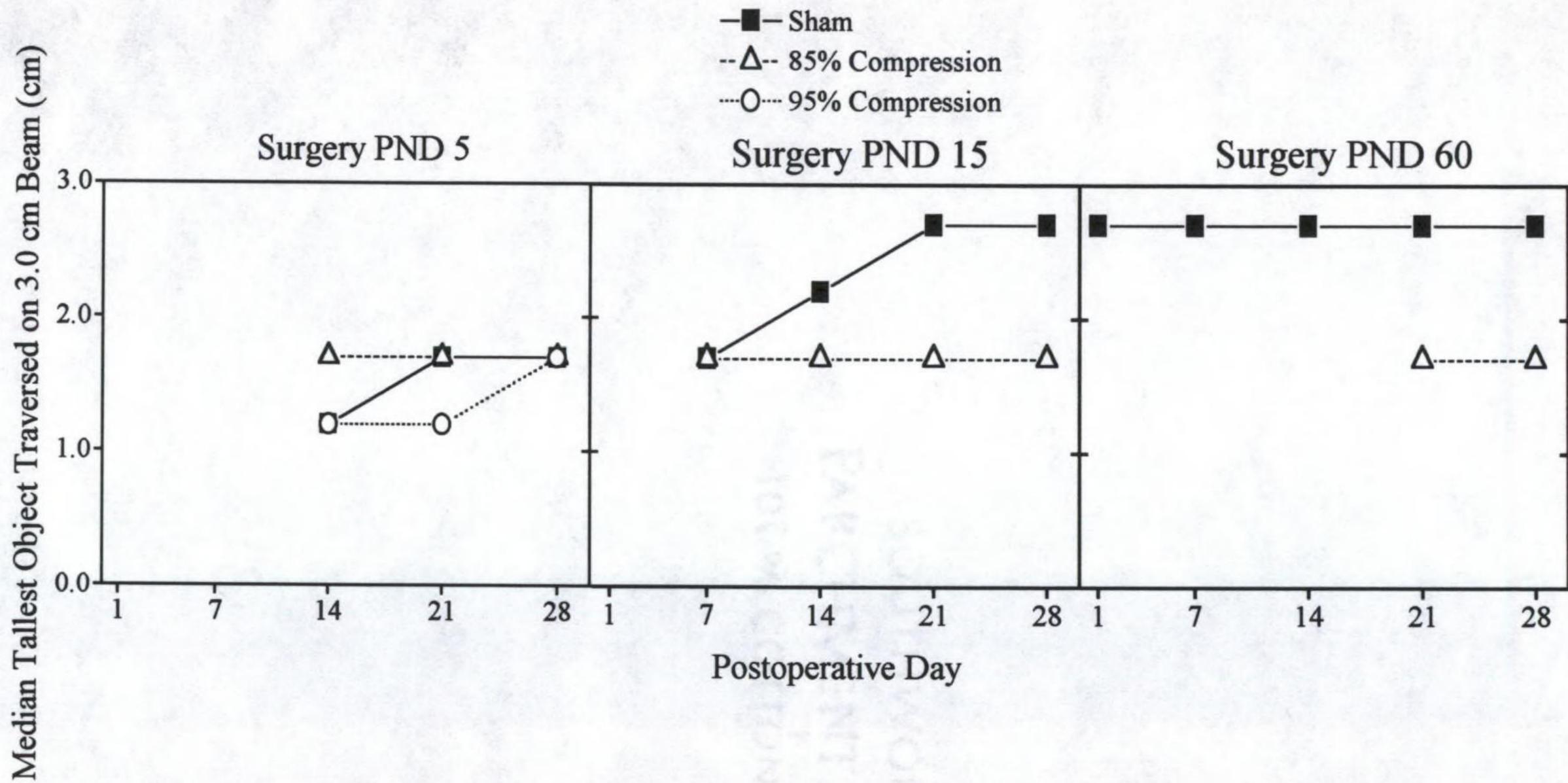


Figure 12. Only rats that successfully traversed the 3.0 cm wide beam with weight-supported steps were tested for obstacle avoidance. This figure depicts the tallest obstacle (0.5, 1.2, 1.7, or 2.7 cm) on the 3.0 cm beam that rats of each experimental group successfully traversed following sham surgery, 85%, or 95% compression of the spinal cord on PND 5, 15, or 60. Only 3 rats of the youngest age group that received 95% compression of the spinal cord and only 3 rats of the oldest age group that received 85% compression of the cord performed this task.

stepped over on PODs 14 [$H(2) = 13.02, p < 0.005$] and 28 [$H(2) = 7.44, p < 0.05$].

Among rats that received the lesser compression, there was no effect of age on the height of the obstacles successfully crossed on POD 14 [$H(1) = 0.86, p > 0.05$] or on PODs 21 and 28 [$Hs(2) < 0.83, ps > 0.05$].

2.2 cm Beam

Only rats that successfully traversed the 3.0 cm wide beam with obstacles were tested for obstacle avoidance on the 2.2 cm wide beam (see Appendix B). Throughout the postoperative period, the maximum height of the obstacles that rats successfully stepped over while traversing the 2.2 cm wide beam was not significantly altered by the compression injury on PND 5 [$Hs(2) < 4.69, ps > 0.05$] or PND 15 [$Hs(1) < 1.08, ps > 0.05$; Fig. 13]. In contrast, rats that received the lesser compression on PND 60 did not step over the tallest two obstacles [$Hs(1) = 6.00, ps < 0.05$]. Only rats that received sham surgery [$H(2) = 9.63, p < 0.01$] or the lesser compression [$H(2) = 12.72, p < 0.005$] on PND 5 stepped over significantly taller obstacles during the postoperative period. On POD 7 when rats that received sham surgery on PND 15 or 60 both traversed the beam, there was an age-related increase in the height of the obstacles that rats successfully stepped over [$H(1) = 9.93, p < 0.005$]. When rats of all three age groups that received sham surgery traversed the beam, there were age-related increases throughout the rest of the postoperative period in the height of the obstacles that rats successfully stepped over [$Hs(2) < 8.54, ps < 0.01$]. Rats that received the lesser compression on PND 15 successfully stepped over taller obstacles on POD 14 than rats that received a similar compression on PND 5 [$H(1) = 9.05, p < 0.005$]. When rats of all three age groups that received the lesser compression traversed the beam, there were no significant effects of

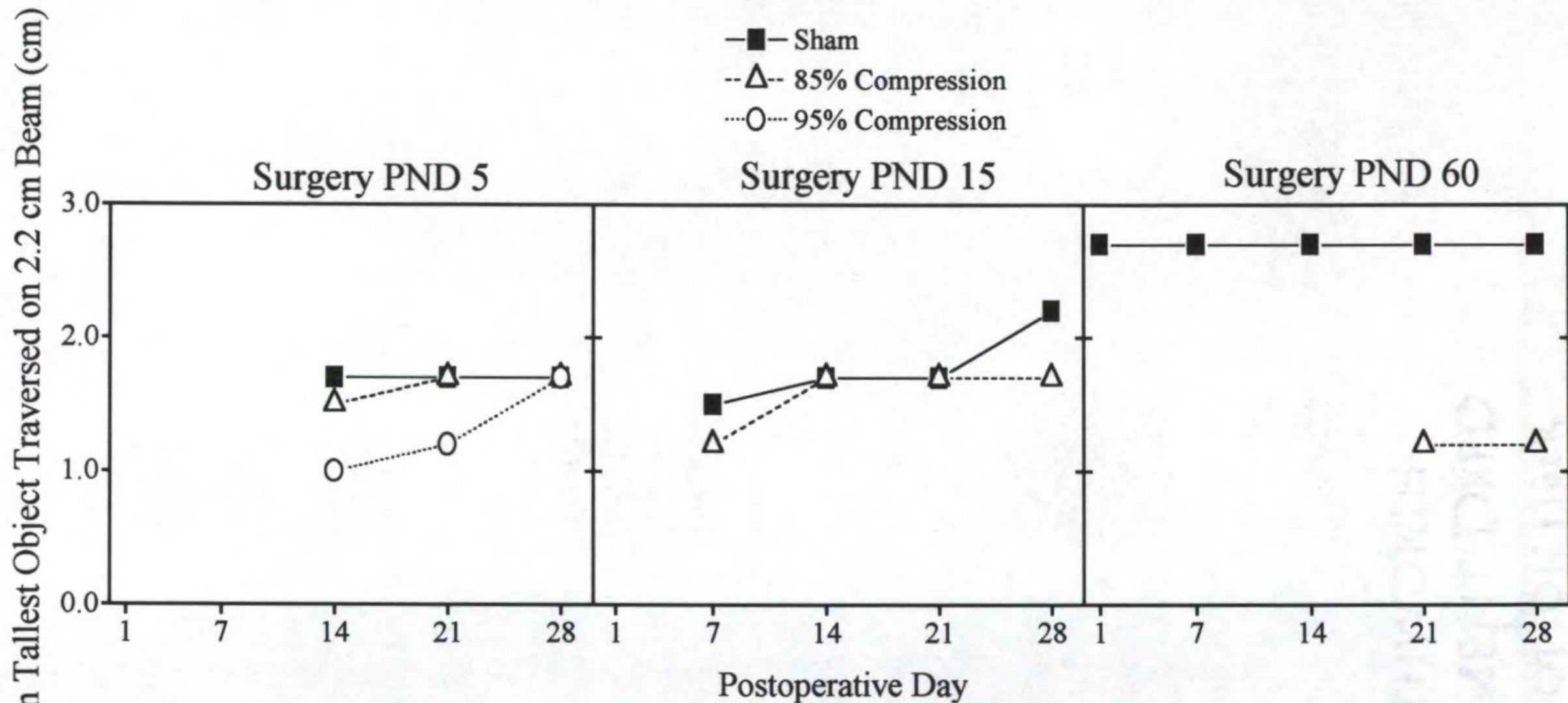


Figure 13. Only rats that successfully traversed the 2.2 cm wide beam with weight-supported steps were tested for obstacle avoidance. This figure depicts the tallest obstacle (0.5, 1.2, 1.7, or 2.7 cm) on the 2.2 cm beam that rats of each experimental group successfully traversed following sham surgery, 85%, or 95% compression of the spinal cord on PND 5, 15, or 60. Only 3 rats of the youngest age group that received 95% compression of the spinal cord and only 3 rats of the oldest age group that received 85% compression of the cord performed this task.

age on the height of the obstacles that rats stepped over while traversing the 2.2. cm beam [$H_s(2) < 2.63$, $p_s > 0.05$].

Parallel Bar Walking

All rats were tested for parallel bar walking, but not all rats successfully traversed the bars. Although rats that received sham surgery or the lesser compression on PND 5 traversed parallel bars between PODs 14 and 28 that were increasingly far apart [$H_s(2) > 8.26$, $p_s < 0.05$], there was no significant effect of surgery when each POD was examined separately [$H_s(2) < 4.03$, $p_s > 0.05$; Fig. 14]. Surgery on PND 15 did significantly affect performance on the parallel bars between PODs 7 and 28 [$H_s(2) < 13.54$, $p_s < 0.005$]. Specifically, rats that received the greater compression on PND 15 did not traverse the parallel bars throughout the postoperative period ($p_s < 0.05$), whereas rats of the other two groups traversed parallel bars that were of similar distances apart ($p_s > 0.05$). Rats of the latter two groups traversed parallel bars throughout the postoperative period that were increasingly far apart [$H_s(4) > 15.02$, $p_s < 0.005$]. Most rats that received a spinal cord compression on PND 60 did not traverse the parallel bars throughout the postoperative period, resulting in significant effects of surgery [$H_s(2) > 11.48$, $p_s < 0.005$]. Rats that received sham surgery on PND 60 crossed parallel bars during the postoperative period that were increasingly far apart [$H(4) = 23.51$, $p < 0.005$].

