

Objective clinical assessment of motor function after experimental spinal cord injury in the rat

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✓ A new method was developed for the clinical assessment of motor function in rats after experimental spinal cord injury. The method consists of placing the animal on an inclined plane which can be adjusted to provide a slope of varying grade, and then assessing the maximum angle of the plane at which the animal can maintain its position without falling. The method was used to quantitate motor function in normal rats and in rats subjected to myelotomy, and consistently showed major differences between the two groups. The method has many positive features: the plane is easy to construct and of low cost; and the test is rapid, non-invasive, repeatable, and consistent.

KEY WORDS • experimental spinal cord injury • rats • method of assessment

DURING the past several years our laboratory has been conducting research on acute experimental spinal cord injury. Monkeys were used in an attempt to make the research as relevant as possible to human cord injury.¹⁴ Clinical assessment of the degree of cord injury in the monkey was accomplished reliably by a double-blind experimental protocol. In contrast to lower species such as cats and dogs, primates do not develop "spinal walking" or other forms of reflex limb movements that can obscure clinical assessment of limb function.¹⁶ Because of the major increase in the cost of monkeys and the uncertainties of supply, it was decided to use for our studies rats which are inexpensive and readily available. However, one of the major problems in the use of lower animals such as rats is the unreliability of the clinical assessment of the degree of spinal cord injury.

There have been several other studies of ex-

perimental spinal cord injury in lower animals such as rodents and rabbits, but with one exception, all used either a variation of the Tarlov system for grading clinical recovery or a non-quantitative method such as motion pictures or simple observation. The exception is a recent report by Eidelberg, *et al.*,⁴ who have successfully developed a quantitative method for clinical assessment of cord injury in the ferret. Their method has certain similarities to our inclined plane method to be reported here.

Materials and Methods

Animals

Male albino rats of the Wistar strain, weighing 350 to 450 gm, were employed in the experiments.*

*The rats were obtained from Woodlyn Laboratory Ltd., Guelph, Ontario, Canada.

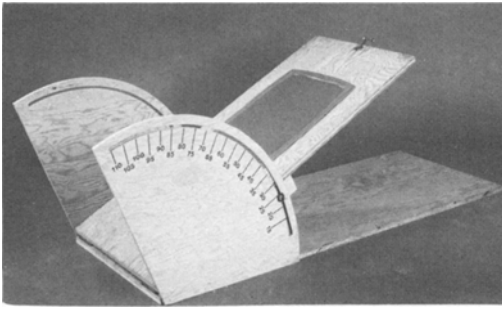


FIG 1. The inclined plane is shown with the various angles marked on the protractor-like sides. The ridged rubber mat is seen on the movable plane which in this view was set at 30°.

Development of the Inclined Plane Method

After considering numerous possible methods and constructing various tracks and devices, we constructed an inclined plane and tested the rats' ability to maintain their position on it. The plane consists of two rectangular plywood boards connected at one end by a hinge (Fig. 1). One of the boards serves as the base and the other as the

movable inclined plane. Two protractor-like plywood side panels with degrees marked on their faces are fixed on the base. A rubber mat with ridges 0.6 cm in height was fixed to the surface of the movable plane. For clinical assessment rats were placed in such a position on the mat that their body axis was perpendicular to the axis of the inclined plane (Fig. 2). To maintain themselves on the plane the animals used both fore and hind limbs.

The maximum inclination of the plane at which a rat could maintain itself for 5 seconds was recorded and taken to represent the rat's functional ability. In practice, the angle was either increased at 5° intervals or decreased at 5° intervals until the rat could maintain its position on the inclined plane for 5 seconds without falling. For example, if the rat fell, then the angle was reduced at 5° steps until it could maintain its position for 5 seconds (Fig. 3).

Experimental Protocol

For testing the inclined plane method of clinical assessment, we studied 15 rats subjected to myelotomy and compared the

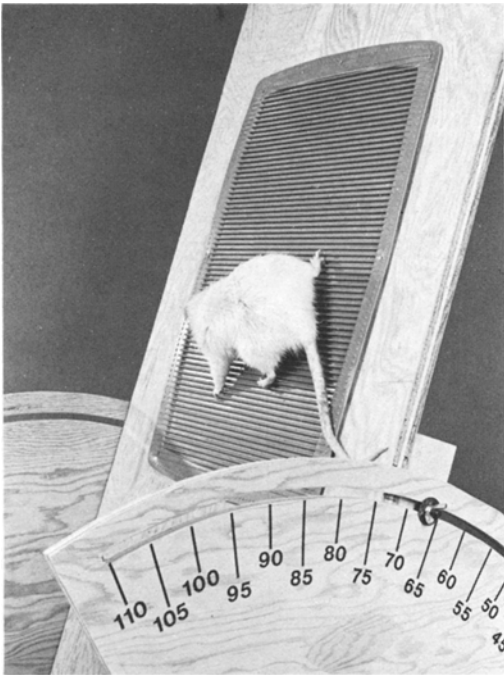


FIG. 2. A normal rat is seen maintaining its position with the inclined plane at approximately 73°.