On POD 7 when rats that had received sham surgery on PND 15 or 60 traversed the parallel bars, there was an age-related increase in the distance between the parallel bars that rats traversed [$H(1) = 7.17$, $p < 0.01$]. The age-related increase in the distance between the parallel bars successfully traversed by rats that received sham surgery

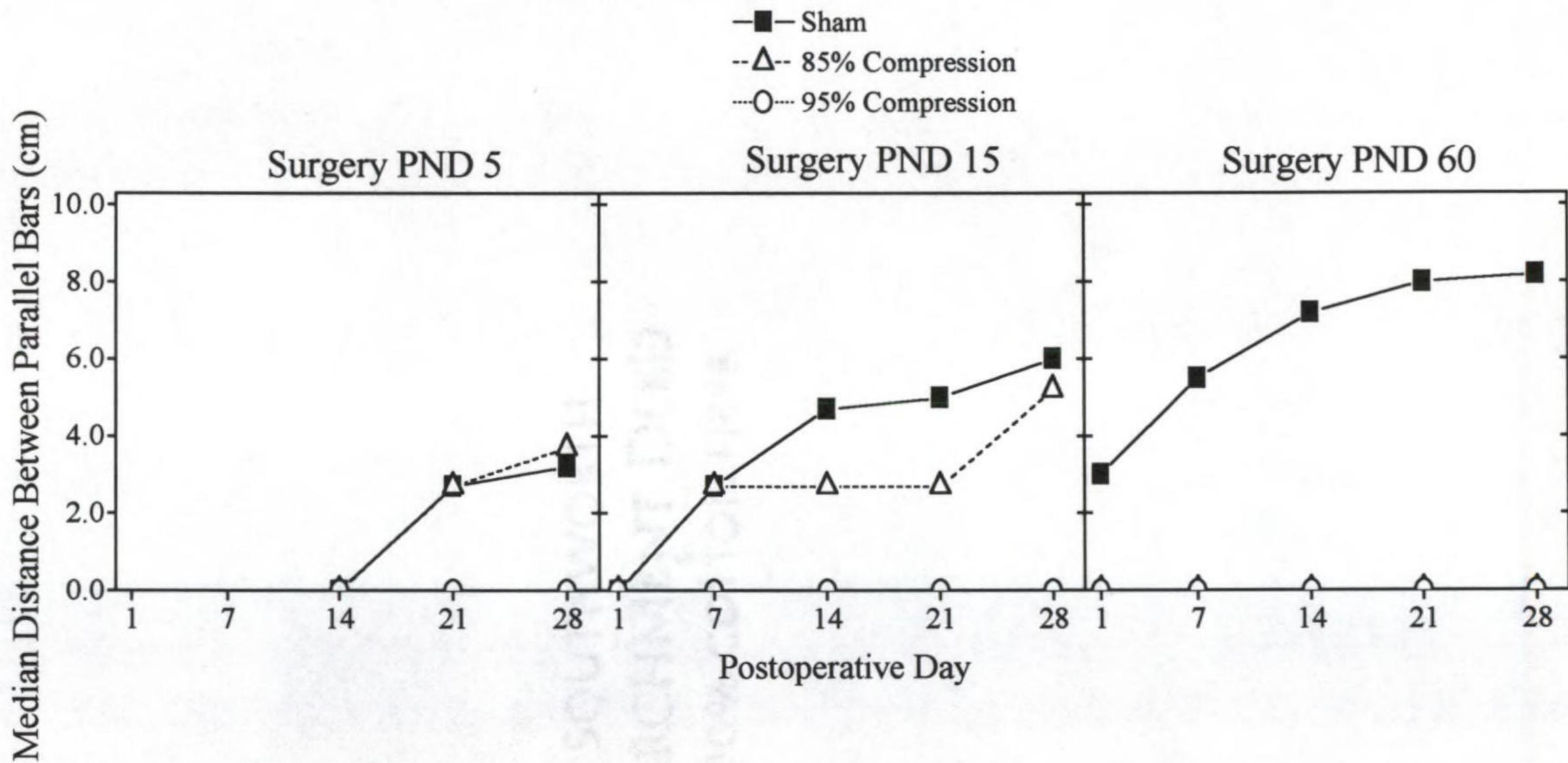


Figure 14. This figure depicts the maximum distance between the parallel bars that was crossed on each postoperative day by rats that received sham surgery, 85%, or 95% compression of the spinal cord on PND 5, 15, or 60.

continued was also evident between PODs 14 and 28 when rats of all three age groups traversed the bars [$H_s(2) > 13.04$, $ps < 0.005$]. Among rats that received the lesser compression, there was an effect of age on the distance between the parallel bars traversed between PODs 14 and 28 [$H_s(2) > 7.17$, $ps < 0.05$]. Specifically, rats that received the lesser compression on PND 15 crossed parallel bars that were further apart on POD 14 than did rats of the other two age groups ($ps < 0.05$). Rats that received the lesser compression on PND 5 or 15 traversed parallel bars on PODs 21 and 28 that were further apart than rats that received similar compressions on PND 60 ($ps < 0.05$), with no significant difference between rats of the former two groups ($ps > 0.05$).

Inclined Plane

Between PODs 14 and 28 for rats that received surgery on PND 5 [$F(2, 21) = 7.55$, $p < 0.01$] and throughout the postoperative period for rats that received surgery on PND 15 [$F(2, 20) = 56.98$, $p < 0.01$], performance on the inclined plane was surgery-dependent (Fig. 15). Rats of both age groups that received sham surgery or the lesser compression maintained balance on a steeper incline than age-matched rats that received the greater compression ($ps < 0.05$), with little difference between the former two groups ($p > 0.05$). Performance on the inclined plane also improved during the postoperative period in rats that received surgery on PND 5 [$F(2, 42) = 28.51$, $p < 0.01$] or 15 [$F(4, 80) = 25.54$, $p < 0.01$]. Following surgery on PND 5 or 15, balance on successively higher inclines was achieved between the first two test sessions (regardless of POD; $ps < 0.05$), with little change thereafter ($ps > 0.05$). Performance on the inclined plane following surgery on PND 60 resulted in significant effects of surgery [$F(2, 20) = 21.40$, $p < 0.01$], POD

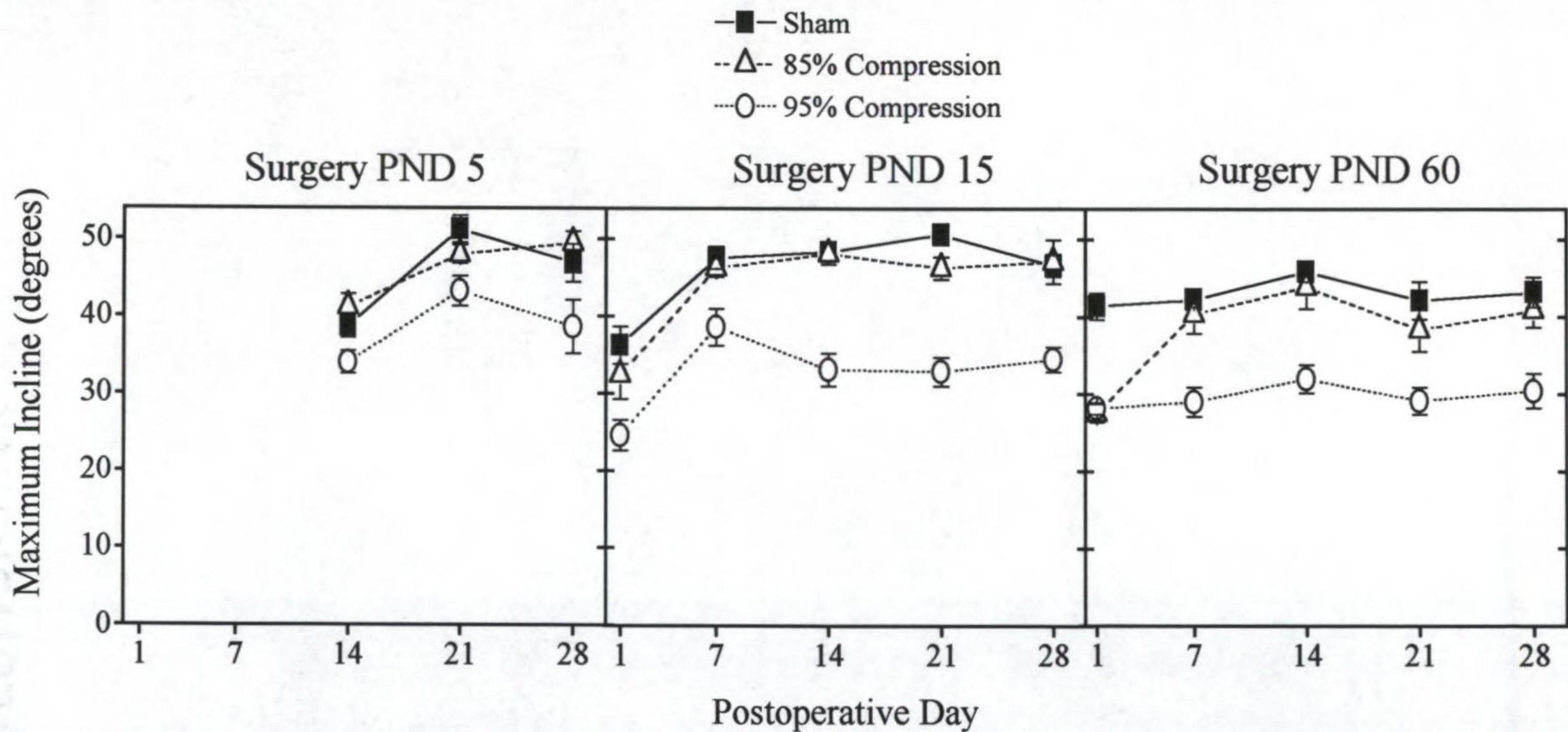


Figure 15. Following sham surgery, 85% compression, or 95% compression on postnatal days 5, 15, or 60, rats were tested throughout the 4-week postoperative period for the maximum degree of incline (\pm SEM) that the rats maintained posture and balance for at least 5 s on the inclined plane.

[$F(4, 80) = 10.85, p < 0.01$], and surgery x POD [$F(8, 80) = 3.56, p < 0.05$]. Specifically, the maximum incline on which rats of that age group maintained their balance was higher throughout the postoperative period in rats that received sham surgery than in rats that received the greater compression ($ps < 0.05$). In contrast, rats that received the lesser compression, performed similarly to rats that received the greater compression on POD 1 ($p > 0.05$), but were similar to rats that received sham surgery throughout the remainder of the testing period ($ps > 0.05$).

The maximum degree of incline on which rats maintained balance and position throughout the postoperative period was dependent on the age of the rat at the time of surgery. Age-related changes in performance on the inclined plane following sham surgery on PND 15 or 60 resulted in a significant effect of age x POD [$F(4, 56) = 5.85, p < 0.01$]. On POD 1, rats that received sham surgery on PND 15 maintained balance on a shallower incline than rats that received the surgery on PND 60 ($p < 0.05$), whereas the opposite was true on PODs 7 and 21 ($ps < 0.05$). An effect of age was also noted between PODs 14 and 28 when rats of all three ages were tested on the inclined plane [$F(4, 42) = 8.39, p < 0.01$]. Specifically, rats that received sham surgery on PND 5 maintained balance on a shallower incline on POD 14 than rats of the other two age groups ($ps < 0.05$). Rats of the youngest two age groups maintained position on a higher incline on POD 21 than rats of the oldest age group ($ps < 0.05$), with little difference between the former two groups ($ps > 0.05$). Rats that received the lesser compression on PND 15 or 60 maintained balance and position on a lower incline on POD 1 than on any other day [$F(4, 52) = 24.07, p < 0.01$], but rats that received the lesser compression on PND 15 generally maintained position and balance on a higher incline than rats that

received a similar compression on PND 60 [$F(1, 13) = 6.46, p < 0.05$]. When rats of all three ages were tested on the inclined plane, performance resulted in a significant age x POD interaction [$F(4, 40) = 4.18, p < 0.01$]. Except on POD 14 when rats that received the lesser compression on PND 15 maintained balance on a higher incline than rats that received a similar compression on PND 5 ($ps < 0.05$), rats of those two age groups maintained position on a higher incline than rats of the oldest age group ($ps < 0.05$), with little difference between the former two groups ($ps > 0.05$). Following the greater compression on PND 15 or 60, performance on the inclined plane resulted in a significant effect of age x POD [$F(4, 52) = 3.15, p < 0.05$]. Specifically, rats that received the greater compression on PND 15 maintained position and balance on a higher incline on POD 7 ($p < 0.05$), than rats that received a similar compression on PND 60, with little difference throughout the rest of the postoperative period ($ps > 0.05$). Among rats of all three age groups, performance on the inclined plane following the greater compression resulted in a significant effect of age x POD [$F(4, 40) = 3.13, p < 0.05$]. Rats that received the greater compression on PND 5 maintained balance on a steeper incline on POD 21 than rats of the other two age groups ($ps < 0.05$) and on POD 28 than rats of the oldest age group ($p < 0.05$). Because absolute weight [$F(2, 61) = 0.34, p > 0.05$] and relative weight gain [$F(2, 61) = 1.74, p > 0.05$] of the rats were not significantly affected by surgery, decreased performance on the elevated beams and the inclined plane by rats that received midthoracic spinal cord compression was not due to surgery-related differences in the weights of the rats (see Appendix C).