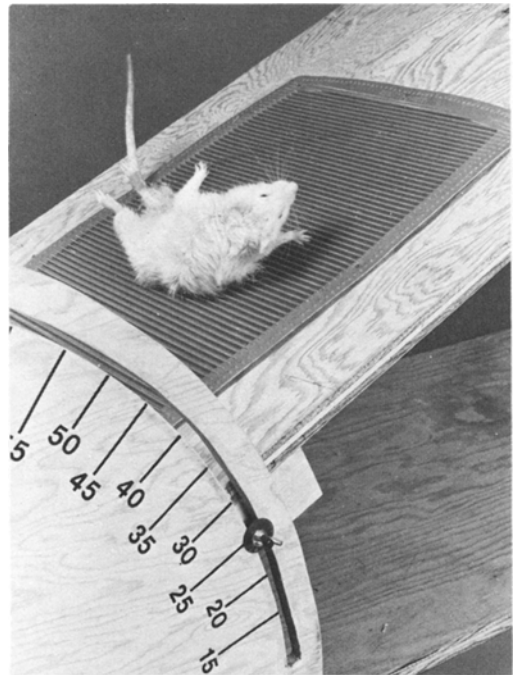


FIG. 3. A rat is shown unable to maintain its position, and tumbling down the ramp which was at 30°. This animal had been subjected to myelotomy 8 weeks before.

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results with 10 normal rats. Myelectomy was performed after a laminectomy of C-7 and T-1. A complete transverse segment of the cervical spinal cord approximately 5 mm in longitudinal extent was removed with scissors at this level. This was done under the operating microscope to be absolutely sure that the continuity of the cord was completely interrupted by the myelectomy. The 10 normal animals did not undergo any operative procedure. The functional ability of each of the 25 rats was assessed weekly with the inclined plane for 8 weeks after surgery.

For comparison, the motor activity of the rats was assessed by the modified Tarlov system which we had used previously in the monkeys,¹⁴ as follows:

Grade 0 = complete paralysis of legs

Grade 1 = flicker of movement

Grade 2 = good movement at all joints but without walking or weight-bearing

Grade 3 = walking and weight-bearing, but not normally

Grade 4 = normal.

Movies were made of some of the animals to demonstrate spinal walking and other movements of the hind limbs.

Statistical Analysis

The change within a group from week to week was assessed by comparing the average weekly maximum angle by means of a t-test, and comparisons were made between the normal and myelectomy groups by means of a t-test.

Care of Rats with Spinal Cord Injury

Postoperative care after myelectomy was very difficult, mainly due to urinary tract dysfunction. During the initial stage of spinal shock after injury, the urinary bladder was flaccid and unresponsive. At this time, it was necessary to manually compress the bladder approximately every 6 hours. At about 10 days, the voiding reflex returned and the manual compressions could be gradually discontinued. It was very important to keep the room temperature at about 25° to 27° C, and to prevent sudden changes of temperature. Soiled animals were bathed frequently. Urinary tract infection occurred in some animals, usually due to *E. coli* or *Proteus* bacteria. These were sensitive to gentamicin

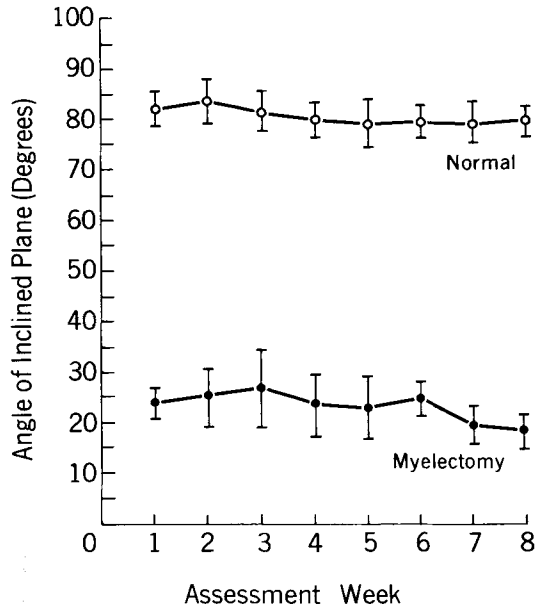


FIG. 4. The average maximum angles attained by normal rats and rats subjected to myelectomy are plotted for each week of assessment. The standard deviations are shown.

which was given intramuscularly in doses of 0.2 mg/100 gm body weight. Even with these measures four of the 15 rats subjected to myelectomy died because of urinary tract dysfunction including hematuria and bladder obstruction.