Hot Plate

In rats of all three groups that received surgery on PND 5, latencies to withdraw or to lick one hindpaw resulted in significant effects of POD [$F(4, 84) = 144.40, p < 0.01$] and surgery \times POD [$F(8, 84) = 4.72, p < 0.01$; Fig. 16]. Except for rats that received the greater compression, latencies to withdraw one hindpaw increased between PODs 1 and 7 for rats that received the lesser compression or sham surgery on PND 5 ($ps < 0.05$), with no significant difference between those two groups ($ps > 0.05$). Latencies to lick one hindpaw decreased significantly between PODs 14 and 28 for rats of those two surgery groups ($ps < 0.05$), with no difference between surgery groups ($ps > 0.05$). In contrast, latencies to withdraw one hindpaw were shorter on POD 7 ($ps < 0.05$) and latencies to lick one hindpaw were longer on POD 28 ($ps < .05$) for rats that received the severe compression, relative to rats of the other two surgery groups. Latencies to lick one hindpaw following surgery on PND 15 resulted in significant effects of surgery [$F(2, 20) = 54.28, p < 0.01$], POD [$F(4, 80) = 38.25, p < 0.01$], and surgery \times POD [$F(8, 80) = 10.52, p < 0.01$]. Hindpaw licking on the hot plate did not emerge during the 30 s time limit in rats of that age group until POD 7, at which time lick latencies were longer in rats that received the spinal cord compression than in rats that received sham surgery ($ps < 0.05$). Latencies to lick one hindpaw then decreased during the postoperative period for rats that received the lesser compression or sham surgery ($ps < 0.05$), with no difference them ($ps > 0.05$). In contrast, rats that received the greater compression on PND 15 never licked the hindpaws within the 30 s time limit ($ps < 0.05$). Latencies to lick one hindpaw following surgery on PND 60 also resulted in significant effects of surgery [$F(2, 20) = 78.89, p < 0.01$], POD [$F(4, 80) = 6.29, p < 0.01$], and surgery \times POD [$F(8, 80) = 2.56,$

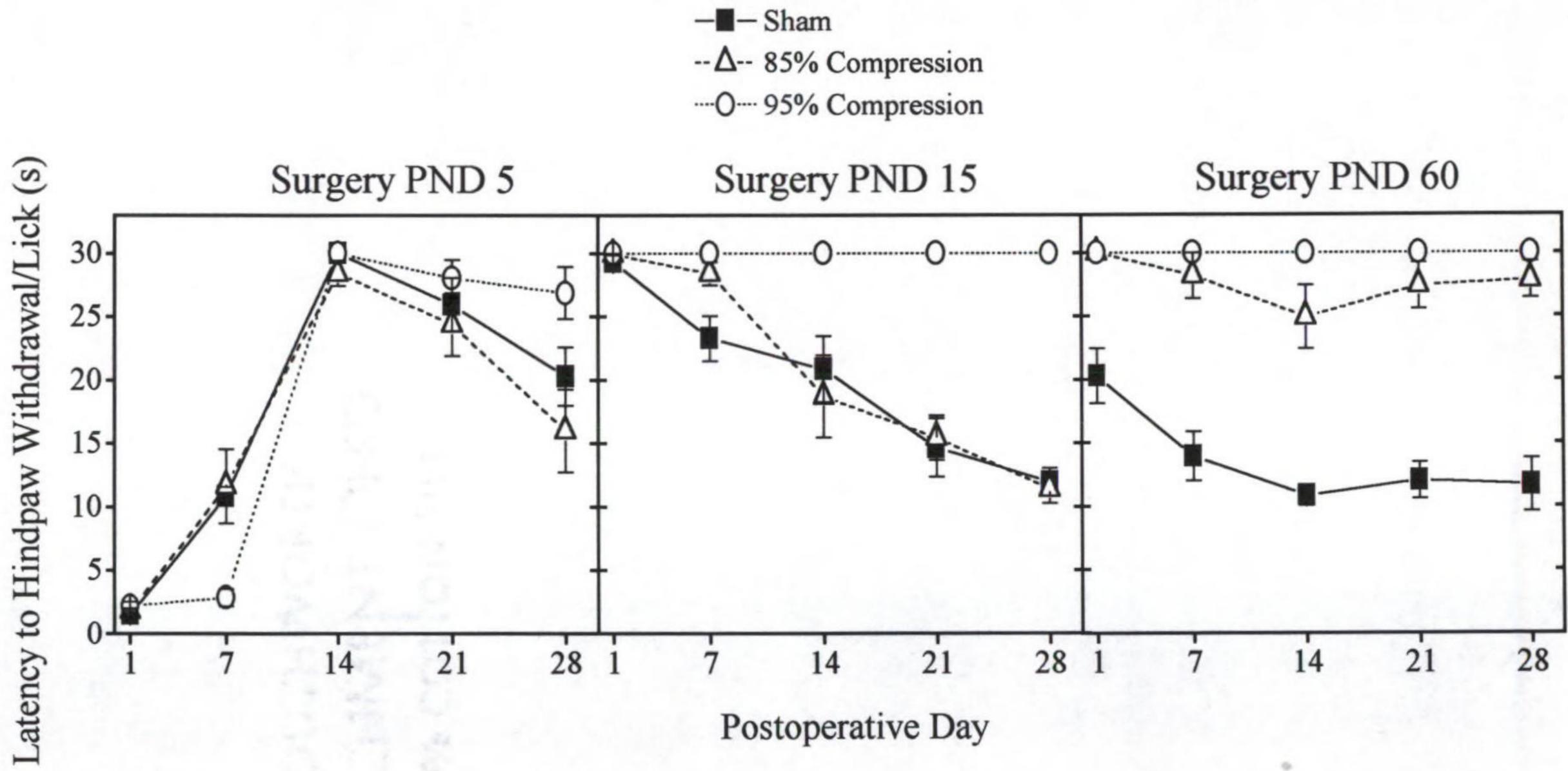


Figure 16. Following sham surgery, 85%, or 95% compression of the spinal cord on postnatal day 5, 15, or 60, rats were primarily tested throughout the 4-week postoperative period for the latency (\pm SEM) to lick one of the hindpaws on a hot plate. Data on postoperative days 1 and 7 of rats of the youngest age group represent the latencies (\pm SEM) to withdraw one hindpaw from the surface of the hot plate.

$p < 0.05$]. Latencies to lick one hindpaw were much longer throughout the postoperative period in rats of that age group that received a spinal cord compression than in rats that received sham surgery ($ps < 0.05$), with little difference between the two compression groups ($ps > 0.05$). Lick latencies decreased between PODs 1 and 7 in young-adult rats that received sham surgery ($p < 0.05$) with little change thereafter ($ps > 0.05$).

Performance on the hot plate also resulted in a significant age x POD interaction for rats that received sham surgery [$F(8, 84) = 33.02, p < 0.001$], the lesser compression [$F(8, 80) = 25.95, p < 0.001$], or the greater compression [$F(8, 80) = 145.48, p < 0.001$]. On PODs 1 and 7, lick latencies were shorter rats that received sham surgery on PND 60 than on PND 15 ($ps < 0.05$). When lick latencies were assessed in rats of all three age groups, lick latencies were increasingly short on each POD with increased age of the rat ($ps < 0.05$), with little difference on PODs 21 and 28 between rats that received sham surgery on PND 15 or 60 ($ps > 0.05$). On PODs 1 and 7, there were no significant differences in the latencies to lick one hindpaw by rats that received the lesser compression on PND 15 or 60 ($ps > 0.05$). However, lick latencies of rats that received the lesser compression on PND 15 decreased throughout the rest of the postoperative period and were generally shorter than lick latencies of rats of the other two age groups that received the lesser compression ($ps < 0.05$). Except on POD 28 when rats that received the lesser compression on PND 5 licked the hindpaws sooner than rats that received the injury on PND 60 ($p < 0.05$), lick latencies of rats of those two age groups were not different from each other ($ps > 0.05$). Except on POD 28 in rats of the youngest age group ($ps < 0.05$), rats that received the greater compression of the spinal cord did not lick the hindpaws within the 30 s time limit ($ps > 0.05$).

Lesion Size

The rostral-most lesion was located between vertebral T₄-T₅ and the caudal-most lesion was located between vertebral T₈-T₉. Except for rats that received the greater compression on PND 5 [$R = -0.72$, $p < 0.05$], hindlimb sensorimotor function on POD 28 was not dependent on lesion location [$-0.13 < R_s < 0.38$, $p_s > 0.05$; Fig. 17]. Although lesion length was not quantified, no lesion extended further than two segments rostral to, or caudal from, the lesion epicenter. The lesion epicenter was characterized by preferential loss of spinal cord gray matter, with the amounts of gray and white matter present depending on severity of compression (Figs. 18, 19, & 20). Generally, there was almost complete loss of central gray matter, accompanied by a small central cavitation, in rats that received the lesser compression on PND 5 or 15. When age-matched rats received the greater compression injury, there was complete elimination of central gray matter and significant degeneration of white matter. In those rats, the white matter generally consisted either of a thin strip or a thin ring of tissue that ranged in thickness between 12.5 μm and 350 μm or between 8.25 μm and 175 μm for rats that received the greater compression on PND 5 or 15, respectively. Following compression of the 60-day-old spinal cord, the lesion epicenter of rats of both injury groups was largely filled with fibrous scar tissue and contained little CNS tissue that was not undergoing Wallerian degeneration.

Although there was significant spinal cord damage in rats of both compression groups, the cross-sectional area of the tissue at the lesion epicenter resulted in a significant age x surgery interaction [$F(4, 61) = 41.48$, $p < 0.001$; Fig. 21]. Following the lesser spinal cord compression, the amount of tissue at the lesion epicenter was

Individual Locomotor Ratings on POD 28 (BBB)

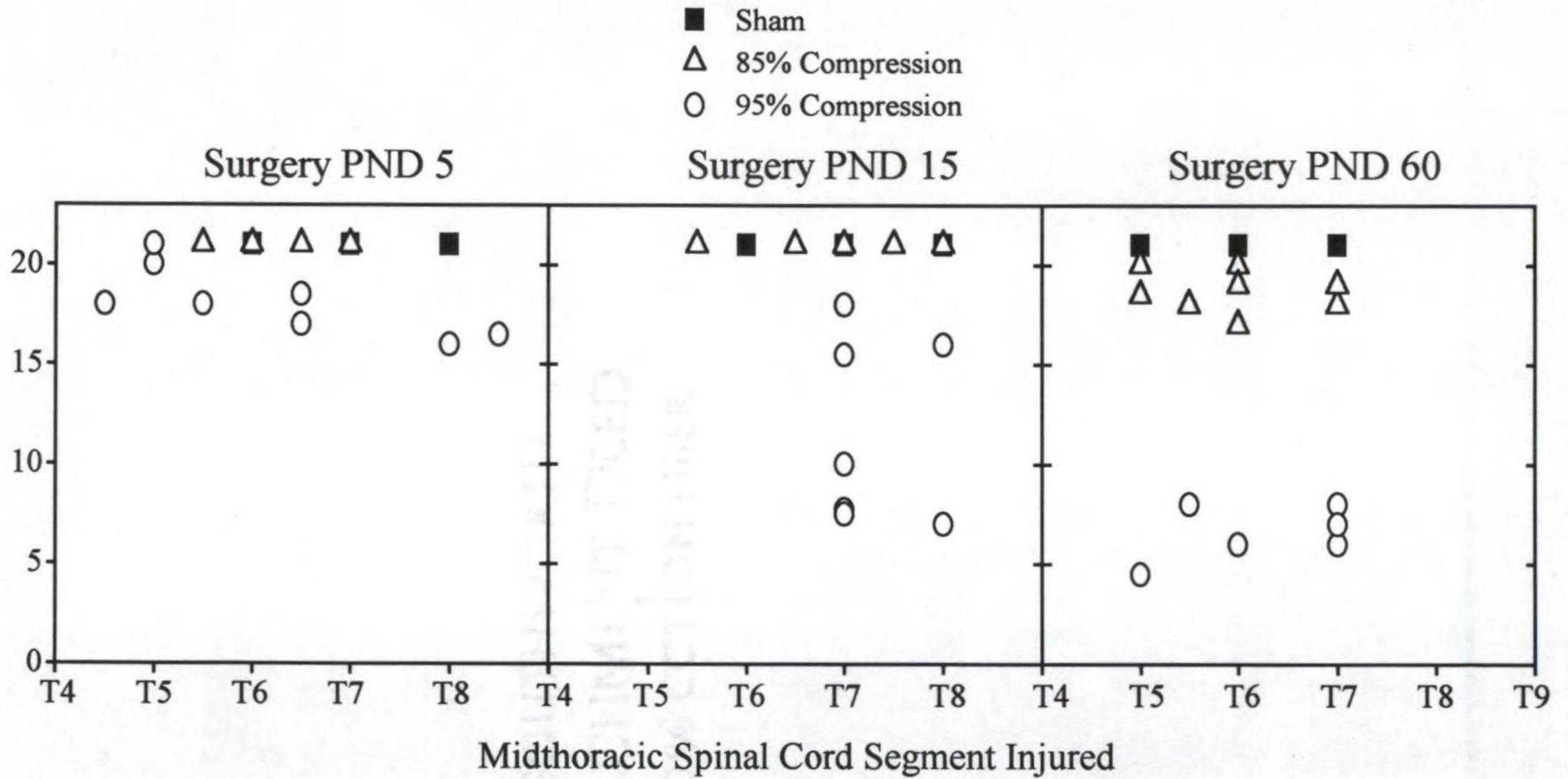


Figure 17. Ratings of overground locomotion on postoperative day 28 are depicted as a function of the midthoracic spinal cord segment that contained the lesion epicenter for each rat that received sham surgery, the 85% compression, or the 95% compression on postnatal day 5, 15, or 60. A score of 21 represents the overground walking of an uninjured, adult rat.



Figure 18. Representative photomicrographs of the lesion epicenter, 29 days following surgery to the midthoracic spinal cord of 5-day-old rats. Calibration bar = 500 μm .

A) sham; B) 85% compression; C) 95% compression.

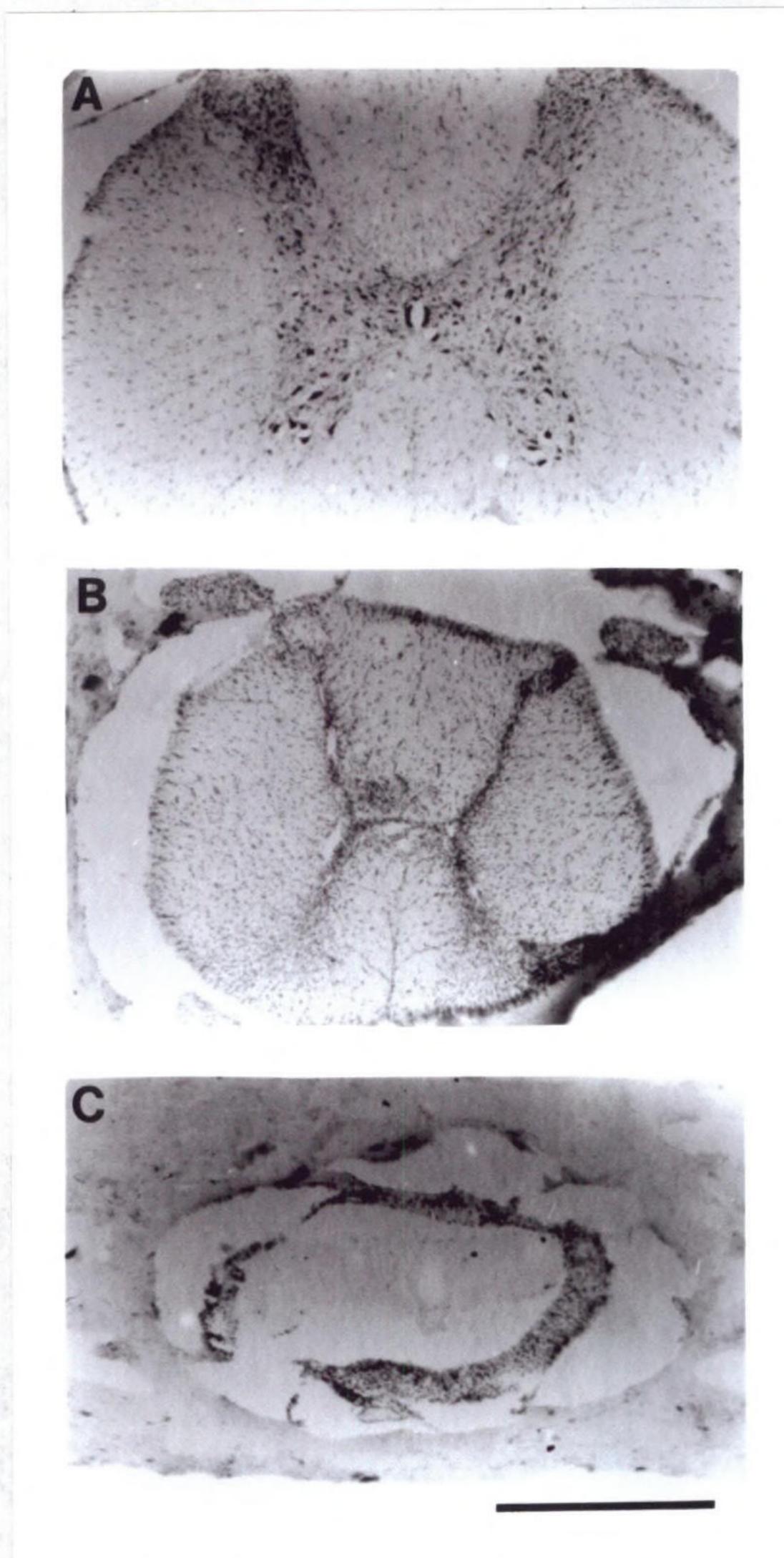


Figure 19. Representative photomicrographs of the lesion epicenter, 29 days following surgery to the midthoracic spinal cord of 15-day-old rats. Calibration bar = 500 μ m. A) sham; B) 85% compression; C) 95% compression.

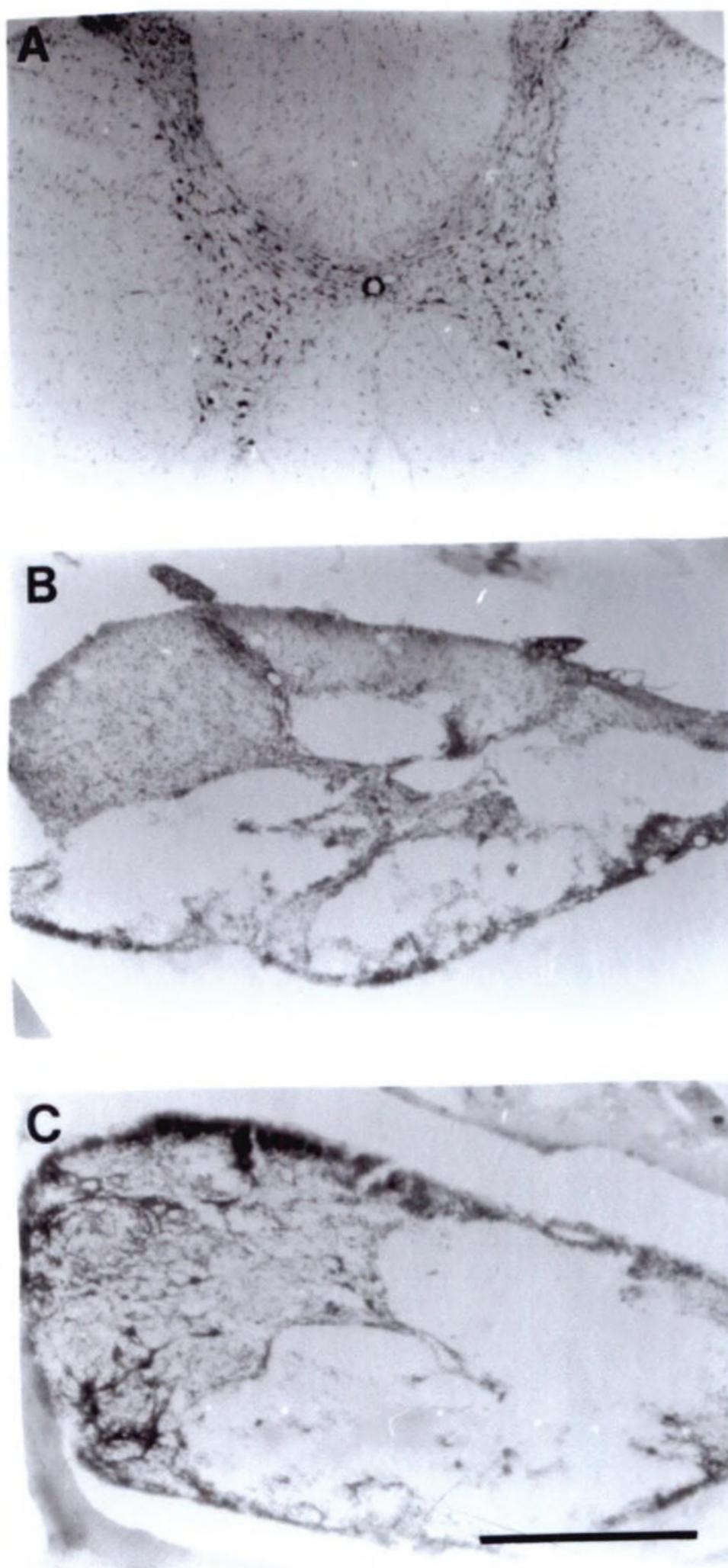


Figure 20. Representative photomicrographs of the lesion epicenter 29 days following surgery to the midthoracic spinal cord of 60-day-old rats. Calibration bar = 500 μm .

A) sham; B) 85% compression; C) 95% compression.

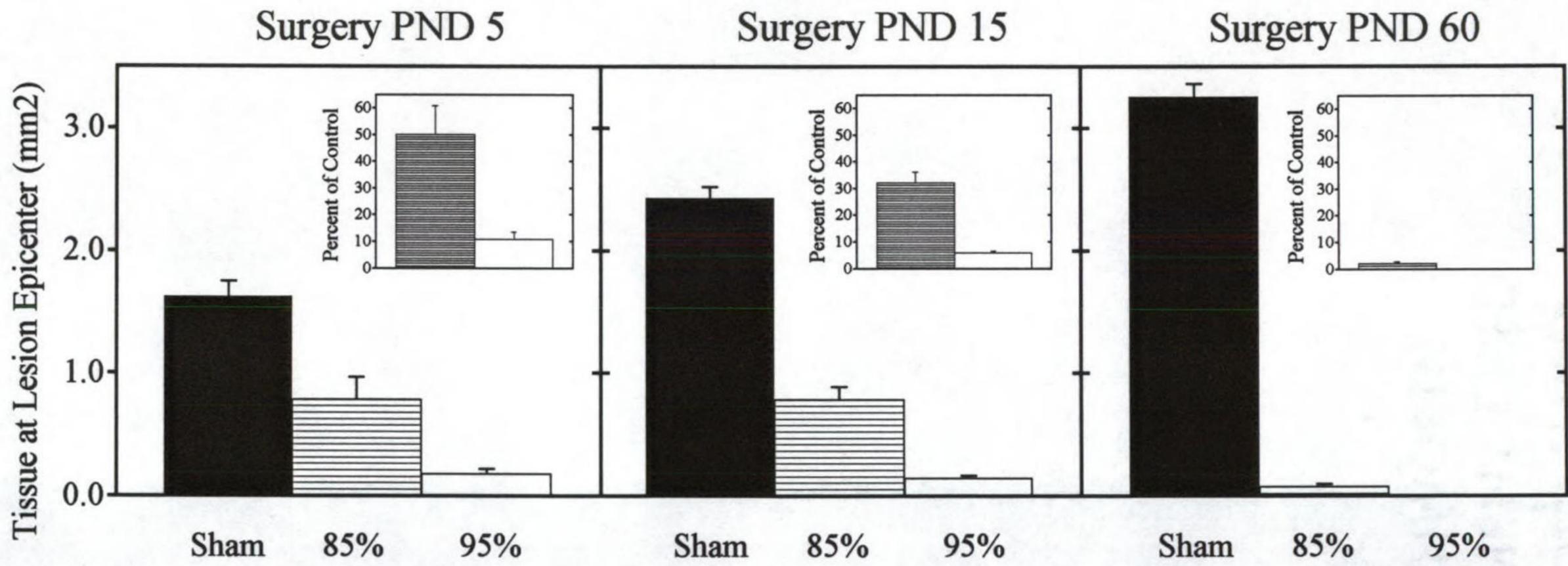


Figure 21. This figure depicts the amount of spinal cord tissue at the lesion epicenter (\pm SEM) following sham surgery, 85% compression, or 95% compression on postnatal day 5, 15, or 60. The cross-sectional area of the spinal cord increased with age. Therefore, the amount of tissue at the lesion epicenter of rats that received 85% or 95% compression of the spinal cord was also calculated as a percentage of the size of the spinal cord of age-matched controls (insets).

significantly decreased in rats of the oldest age group than in rats of the other two age groups ($p_s < 0.05$). However, there was no significant difference in the amount of tissue at the lesion epicenter of rats of the three age groups that received the greater compression ($p_s > 0.05$). Within each injury group, there was little difference in the amount of tissue at the lesion epicenter of rats that received the compression as neonates ($p_s > 0.05$). Spinal cord size of rats that received sham surgery increased during ontogeny ($p_s < 0.05$). When the absolute amount of tissue at the lesion epicenter was expressed as a percentage of control tissue size, the percentage of tissue at the lesion epicenter was also larger in rats that received the lesser compression than in rats that received the greater compression [$F(1, 40) = 31.64, p < 0.001$]. However, the age x surgery interaction was also significant [$F(2, 40) = 7.25, p < 0.01$]. Subsequent analyses revealed that the percentage of tissue at the lesion epicenter decreased with increased severity of compression in rats that received surgery on PND 5 or 15 ($p_s < 0.05$), but did not significantly differ between the two groups that received a spinal cord compression on PND 60 ($p > 0.05$). The percentage of tissue at the lesion epicenter decreased with increased age in rats that received the lesser compression ($p_s < 0.05$). However, there was no significant difference in the percentage of tissue at the lesion epicenter of rats of the three age groups that received the greater compression ($p_s > 0.05$). Except for rats that received the greater compression on PND 15 [$R = 0.77, p < 0.05$], the amount of spinal cord tissue at the lesion epicenter was not dependent on the spinal cord level [$-0.53 < R_s < 0.33, p_s > 0.05$; Fig. 22].

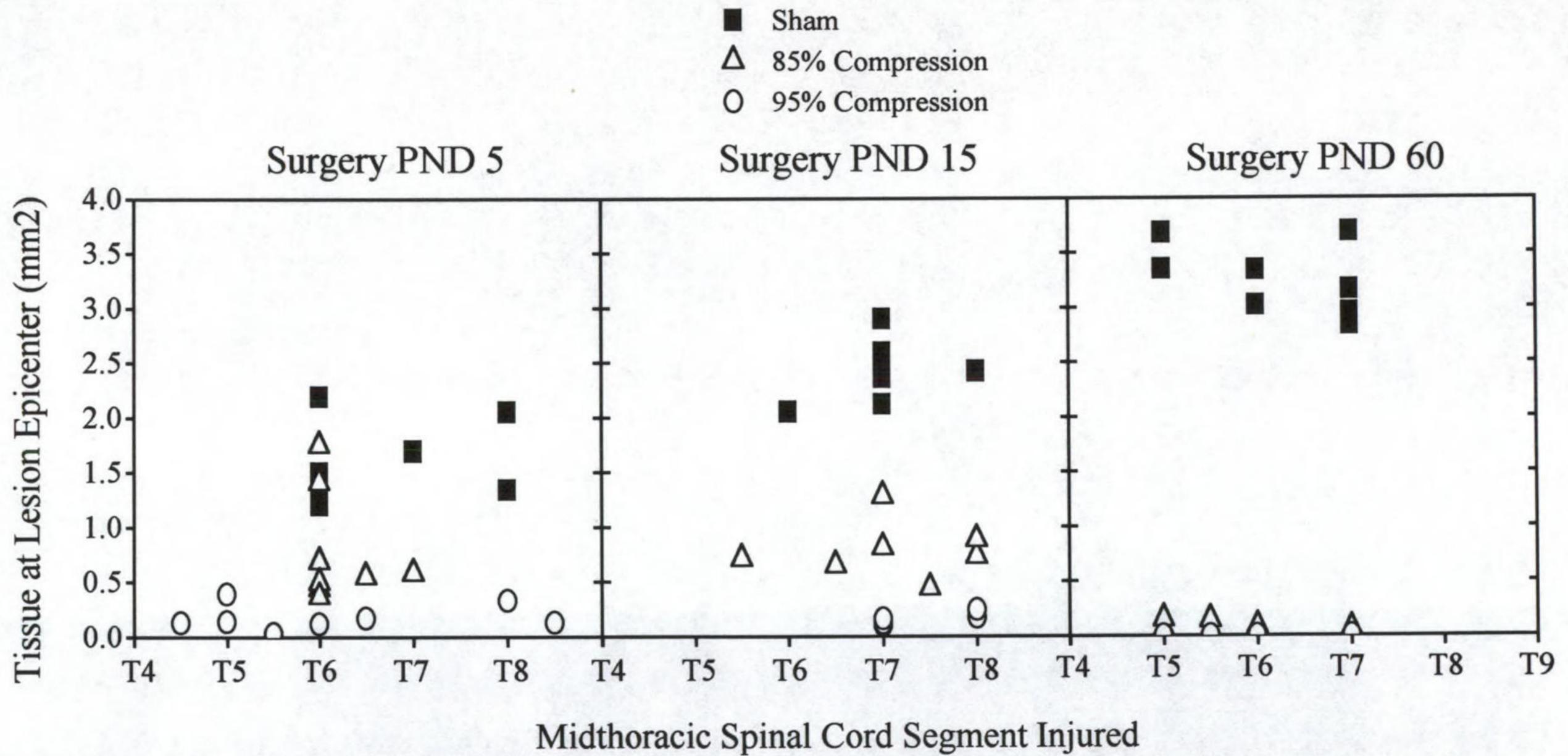


Figure 22. This figure shows the amount of spinal cord tissue at the lesion epicenter, for individual rats that received sham surgery, the 85% compression, or the 95% compression, as a function of the midthoracic spinal cord segment that was injured on postnatal day 5, 15, or 60.