Results

In practice the inclined plane method was extremely easy to use because of the rapidity and ease with which the maximum angle could be determined for each animal. Figure 4 shows the results during the 8-week assessment period for each group of animals. The normal animals showed consistent results from week to week and there was no tendency for them to improve their performance with experience. At the end of the 8th week, the 10 normal animals had an average response of $80.5^\circ \pm 3.6^\circ$ (SD). When the results were averaged over the 8-week assessment period, the normal animals could maintain themselves at an angle of $81.0^\circ \pm 1.6^\circ$.

The animals subjected to myelectomy showed a slight tendency toward improvement during the first 3 weeks, but then their performance tended to deteriorate (Fig. 4).

However, there was no significant variation in their response during the 8 weeks of postoperative assessment. At the end of the 8th week the 11 surviving animals subjected to myectomy had an average response of $18.2^\circ \pm 3.5^\circ$, which is significantly different from the normal ($p < 0.01$). When averaged over the entire 8-week assessment period, these animals could maintain themselves at $23.0^\circ \pm 2.9^\circ$, which is also significantly different from the normal ($p < 0.01$).

All animals in the myectomy group showed modified Tarlov ratings of either Grade 2 or Grade 3. Eight of the animals could walk on all four limbs and this was documented by moving pictures.

Discussion

Most studies of experimental cord trauma have used a system like that devised by Tarlov¹³ for quantitating voluntary somatic motor function below the level of the lesion. We agree with Eidelberg³ that such a system has major shortcomings and is probably inaccurate in lower species. However, in primates the Tarlov system combined with a double-blind experimental protocol¹⁵ is accurate because primates with cord transection are incapable of complex motor function such as walking or stepping.^{1,6}

Sherrington¹¹ found that the reflexes in cats and dogs soon after spinal cord transection were mainly of a phasic nature that resembled some patterns of movement used during locomotion, but initially locomotion itself could not be induced. However, when the survival period was extended over several weeks, muscular tonus gradually increased. If the animals were then suspended in the air with the trunk nearly vertical, alternating rhythmic movements of the hind limbs were seen, and these were termed spinal stepping. Philippon¹⁰ also demonstrated stepping movements of the hind limbs of the cord-transected dog if the forelimbs were supported, and these results generated a great deal of controversy. One group of investigators claimed that, after cord transection, animals were unable to maintain their equilibrium and were incapable of coordinated walking.^{1,6,9,11} However, a second group^{7,8,12,13} showed that cord-transected cats and dogs could in fact support their body weight on the hind limbs.

These investigators employed a wide variety of techniques to assess locomotor ability in cord-transected animals including motion pictures,⁷ treadmills,⁵ electromyography,¹ and simple observation.^{9,10,16} However, none of their techniques provided quantitative assessment of function in cord-injured animals. For lower species, there are at least three methods of quantitative assessment of residual cord function. First, histological methods could be used but would likely entail detailed axonal counts in the motor tracts, and might even require that the counts be performed by electron microscopy. A second method would include neurophysiological techniques² such as corticospinal tract stimulation or measurement of the H-reflex, which depends on descending impulses in the cord. Somatosensory evoked responses do not reflect persisting motor function, and cord dorsum evoked potentials² have so far only been used to assess afferent cord conduction. The third method is that of clinical functional assessment exemplified by the inclined plane technique that we developed. This method has several advantages. The apparatus was extremely easy to construct and was inexpensive. The test on each animal required 1 minute to perform, was completely non-invasive, and could be repeated as frequently as desired. Spinal-injured animals have diminished ability to maintain their equilibrium.⁷ The inclined plane method quantitatively assesses the animals' ability to prevent themselves from falling over, in addition to assessing the residual strength in the upper and lower limbs.

The value of the inclined plane method was tested in animals subjected to myectomy so that we would be certain that it would clearly record and quantitate the functional disability due to major spinal cord injury. The large difference in the results between the myectomy group and the normal group shows that the test is capable of recording and quantitating function, and the consistency of the results over an 8-week period proves the reliability of the test (Fig. 4). In contrast, the attempt to apply Tarlov ratings to the myectomy group shows the inaccuracy of this method for quantitating somatic motor function in rats. Almost all the animals in the myectomy group were capable of performing complex motor activity and some showed

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spinal stepping and spinal walking. All achieved ratings of Grade 2 and 3 on our modification of the Tarlov scale. Clearly, such a system cannot be applied to lower animals.

Our technique may be applicable to other species, but we have not determined this. Eidelberg, *et al.*,⁴ recently developed a very good method of functional assessment of ferrets with spinal cord injuries which also utilizes an inclined plane. However, his technique requires conditioning of the animals and motivation. Our inclined plane method does not require conditioning and does not depend on a reward situation involving motivation of the animals.

At the present time, we are using the inclined plane method to assess various therapeutic modalities after graded spinal cord injuries in the rat. The results, which will be reported separately, indicate that this method of assessment is reliable and consistent.

Acknowledgments

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