CHAPTER 4 DISCUSSION

Because the spinal cord of adult humans is rarely transected during injury, animal models of incomplete SCI have been developed to elucidate the behavioral and neuroanatomical sequelae postinjury. Although human infants are not exempt from traumatic SCI, the effects of incomplete injury to the developing spinal cord have not been thoroughly investigated. Instead, most knowledge regarding the effects of SCI during development has been obtained from infant animals with partial or complete spinal cord transections. The consequences of injury to the neonatal central nervous system are not necessarily the same as the consequences of injury to the mature nervous system (see Kolb, Holmes, & Whishaw, 1987; Kolb & Whishaw, 1985; Yager, Shuaib, & Thornhill, 1996; Stelzner et al., 1975; Weber & Stelzner, 1977). Recovery processes invoked following traumatic injury to the immature nervous system act on a background of processes important for normal growth and development. For those reasons, efficacy of treatments designed for adults following traumatic SCI may not be the same in infants and obligates elucidation of the behavioral, neuroanatomical, and neurophysiological effects of pediatric SCI. The first aim of the present experiments was to quantify age-related changes in the behavioral and neuroanatomical consequences of midthoracic spinal cord compression. Rating scales are commonly used with adult animals with SCIs to describe locomotor function during overground walking. However, ratings of overground walking provide only a subjective assessment of gross locomotor skill.

Therefore, the second aim of the present experiments was to compare ratings of overground walking with performance on a quantitative battery of tasks that challenge hindlimb sensorimotor function postinjury. Results of the present experiments suggest (1) an age-dependent decrease in hindlimb sensorimotor function following midthoracic spinal cord compression, (2) that ratings of overground locomotion do not accurately depict deficits in hindlimb function postinjury, and (3) that numerous tests should be employed to fully characterize injury-related sensorimotor deficits.

Age-Dependent Effects of Midthoracic Spinal Cord Compression

In adult animals, one factor that contributes to neurologic outcome following SCI is the amount of compression that the spinal cord undergoes during the traumatic event (Gruner et al., 1996). Elucidation of age-related changes in the behavioral and neuroanatomical effects of midthoracic spinal cord compression is complicated by an age-dependent increase in the size of the spinal cord. Therefore, spinal cords of rats of the present experiments were compressed by a percentage of the uncompressed width of the spinal cord to produce compressions of the same relative amount in rats of the three age groups. Regardless of age, increasing the amount of compression decreased hindlimb function on the sensorimotor battery of tasks. Although spinal cords of rats of the present experiments were compressed by the same relative amount (within an injury group) during ontogeny, hindlimb sensorimotor function postinjury decreased with increased age of the rat. Previous reports with adult animals suggest that there is preferential degeneration of spinal cord gray matter following traumatic SCI and that the amount of white matter spared depends on the severity of the injury (e.g., Blight, 1983; Noble &

Wrathall, 1985). Furthermore, the quality of hindlimb sensorimotor function is proportional to the amount of tissue spared at the lesion epicenter (e.g., Basso et al., 1996; Noble & Wrathall, 1985). In the present experiments, the amount of tissue at the lesion epicenter decreased with increased compression of the spinal cord in rats of the youngest two age groups. However, there was no significant difference in the amount of tissue at the lesion epicenter between the two groups of rats that received spinal cord compression on PND 60. Furthermore, an age-related decrease in the amount of tissue at the lesion epicenter was found only among the three groups of rats that received the lesser compression. Thus, results of the present experiments do not completely support a direct relationship between the amount of tissue at the lesion epicenter and the quality of hindlimb sensorimotor function.

Performance on the sensorimotor battery of tasks of rats of the present experiments that received the lesser compression on PND 5 was indistinguishable from sham controls throughout the postoperative period. Performance of rats that received the lesser compression on PND 15 was only marginally different from sham controls during beam walking. Rats of that age group that received the injury had a higher percentage of hindlimb slips during beam walking than did age-matched controls. Those rats were also more likely to traverse the beams with all four feet in stance than age-matched controls, which would afford rats that received the compression greater stability during beam walking. Speed of walking across the beams was not measured in the present experiments because forward progression by rats of all ages was interrupted by pauses. Footfall patterns vary with speed of locomotion (Hildebrand, 1989), so greater use of a 4-limb footfall pattern by rats that received the lesser compression on PND 15 may reflect a

slower walking speed on the beam, relative to sham controls. Because rats often paused with all four feet on the beam following a slip or fall, elevations in the percentage of footfalls with 4 limbs in stance may be confounded by the injury-related increase in the percentage of hindlimb slips.

Despite few changes to sensorimotor functioning in rats that received the lesser compression on PND 5 or 15, midthoracic gray matter had completely degenerated in most rats and, in some cases, resulted in the formation of a small central cavity. The cross-sectional area of tissue at the lesion epicenter decreased between rats of these two age groups. Degeneration of midthoracic gray matter would mainly cause loss of sensory input from a few dermatomes and loss of interneurons and motoneurons important for control of axial muscles or for autonomic functions. Therefore, few alterations to hindlimb sensorimotor function would be expected with minimal impingement of the lesion on white matter tracts. In support of this notion, kainic acid, which selectively kills neurons without damaging fibers of passage (e.g., Coyle & Schwarcz, 1976), produces minor locomotor deficits in ratings of overground walking when injected into the midthoracic spinal cord of rats (Magnuson, Trinder, Zhang, Burke, Morassutti, & Shields, 1999). Thus, the failure of all behavioral tasks of the present experiments to clearly distinguish between rats that received the lesser compression or sham surgery on PND 5 suggests that descending, ascending, propriospinal, and intraspinal systems of rats that received the lesser compression were functioning in a near-normal capacity. Because the corticospinal tract is important for accuracy of limb placement during walking (Hicks & D'Amato, 1975), elevations in the percentage of hindlimb slips during beam walking by rats that received the lesser compression on PND 15 suggests that the

spinal cord lesion in those rats impinged upon axons of that descending, supraspinal system.

In contrast to the effects of injury to the neonatal spinal cord, behavioral performance of rats that received the lesser compression on PND 60 was severely disrupted on all tasks, except overground walking and the inclined plane. Rats of that experimental group suffered an initial loss of hindlimb joint movement and weight support following spinal cord compression. Hindlimb weight support, stepping, and coordination between brachial and pelvic girdles dramatically improved in those rats during the first postoperative week. By the end of the postoperative period, there were only deficiencies in foot position at lift off, tail position, and trunk stability. Except for an initial deficit on POD 1, performance of those rats on the inclined plane was also similar to sham controls throughout the remainder of the postoperative period.

As expected from the behavioral findings, the percentage of tissue at the lesion epicenter was smaller in rats that received the lesser compression on PND 60 than in rats that received a similar compression on PND 5 or 15. In fact, there was almost complete loss of all CNS tissue at the lesion epicenter of rats of the oldest age group. The spinal cord cavity mainly contained astrocytic scarring and the small patches of CNS tissue that remained were undergoing Wallerian degeneration. However, it is likely that there were functional axons coursing through this necrotic tissue that contributed to hindlimb motor function following compression of the 60-day-old spinal cord. Although cats can be trained to support the weight of the hindquarters following spinal cord transection (see Hodgson, Roy, de Leon, Dobkin, & Edgerton, 1994; Lovely, Gregor, Roy, & Edgerton, 1986), rats that receive no special training following spinal cord transection do not use

the hindlimbs for weight support (Weber & Stelzner, 1977). Pathways of the ventral and ventrolateral spinal cord seem to be important for locomotor control (see Eidelberg, 1981; Eidelberg, Story, Walden, & Meyer, 1981; Steeves & Jordan, 1980). Lesions of the motor cortex of rats causes the limbs to slip off of beams, whereas walking over a flat surface is not disrupted (Hicks & D'Amato, 1975). More recent evidence shows that animals walk with the hindlimbs following complete transection of the ventral and ventrolateral tracts, but hindlimb posture and weight support is severely reduced (for review see Rossignol, Chau, Brustein, Belanger, Barbeau, & Drew, 1996). The initial loss of hindlimb weight support and stepping in rats of the oldest age group was probably due to spinal shock. The mature spinal cord is dependent on inputs from descending systems for normal functioning (see Weber & Stelzner, 1977). Spinal shock is a decreased responsivity of motor circuits following denervation and may be due, at least in part, to overactivity of intraspinal inhibitory system (see Robinson & Goldberger, 1986b; Simpson, Robertson, & Goodman, 1993). Recovery of overground walking in rats that received the lesser compression on PND 60 suggests that some fibers of tracts of the ventral and ventrolateral spinal cord survived the injury and contributed to overground walking. However, it is important to note the importance of sensory influences on motor function (see Delcomyn, 1980). Stretching of the skin during overground walking may have indirectly activated locomotor circuits caudal to the lesion, which could enhance locomotor function. Although such mechanisms would aid locomotor function in rats of all age and injury groups, the effect may be more dramatic in rats in which the amount of tissue at the lesion epicenter was severely reduced, which presumably reflects severe impairment in communication between supraspinal and spinal sensorimotor systems.

Increased sparing of neurons of the rubrospinal (Fehlings & Tator, 1995; Midha, Fehlings, Tator, Saint-Cyr, & Guha, 1987), vestibulospinal, reticulospinal, and raphespinal (Midha et al., 1987) systems have been correlated with higher scores on the inclined plane. Because performance on the inclined plane was largely intact in rats of this experimental group, hindlimb sensorimotor performance following the lesser compression on PND 60 was likely influenced by contributions from those supraspinal, descending systems. In contrast to the near-normal performances during overground walking and on the inclined plane, only 3 rats that received the lesser compression on PND 60 walked on the elevated beams or parallel bars by PODs 21 and 28. Severe impairments in beam- and parallel bar walking suggest that the spinal cord lesion disrupted fibers of the corticospinal tract because performance on those tasks require greater accuracy of limb placement than does overground walking. In addition, most rats that received the lesser compression on PND 60 did not lick the hindpaws within the 30 s time limit on the hotplate. Nociceptive pathways for cutaneous pain largely ascend in the ventrolateral spinal cord (Willis & Westlund, 1997) and forebrain circuits are required for the hindpaw lick response on the hotplate (Woolf, 1984). Therefore, results of the present experiments suggest functional disruption of ascending nociceptive pathways or descending motor pathways important for that behavioral response.

In rats of all three age groups that received the greater compression of the spinal cord, performance on all behavioral tasks was disrupted. Ratings of overground walking most clearly illustrate an age-related decrease in sensorimotor function in these rats. Overground walking of rats of the youngest age group was rated near-normal by the end of the postoperative period. However, those rats retained deficiencies in foot position at

lift off, tail position, and trunk stability. Rats that received a similar compression on PND 15 were also deficient for toe clearance during the swing phase of the step cycle. Following compression of the 60-day-old spinal cord, the hindquarters of most rats no longer supported weight and the hindlimb movements were not apparently coordinated with forelimb movements. Among all rats that received the greater compression, only 3 rats that received the injury on PND 5 traversed the elevated beams and parallel bars. Furthermore, only rats that received the compression on PND 5 licked the hindpaws on the hotplate within the 30 s time limit (on POD 28 only).

Only a thin strip or thin ring of white matter remained at the lesion epicenter of rats that received the greater compression on PND 5 or 15, whereas the lesion epicenter of rats of the oldest age group contained astrocytic scarring and was devoid of CNS tissue. However, some functional axons may have been dispersed throughout the necrotic tissue and contributed to hindlimb sensorimotor functioning. The age-dependent decrease in the quality of overground walking of rats of that received the greater compression suggests an age-dependent impingement of the lesion on descending fibers of the ventral and ventrolateral tracts, as well as on ascending and propriospinal systems. However, the cross-sectional area of tissue at the lesion epicenter of rats of this injury group did not differ significantly with age. Thus, reorganization of intraspinal systems may have played a greater role in the recovery of locomotor function of rats of the youngest age groups than in rats of the oldest age group.

Neuroanatomical Contributions to Recovery of Function

Regardless of the age of the rat at the time of surgery, there was evidence for improvement in hindlimb sensorimotor function during the 4-week postoperative period, as has been reported following injury to the adult spinal cord (e.g., Gale et al., 1985; Gruner et al., 1996). Rats of the present experiments that received a spinal cord compression on PND 5 or 15 had greater locomotor function immediately postinjury (sparing), as well as greater recovery of hindlimb sensorimotor function during the postoperative period than rats that received similar compressions on PND 60. This age-dependent decrease in sparing and recovery of function postinjury exemplifies the infant lesion effect (e.g., Bregman & Goldberger, 1982; 1983a; Robinson & Goldberger, 1986a). Behavioral recovery following injury to the developing spinal cord occurs on a background of processes active for normal growth and maturation. Thus, performance of rats of the two youngest age groups on the battery of tasks of the present experiments was largely influenced by sensorimotor maturation.

Rats are altricial mammals that rapidly develop the raised, quadrupedal posture for walking within the first two weeks of postnatal life (Altman & Sudarshan, 1975). However, locomotor skills continue to develop throughout the first postnatal month (see Altman & Sudarshan, 1975; Clarke & Williams, 1994). In the present experiments, a rating of '21' on the BBB locomotor recovery scale represents the hindlimb locomotor function of intact, adult rats. Although the BBB locomotor recovery scale was not ideal for describing locomotor function of neonatal rats, the scale enabled a rough description of age-related changes in locomotor function. Relative to control rats of the oldest age group, low scores on PODs 1, 7, and 14 of rats that received the lesser compression or

sham surgery on PND 5 and the low score on POD 1 of rats that received the lesser compression or sham surgery on PND 15 reflect locomotor immaturity, rather than surgery-related deficits. Generally, those rats continued to show outward rotation of the hindfeet at lift off after all other characteristics of the gait (including toe clearance, tail elevation, and trunk stability) were mature. The smooth surface of the inclined beams and parallel bars may have also revealed the immature locomotor skills of the young rats (also see Altman & Sudarshan, 1975). The percentage of hindlimb slips during beam walking was initially high and scores on the inclined plane were initially low in rats that received sham surgery as neonates. Rats that received surgery as neonates also stepped over increasingly tall objects on the beams and traversed parallel bars that were increasingly far apart during the postoperative period. Those latter two findings probably reflect ontogenetic increases in the size of the rat.

Sensorimotor immaturity of rats of the youngest two age groups was also revealed on the hot plate task. Tailflick (withdrawal) latencies in response to noxious heating of the tail of an adult rat reflect activation of spinal circuits (see Irwin et al., 1951). Relative to adult rats, cutaneous reflexes of young rats are exaggerated in amplitude and duration (e.g., Stelzner, 1971), suggesting that inhibition of spinal circuits by supraspinal systems is immature in young animals. Inhibitory systems, such as those that descend as the dorsolateral funiculus (Fields & Basbaum, 1978), mature anatomically (Leong et al., 1984) and functionally (e.g., Fitzgerald & Koltzenberg, 1986; Van Praag & Frenk, 1991) during the first three postnatal weeks. In the present experiments, latencies to withdraw one hindpaw from the surface of the hot plate increased between PODs 1 and 7 in rats that received sham surgery on PND 5, reflecting the age-related increase in descending

inhibition of spinal reflexes. Forebrain circuits are required to lick the hindpaws on the hot plate (Woolf, 1984). Therefore, failure of rats of the youngest two age groups (regardless of surgery group) to lick one hindpaw on the hot plate on the first day of testing (regardless of POD) reflects immaturity of sensory or motor systems important for that behavioral response to noxious heat (also see McEwen & Tucker, 2000). Adult rats that are familiar with the hot plate testing environment have shorter lick latencies than naive rats, which are due to a reduction in novelty-induced analgesia (Bardo & Hughes, 1979; Gamble & Milne, 1989; Plone, Emerich, & Lindner, 1996). In the present experiments, latencies to lick one hindpaw on the hot plate decreased between PODs 1 and 7 in rats that received sham surgery on PND 60. That finding probably reflects a reduction in novelty-induced analgesia with experience, rather than maturation of sensory or motor systems.

In addition to contribution by normal processes of growth and development, compensatory changes invoked following an injury may lead to greater recovery of function of rats that received an injury during infancy than in adulthood. Descending systems that survive an injury (spared systems) have been shown to contribute to locomotor recovery in adult animals through activation of commissural collaterals (e.g., Harris, Little, & Goldstein, 1994) and through sprouting (e.g., Aoki, Fujito, Satomi, Kurosawa, & Kasaba, 1986). However, injury to the developing nervous system results in sprouting that is more rapid (e.g., Gall & Lynch, 1978; 1981) and more profuse (e.g., Gomez-Pinilla, Villablanca, Sonnier, & Levine, 1986; Hulsebosch & Coggeshall, 1983; Prendergast & Misantone, 1980) than in the mature system. Furthermore, upregulation of postsynaptic receptors following injury to the adult nervous system (denervation

supersensitivity) may also aid locomotor function postinjury by enhancing responsiveness of lumbar motor circuits to diminished descending inputs. However, receptor levels are already elevated in the immature nervous system, relative to adults (e.g., Gonzalez, Fuchs, & Droge, 1993; Kalb, Lidow, Halsted, & Hockfield, 1992), which may provide enhanced responsiveness of lumbar motor circuits of the immature cord and support greater locomotor function immediately postinjury. Those compensatory changes postinjury may partly explain the rapid improvements in functional recovery of rats of the present experiments following compression of the infant spinal cord than following compression of the 60-day-old spinal cord. For example, rats that received the greater compression of the spinal cord on PND 15 showed the largest improvement in overground walking and the largest reduction in the percentage of hindlimb slips during beam walking between PODs 1 and 7. In contrast, the largest improvement in overground walking in rats that received the greater compression on PND 60 occurred within the first two postoperative weeks and the percentage of hindlimb slips during beam walking only decreased slightly during the last two days of the postoperative period.

Although fibers of most descending systems have reached the lumbosacral spinal cord by birth (Leong et al., 1984), some of those projection systems are not mature and continue to expand their innervation of spinal circuits during postnatal life. Therefore, late-developing systems that were not injured by compression of the developing spinal cord may continue to grow and innervate targets caudal to the lesion. Descending projections by catecholaminergic (Aramant, Giron, & Ziegler, 1986; Rajaofetra, Poulat, Marlier, Geffard, & Privat, 1992; Tanaka, Takahashi, Miyamoto, Oki, Cho, & Okuno,

1996) and serotonergic (Bregman, 1987; Rajaofetra, Sandillon, Geffard, & Privat, 1989; Tanaka, Mori, & Kimura, 1992) systems of the brainstem continue to mature in distribution and density during the first 2 to 3 postnatal weeks. In addition, the corticospinal tract, originating from the most posterior cortex, does not reach the midthoracic spinal cord until around PND 7 (Joosten, Gribnau, & Dederen, 1987). Although those projections are normally retracted during development (Joosten et al., 1987), they may continue to grow caudally in the injured spinal cord to form functional synaptic connections with denervated targets. Evidence suggests that fibers of developing tracts that survive an injury do grow around a lesion (e.g., Bernstein & Stelzner, 1983; Bregman & Goldberger, 1983b). In addition, exuberant connections that are already in place at the time of injury may be maintained (see Stanfield, 1989) and contribute to locomotor sparing and recovery of function following injury to the infant spinal cord. The extent to which late-developing fiber systems contributed to locomotor recovery in rats of the present experiments is not clear. Because the spinal cords were harvested 29 days following the midthoracic spinal cord compression, the amount of tissue at the lesion epicenter of rats of the oldest age group reflects the amount of tissue that was spared postinjury. In contrast, the amount of tissue at the lesion epicenter of rats of the two youngest age groups probably reflects growth of late-developing fiber systems, as well as sparing of more mature systems, such as the rubrospinal system (see Shieh, Leong, & Wong, 1983) that survived the injury.

Irrespective of contributions by descending systems to locomotor recovery, changes in intraspinal functioning are also likely to contribute to functional recovery following SCI. Evidence suggests that functional autonomy or adaptability of spinal

motor circuits decreases with age. Following complete spinal cord transection, rats less than 12 days of age recover good hindlimb posture and overground walking, despite isolation of lumbar motor circuits from descending and propriospinal systems. In contrast, complete transection of the spinal cord on PND 15 or later results in increased spinal shock and little recovery of hindlimb function, if any (Stelzner et al., 1975; Weber & Stelzner, 1977). The age-dependent decrease in locomotor recovery from spinal cord transection corresponds to a period of rapid synaptogenesis in the developing lumbar spinal cord (Gilbert & Stelzner, 1979), which suggests that lumbar motor circuits become more dependent on supraspinal inputs for normal functioning after PND 15 (Weber & Stelzner, 1977). The age-related decrease in functional autonomy of the spinal cord corresponds to an age-related increase in intraspinal inhibitory systems, which seem to be dependent on descending input for complete development (see Robinson & Goldberger, 1986b). In contrast, immature lumbar motor circuits may reorganize following spinal cord transection and functionally compensate for the missing inputs (Weber & Stelzner, 1977). Reorganization may be accomplished by sprouting of dorsal roots or intact intraspinal fiber systems of the caudal spinal cord (see Stelzner & Cullen, 1991).

The importance of spinal motor circuits in the recovery of hindlimb function following injury to the midthoracic spinal cord is further illustrated by the finding that locomotor function of infant rats with SCIs deteriorates on tasks that test the functional efficacy of descending motor circuits (McEwen & Stehouwer, 1998b). L-DOPA-induced air-stepping has been utilized to study locomotor development in newborn rats because postural demands are eliminated and locomotor development can be studied *in vivo*. Briefly, neonatal rats were suspended in harnesses to eliminate postural demands and

administered L-DOPA (sc) to activate locomotor circuits. Under those conditions, L-DOPA reliably induces coordinated air-stepping of all four limbs of 5- to 20-day-old intact rats (McCrea, Stehouwer, & Van Hartesveldt, 1994; Stehouwer, McCrea, & Van Hartesveldt, 1994; Van Hartesveldt et al., 1991). Following midthoracic spinal cord transection, hindlimb air-stepping is virtually eliminated (Iwahara, Van Hartesveldt, Garcia-Rill, & Skinner, 1991; McEwen, Van Hartesveldt, & Stehouwer, 1997), unless excitatory input to lumbar motor circuits is augmented (Arnaiz, Stehouwer, & Van Hartesveldt, 1997; McEwen et al., 1997). Therefore, L-DOPA-induced air-stepping may provide a test of the functional efficacy of descending catecholaminergic, and possibly serotonergic (Commissiong & Sedgwick, 1979; but see Goldstein & Frenkel, 1971; Hollister, Breese, & Mueller, 1979), systems following SCI. Although rats recover good overground walking following transection of the immature spinal cord (Stelzner et al., 1975; Weber & Stelzner, 1977), kinematic analyses of limb movements during L-DOPA-induced air-stepping reveal persistent deficits and progressive loss of locomotor function of infant rats in which the spinal cord was compressed by 95% of the uncompressed width (McEwen & Stehouwer, 1998b). Greater reorganization of lumbar spinal circuits and activation of peripherally driven reflex mechanisms may explain greater recovery of hindlimb function in rats of the present experiments that received such an injury as neonates than as young-adults. This is not to say that there is no reorganization by spinal circuits of the mature system. Rats of the oldest age group in the present experiments that received the greater compression recovered some hindlimb function during the postoperative period, despite presumed elimination of descending and ascending fibers at the lesion epicenter.

However, hindlimb sensorimotor function postinjury depends, not only on the extent and distribution of spared systems, but also on the functional integrity of those systems. Axons that survive an injury lose their myelin sheath (Blight, 1983), which decreases conduction velocity or causes nerve conduction block (Young, 1989). Thus, demyelinated systems that survive the injury would contribute little to behavioral sparing or recovery postinjury. Because demyelination and spontaneous remyelination are slow processes that occur for a year following weight-drop injury to the adult rat spinal cord (Salgado-Ceballos, Guizar-Sahagun, Feria-Velasco, Grijalva, Espitia, Ibarra, & Madrazo, 1998), functional recovery during the 4-week postoperative period of the present experiments was probably not due to remyelination of surviving fiber systems. Although lesion lengths were not quantified in the present experiments, lesions did not extend more than two segments rostral to, or caudal from, the lesion epicenter. Hindlimb rhythm-generating circuits are located within the caudal thoracic and lumbar segments of the spinal cord (Cowley & Schmidt, 1997; Kjaerulff & Kiehn, 1996; Kremer & Lev-Tov, 1997; but see Cazalets, Borde, & Clarac, 1995), but the entire extent of the lesions did not extend beyond the midthoracic cord and probably did not impinge directly on hindlimb motor circuits. However, there is evidence to suggest transsynaptic degeneration of motoneurons caudal to a spinal cord lesion in adults (Eidelberg, Nguyen, Polich, & Walden, 1989; but see Bjugn, Nyengaard, & Rosland, 1997), which could indirectly alter rhythm-generating circuits and impair hindlimb sensorimotor function.

In contrast to the age-related decrease in recovery of function following spinal cord transection (Stelzner et al., 1975; Weber & Stelzner, 1977), hindlimb sensorimotor function was not dramatically reduced between rats of the present experiments that

received the compression on PND 5 or 15. Because spinal cords of rats of the present experiments were not completely severed, ascending, descending, and propriospinal systems that survived the injury remained to contribute to hindlimb sensorimotor function. In adult animals, survival of only 5-10% of spinal cord axons sustain hindlimb function following compressive (e.g., Eidelberg, Straehley, Erspamer, & Watkins, 1977) or contusive (e.g., Blight, 1983) injury to the spinal cord. Descending and propriospinal systems that survived compression of the midthoracic spinal cord on PND 15 probably maintained excitability of lumbar motor circuits above some threshold value and prevented the dramatic reduction in hindlimb sensorimotor function observed following complete transection of the spinal cord.

Sensorimotor Recovery Postinjury is Task-Dependent

In general, hindlimb sensorimotor function was better following compression of the developing spinal cord than following compression of the mature spinal cord. However, the severity of the injury "changes" with the behavioral or neuroanatomical measure. Ratings of overground walking are typically used to assess locomotor function following injury to the spinal cord of adult animals. However, rating scales are subjective and overground walking does not challenge hindlimb function postinjury. In the present experiments, rats that received the lesser compression on PND 60 and rats that received the greater compression on PND 5, 15, or 60 performed considerably better during overground walking than during walking across the elevated beams or parallel bars. Those findings suggest that ratings of overground walking do not accurately depict the severity of hindlimb sensorimotor dysfunction postinjury. In addition to contributions

by specific neural systems, differential denervation and reinnervation of hindlimb muscles or their motoneurons may also explain the task-dependent performances postinjury. Severe compression of the spinal cord transiently causes muscle atrophy in rats (Mayer, Burke, Toop, Walmsley, & Hodgson, 1984), which would result in hindlimb motor paresis. Therefore, tasks which may require greater hindlimb muscle strength for successful completion, such as walking across the inclined beams and parallel bars, or maintaining position and balance on the inclined plane, may reveal hindlimb sensorimotor impairments that are not evident during less challenging tasks, such as overground walking. Anterior horn cells that supply proximal muscles are located ventromedially, whereas those innervating distal muscles are located dorsolaterally (Romanes, 1951; Sharrard, 1955). The spatial distribution of motoneurons that innervate proximal and distal muscles would presumably result in differential denervation of those muscles following spinal cord compression. Humans generally recover use of proximal muscles before distal muscles following SCI (see Ditunno, Graziani, & Tessler, 1997), which may be explained by differential sprouting and reinnervation of motoneurons by descending, propriospinal, or segmental systems that survive the injury. If reinnervation of proximal muscles (see Nakamura, Fujimura, Yato, & Watanabe, 1996) or of motoneurons that supplied proximal muscles occurred sooner or to a greater extent than reinnervation of distal muscles or the motoneurons that supplied distal muscles in rats of the present experiments, recovery of gross motor skills (e.g., overground walking) may occur before fine motor control (e.g., beam walking).

Although recovery of locomotor function following SCI depends on neuroanatomical and neurophysiological changes in the nervous system, postoperative

recovery may also depend on behavioral compensation (see Goldberger, Bregman, Vierck, & Brown, 1990). Rats are quadrupedal animals and could redistribute their body weight onto the uninjured forelimbs to compensate for hindlimb motor paresis following midthoracic SCI. In the present experiments, rats may have redistributed their body weight to the forelimbs during overground walking, but not during tasks that challenged hindlimb sensorimotor functioning postinjury. Redistribution of body weight following midthoracic SCI could be examined by having the animals stand on force plates or walk across a gait mat. Postural adjustments postinjury may also be identified with kinematic analyses of joint movement.

Apparent improvements in hindlimb sensorimotor function postinjury may also be an effect of practice. Following complete isolation from descending circuits, the spinal cord of adult cats can be trained to bear full weight support and to generate reciprocal steps on a treadmill (Lovely et al., 1986). Thus, spinal motor circuits do not merely carry out commands from descending motor circuits, but can learn a motor task. The information used by the spinal cord is task specific because cats trained to stand do not walk on a treadmill and cats trained to walk on a treadmill do not stand for long periods of time (Hodgson et al., 1994). Human infants given daily step training also step more during testing than infants given daily sit training, but do not sit as long as infants trained to sit (Zelazo, Zelazo, Cohen, & Zelazo, 1993). Similar training procedures have improved the locomotor gait of human patients with incomplete SCIs (e.g., Barbeau & Rossignol, 1994; Dietz, Wirz, Curt, & Colombo, 1998; Fung, Stewart, & Barbeau, 1990). Because the spinal cord was not completely transected in rats of the present experiments, supraspinal and spinal systems could participate in motor learning and lead to gradual

improvements in motor function with repeated testing during the 4-week postoperative period. The rats were free to walk around their home cages between test sessions, so better performance during overground walking than during beam walking may be explained by "practice". The effects of practice may also explain the decrease in the percentage of hindlimb slips during beam walking between the first two beam sessions (regardless of POD) in rats of all age and surgery groups that traversed the beams, because beam walking improved despite continued weight gain of the rats and no further improvements in overground walking. Furthermore, there was no decrement in the degree of incline on which the rats maintained balance and position on the inclined plane during the postoperative period, despite continued weight gain by the rats. The inclined plane task has been criticized as a test of undamaged (forelimb) systems (Steeves & Tetzlaff, 1998). In order to maintain position and balance on the inclined plane, the rats must redistribute their weight and shift their center of gravity as the incline is raised. Therefore, the test may provide a better assessment of forelimb muscle strength than hindlimb strength or balance. It seems unlikely that the weekly test sessions in the present experiments significantly increased muscle strength to account for maintained performance, despite the dramatic increase in body weight. Instead, it seems more plausible that rats learned to redistribute their weight during successive tests in which the plane was gradually raised. However, it is noteworthy that the inclined plane used in the present experiments was not covered by a rubber mat (see Rivlin & Tator, 1977), but was modified to have a smooth surface. Therefore, the inclined plane of the present experiments may have provided a more difficult test for sensorimotor deficits postinjury than previous reports because there was nothing for rats to grasp and hold onto during

testing. Because the quality of hindlimb function postinjury varied with the task in the present experiments, numerous tests should be used to fully characterize recovery of hindlimb sensorimotor function postinjury.

Methodological Considerations

Results of the present experiments show an age-related decline in sensorimotor function following midthoracic spinal cord compression, which may be due to age-related changes in neuroanatomical, neurophysiological, or behavioral compensatory mechanisms during recovery. However, procedures of the present experiments may have differentially affected locomotor recovery of rats of the three age groups from the outset. Neurologic outcome following slow compression of the adult spinal cord depends on the amount (Gruner et al., 1996), speed (Tarlov et al., 1953), and duration (Tarlov et al., 1953) of the compression. Because the size of the spinal cord increased during ontogeny, spinal cords of rats of the present experiments were compressed by a percentage of the uncompressed width of the spinal cord. Increasing the amount of compression increased severity of dysfunction. Mechanical properties of the spinal cord or its response to injury may change during ontogeny and contribute to the age-related changes in tissue survival and neurologic outcome. For example, water content of the infant rat brain is high and decreases during postnatal life (Himwich, 1962) and membrane elasticity of dorsal root ganglion neurons decreases during ontogeny in mice (Horie et al., 1990). Although the amount and duration of compression were controlled across experimental groups of the present experiments, viscoelastic properties of the spinal cord tissue may alter the amount of intraspinal pressure during compression and contribute to the age-related changes in

tissue survival postinjury. In addition, 5-day-old rats were anesthetized by hypothermia, which is neuroprotective to central tissue of the adult and newborn (e.g., Hansebout, Kuchner, & Romero-Sierra, 1975; Ikonomidou, Mosinger, & Olney, 1989; Kuchner, Mercer, Pappius, & Hansebout, 1976). Hypothermia may have minimized tissue degeneration postinjury by stabilizing cell membranes and suppressing destructive metabolic mechanisms (see Janssen & Hansebout, 1989). Furthermore, the incision site of rats of all age and injury groups was irrigated with saline, which may have reduced secondary degeneration by diluting extracellular Ca^{2+} and other ions known to exacerbate secondary injury to nervous tissue (see Faden, 1997; Sabel, Labbe, & Stein, 1985). That procedure was performed on rats of all age and injury groups, so an age-dependent effect on behavioral recovery is not likely.

The biochemical cascade that follows traumatic injury to the mature spinal cord and leads to secondary neural degeneration is well known. Those events include elevations in excitatory amino acids and other neurotransmitters, inflammatory and immune responses, production of free radicals, Ca^{2+} and Na^{+} influx, gliosis, and reductions in blood flow, glucose utilization, and oxygen utilization (see Faden, 1996; Janssen & Hansebout, 1989). Although the biochemical changes that follow traumatic injury to the developing spinal cord have not been elucidated, the cascade of biochemical events that follows ischemic injury to the developing brain is similar to the cascade of biochemical events that follows injury to the adult spinal cord (see Berger & Garnier, 1999; Giacoia, 1993). However, age-related changes in the magnitude of the release of various substances or the spinal cord reaction to those biochemical events probably exist. For example, Yager et al. (1996) reported significantly greater total brain damage in 1-

week, 3-week, and adult rats, than in rats of intermediate ages (6- and 9-week-olds) following a hypoxic-ischemic brain insult. In addition, injection of the excitatory amino acid, NMDA, into the striatum produced greater damage in 7-day-old and 3-month-old rats than in adults (McDonald, Silverstein, & Johnston, 1988; also see Ikonomidou, Mosinger, & Salles, 1989). In the adult spinal cord, NMDA receptors are restricted to the substantia gelatinosa, whereas NMDA receptors are distributed throughout the spinal cord gray matter during early postnatal development. The level of NMDA receptor binding increased between birth and approximately PND 8 (Gonzalez et al., 1993), but then decreased in all areas of the spinal cord, except the substantia gelatinosa, until PND 28 (Kalb et al., 1992). Thus, massive efflux of excitatory amino acids from damaged neurons following traumatic SCI (e.g., Faden, 1996) may have greater adverse effects on tissue survival in the developing spinal cord because of the elevated levels of receptor binding sites. Because the reaction of the neonatal nervous system to injury is not the same as in adults, postinjury treatments designed for adults may not have the same therapeutic efficacy for infants. Development of effective treatments for injury to the developing spinal cord is dependent on the elucidation of the biochemical and neuroanatomical events that follow traumatic SCI.

Concluding Remarks and Implications

Limited evidence suggests good recovery of ambulation following injury to the spinal cord of human infants. One infant diagnosed with spastic quadriplegia following injury to the cervical spinal cord at 9 weeks of age eventually gained some ability to walk, crawl, and manipulate toys. Morphology of the cervical spinal cord also returned

to near-normal several months postinjury (Thomas, Robinson, Evans, & Bullock, 1995). Another infant sustained a complete fracture-dislocation of the lumbar cord, which resulted in flaccid paralysis of the lower extremities. However, 12 months later, she regained strength in all lower extremity muscles and walked with orthotic aids (Gabos, Tuten, Leet, & Stanton, 1998). Because injury to the human infant spinal cord is less common than in adults, there is danger of misdiagnosis by physicians (see Dickman, Rekate, Sonntag, & Zabramski, 1989; Hesketh, Eden, Gattamaneni, Campbell, Jenney, & Lashford, 1998; Rossitch & Oakes, 1992). Therefore, behavioral diagnostics of injury to the developing spinal cord are needed.

Further investigations into the effects of pediatric SCI are important because injury to the developing nervous system may result in unique behavioral alterations, not observed following injury to the mature spinal cord. Following complete crush of the spinal cord of neonatal opossum, spinal cord morphology was near-normal and the animals recovered good use of the hindlimbs, as well as coordination between limb girdles. However, the abnormally high hindlimb stepping that was apparent at weaning was exaggerated in adulthood and impeded grid walking and climbing (Saunders, Deal, Knott, Varga, & Nicholls, 1995). Placing responses of kittens were hypermetric and slow following partial hemisection of the spinal cord and never completely matured (Bregman & Goldberger, 1982). In the present experiments, comparatively short latencies to withdraw one hindpaw from the hotplate surface on POD 7 by rats that received the greater compression on PND 5 reflects the removal of descending inhibitory systems and supersensitivity of hindlimb sensorimotor circuits to the thermal/noxious stimulus. Furthermore, rats that received the greater compression on PND 5 stood with ventrum

unusually high off of the table surface following episodes of righting on POD 7.

Although overground walking of those rats was rated as similar to sham controls by the end of the postoperative period, those rats continued to walk with the ventrum held higher off of the table surface than sham controls. Such behavioral changes postinjury must be considered in the design of rehabilitative strategies following injury in infancy.

Pediatric SCI may also change how the nervous system is constructed.

Motoneuron dendrite bundles develop postnatally in kittens and their maturation parallels the emergence of mature stepping, walking, and weight-bearing (Scheibel & Scheibel, 1970). However, motoneuron dendrite bundles do not mature following spinal cord transection (Reback, Scheibel, & Smith, 1982). Transection of the midthoracic spinal cord of kittens alters the biochemical, histochemical, and contractile properties of the hindlimb muscles (Johnson, Smith, Eldred, & Edgerton, 1982). Although spinal cord transection causes muscle spasticity in both kittens and cats, kittens are more prone to develop skeletoarticular disorders following SCI than are cats (see Smith, Smith, Zernicke, & Hoy, 1982). Furthermore, supraspinal systems are required for postnatal fine-tuning of spinal nociceptive systems, which is altered following damage to the developing spinal cord (Levinsson, Luo, Holmberg, & Schouenborg, 1999). Because the consequences of such changes are not completely understood, greater understanding of the behavioral, neuroanatomical, and neurophysiological effects of injury to the developing spinal cord is required.

APPENDIX A

BASSO, BEATTIE, AND BRESNAHAN (BBB) LOCOMOTOR RECOVERY SCALE

- 0 No observable HL movement
- 1 Slight movement of one or two joints, usually the hip and/or knee
- 2 Extensive movement of one joint or extensive movement of one joint *and* slight movement of one other joint
- 3 Extensive movement of two joints
- 4 Slight movement of all three joints of the HL
- 5 Slight movement of two joints *and* extensive movement of the third
- 6 Extensive movement of two joints *and* slight movement of the third
- 7 Extensive movement of all three joints of the HL
- 8 Sweeping with no weight support or plantar placement of the paw with no weight support
- 9 Plantar placement of the paw with weight support in stance only (i.e., when stationary) or occasional, frequent or consistent weight-supported dorsal stepping and no plantar stepping
- 10 Occasional weight-supported plantar steps; no FL-HL coordination
- 11 Frequent to consistent weight-supported plantar steps *and* no FL-HL coordination
- 12 Frequent to consistent weight-supported plantar steps *and* occasional FL-HL coordination
- 13 Frequent to consistent weight-supported plantar steps *and* frequent FL-HL coordination
- 14 Consistent weight-supported plantar steps, consistent FL-HL coordination *and* predominant paw position during locomotion is rotated (internally or externally) when it makes *initial contact* with the surface as well as just before it is *lifted off* at the end of stance; or frequent plantar stepping, consistent FL-HL coordination and occasional dorsal stepping
- 15 Consistent plantar stepping and consistent FL-HL coordination *and* no toe clearance or occasional toe clearance during forward limb advancement; predominant paw position is parallel to the body at initial contact
- 16 Consistent plantar stepping and consistent FL-HL coordination during gait and toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact and rotated at lift off
- 17 Consistent plantar stepping and consistent FL-HL coordination during gait *and* toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact and lift off
- 18 Consistent plantar stepping and consistent FL-HL coordination during gait *and* toe clearance occurs consistently during forward limb advancement; predominant paw position is parallel at initial contact and rotated at lift off

- 19 Consistent plantar stepping and consistent FL-HL coordination during gait, toe clearance occurs consistently during forward limb advancement, predominant paw position is parallel at initial contact *and* lift off, and tail is down part or all of the time
- 20 Consistent plantar stepping and consistent coordinated gait, consistent toe clearance, predominant paw position is parallel at initial contact and lift off and trunk instability; tail consistently up
- 21 Consistent plantar stepping and coordinated gait, consistent toe clearance, predominant paw position is parallel throughout stance, and consistent trunk stability; tail consistently up

APPENDIX B
NUMBER OF RATS IN EACH STATISTICAL ANALYSIS

Surgery on PND 5

Analyses	Compression	Postoperative Day					
		1	7	14	21	28	29
Righting	0%	8	8	-	-	-	-
	85%	8	8	-	-	-	-
	95%	8	8	-	-	-	-
Overground Walking	0%	8	8	8	8	8	-
	85%	8	8	8	8	8	-
	95%	8	8	8	8	8	-
Slips – 3.0 cm Beam	0%	-	-	8	8	8	-
	85%	-	-	8	8	8	-
	95%	-	-	3	3	3	-
Footfalls – 3.0 cm Beam	0%	-	-	8	8	8	-
	85%	-	-	8	7	7	-
	95%	-	-	2	2	1	-
Slips – 3.0 cm Beam	0%	-	-	8	8	8	-
	85%	-	-	8	8	8	-
	95%	-	-	3	3	3	-
Footfalls – 2.2 cm Beam	0%	-	-	2	7	8	-
	85%	-	-	5	7	7	-
	95%	-	-	0	2	1	-
Obstacles-3.0 cm Beam	0%	-	-	8	8	8	-
	85%	-	-	8	8	8	-
	95%	-	-	3	3	3	-
Obstacles-2.2 cm Beam	0%	-	-	8	8	8	-
	85%	-	-	8	8	8	-
	95%	-	-	3	3	3	-
Parallel Bar Walking	0%	-	-	8	8	8	-
	85%	-	-	8	8	8	-
	95%	-	-	8	8	8	-
Inclined Plane	0%	-	-	8	8	8	-
	85%	-	-	8	8	8	-
	95%	-	-	8	8	8	-
Hot Plate	0%	8	8	8	8	8	-
	85%	8	8	8	8	8	-
	95%	8	8	8	8	8	-
Lesion Size	0%	-	-	-	-	-	8
	85%	-	-	-	-	-	8
	95%	-	-	-	-	-	8

Surgery on PND 15

Analyses	Compression	Postoperative Day					
		1	7	14	21	28	29
Righting	0%	-	-	-	-	-	-
	85%	-	-	-	-	-	-
	95%	-	-	-	-	-	-
Overground Walking	0%	8	8	8	8	8	-
	85%	7	7	7	7	7	-
	95%	8	8	8	8	8	-
Slips – 3.0 cm Beam	0%	8	8	8	8	8	-
	85%	6	7	7	7	7	-
	95%	0	0	0	0	0	-
Footfalls – 3.0 cm Beam	0%	6	8	8	8	8	-
	85%	1	6	6	6	7	-
	95%	0	0	0	0	0	-
Slips – 2.2 cm Beam	0%	8	8	8	8	8	-
	85%	4	7	7	7	7	-
	95%	0	0	0	0	0	-
Footfalls – 2.2 cm Beam	0%	-	8	8	6	8	-
	85%	-	5	6	5	6	-
	95%	-	0	0	0	0	-
Obstacles-3.0 cm Beam	0%	-	8	8	8	8	-
	85%	-	7	7	7	7	-
	95%	-	0	0	0	0	-
Obstacles-2.2 cm Beam	0%	-	8	8	8	8	-
	85%	-	7	7	7	7	-
	95%	-	0	0	0	0	-
Parallel Bar Walking	0%	8	8	8	8	8	-
	85%	7	7	7	7	7	-
	95%	8	8	8	8	8	-
Inclined Plane	0%	8	8	8	8	8	-
	85%	7	7	7	7	7	-
	95%	8	8	8	8	8	-
Hot Plate	0%	8	8	8	8	8	-
	85%	7	7	7	7	7	-
	95%	8	8	8	8	8	-
Lesion Size	0%	-	-	-	-	-	8
	85%	-	-	-	-	-	7
	95%	-	-	-	-	-	8

Surgery on PND 60

Analyses	Compression	Postoperative Day					
		1	7	14	21	28	29
Righting	0%	-	-	-	-	-	-
	85%	-	-	-	-	-	-
	95%	-	-	-	-	-	-
Overground Walking	0%	8	8	8	8	8	-
	85%	8	8	8	8	8	-
	95%	7	7	7	7	7	-
Slips – 3.0 cm Beam	0%	8	8	8	8	8	-
	85%	0	0	0	3	3	-
	95%	0	0	0	0	0	-
Footfalls – 3.0 cm Beam	0%	7	8	8	8	8	-
	85%	0	0	0	0	1	-
	95%	0	0	0	0	0	-
Slips – 2.2 cm Beam	0%	7	8	8	8	8	-
	85%	0	0	0	3	3	-
	95%	0	0	0	0	0	-
Footfalls – 2.2 cm Beam	0%	5	8	8	8	8	-
	85%	0	0	0	0	1	-
	95%	0	0	0	0	0	-
Obstacles-3.0 cm Beam	0%	7	8	8	8	8	-
	85%	0	0	0	3	3	-
	95%	0	0	0	0	0	-
Obstacles-2.2 cm Beam	0%	7	8	8	8	8	-
	85%	0	0	0	3	3	-
	95%	0	0	0	0	0	-
Parallel Bar Walking	0%	8	8	8	8	8	-
	85%	8	8	8	8	8	-
	95%	7	7	7	7	7	-
Inclined Plane	0%	8	8	8	8	8	-
	85%	8	8	8	8	8	-
	95%	7	7	7	7	7	-
Hot Plate	0%	8	8	8	8	8	-
	85%	8	8	8	8	8	-
	95%	7	7	7	7	7	-
Lesion Size	0%	-	-	-	-	-	8
	85%	-	-	-	-	-	8
	95%	-	-	-	-	-	7

APPENDIX C
BODY WEIGHT IN GRAMS (\pm SEM) ON EACH POSTOPERATIVE DAY

POD	Compression	Age on the Day of Surgery		
		5 days	15 days	60 days
1	0%	15.2 \pm 0.5	42.0 \pm 0.9	323.0 \pm 32.7
	85%	14.9 \pm 0.6	45.4 \pm 0.8	322.7 \pm 28.2
	95%	15.9 \pm 0.4	43.0 \pm 1.3	316.0 \pm 33.0
7	0%	33.7 \pm 0.9	69.8 \pm 1.5	350.5 \pm 35.0
	85%	30.6 \pm 1.0	77.5 \pm 2.3	346.0 \pm 26.6
	95%	30.1 \pm 0.8	68.7 \pm 2.2	322.8 \pm 28.9
14	0%	55.8 \pm 1.7	119.8 \pm 4.0	376.3 \pm 38.0
	85%	51.6 \pm 1.4	128.6 \pm 5.1	399.0 \pm 33.5
	95%	50.9 \pm 1.7	119.3 \pm 3.4	358.7 \pm 31.9
21	0%	103.7 \pm 2.7	178.8 \pm 7.6	401.9 \pm 40.9
	85%	94.2 \pm 3.2	185.7 \pm 9.1	433.9 \pm 41.7
	95%	98.2 \pm 3.4	169.5 \pm 6.9	389.4 \pm 39.4
28	0%	161.8 \pm 5.2	228.4 \pm 12.8	422.9 \pm 43.1
	85%	155.5 \pm 5.4	234.5 \pm 15.8	462.6 \pm 46.5
	95%	151.2 \pm 6.3	216.6 \pm 11.4	423.3 \pm 45.9

SOUTHWORTH
ARCHMENT DEED
100% COTTON FIBER

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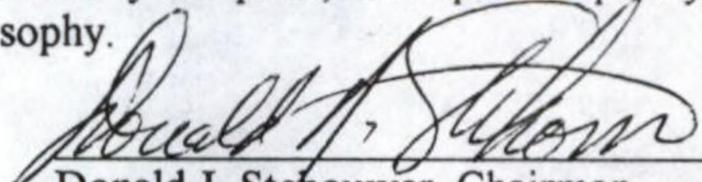
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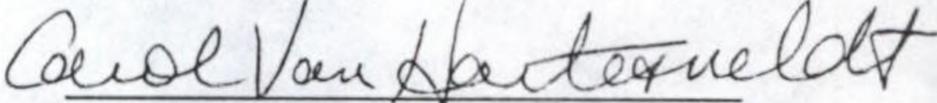
BIOGRAPHICAL SKETCH

Melanie Lynn McEwen was born in St. Charles, Missouri, to James Albert McEwen and Carol Marie (Orf) McEwen on August 23, 1969. She graduated from St. Charles West High School in June 1987 and enrolled at the University of Missouri-Columbia as a psychology major. Melanie began her research career during her junior year when she worked with Dr. Douglas Anger on the effects of reinforcement and extinction on bar pressing in rats. That summer, Melanie worked with Dr. George T. Taylor at the University of Missouri-St. Louis on neuroendocrine function and muscle strength in rats. Upon returning to the University of Missouri-Columbia for her senior year, she worked with Dr. Todd Schachtman on conditioned taste aversion in rats and mice. Melanie graduated from the University of Missouri-Columbia in May 1991 with her B.A. in psychology. Subsequently, she attended the University of Missouri-St. Louis and Washington University in St. Louis as a nondegree student, while working as a laboratory research technician in the Department of Psychiatry at the Washington University School of Medicine. Melanie worked in the laboratory of Dr. John Olney, under the guidance of Dr. David F. Wozniak, on excitotoxicity and learning and memory in rats and mice. In 1993, she began her graduate career in psychobiology at the University of Florida in Gainesville with Dr. Donald J. Stehouwer, where she studied the development of locomotor systems in rats. Melanie received her M.S. in psychology in December 1996 and plans to continue her research career as a postdoctoral fellow following graduation with her Ph.D. in May 2000.

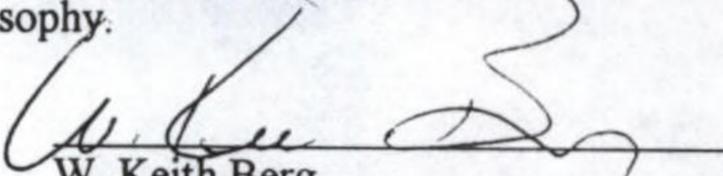
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Donald J. Stehouwer, Chairman
Professor of Psychology

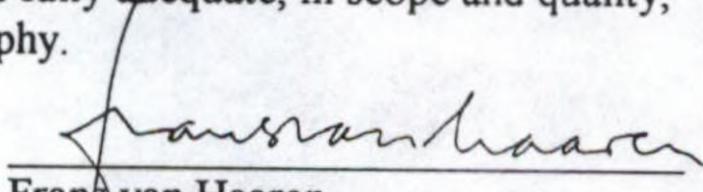
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Carol Van Hartesveldt
Professor of Psychology

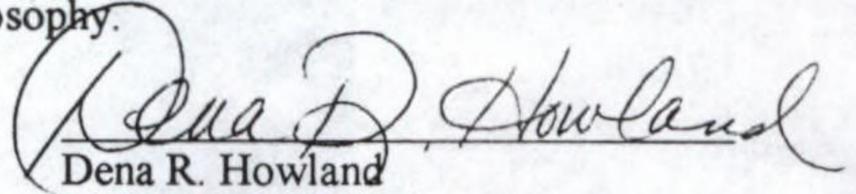
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Professor of Psychology

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Frans van Haaren
Associate Scientist of Psychology

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Dena R. Howland

Assistant Professor of Neuroscience

This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2000

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