Experimental Neurology **168,** 192–195 (2001)
doi:10.1006/exnr.2000.7600, available online at http://www.idealibrary.com on IDEAL®

BRIEF COMMUNICATION

Quantification of Functional Recovery Following Rat Sciatic Nerve Transection

Rahul Koka and Tessa A. Hadlock[1]

Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, and Harvard Medical School, Boston, Massachusetts

Received May 5, 2000; accepted October 31, 2000

Functional recovery following experimental nerve injury has been notoriously difficult to quantify precisely. The current gold standard in the rat sciatic nerve model involves analysis of footprints of the recovering animal, and computation of the sciatic function index (SFI). We performed transection injuries and measured recovery both by walking track analysis and by a newer, simpler, more quantitative test of motor recovery, the extensor postural thrust (EPT). We demonstrate a high correlation between both testing modalities and suggest a role for EPT measurements as an easier, more consistent measure of motor recovery following experimental rat sciatic nerve transection. © 2001 Academic Press

Key Words: **rat sciatic nerve; transection; behavioral testing.**

Peripheral nerve injuries are a commonly encountered clinical problem and often result in long-term functional deficits. Extensive investigation toward the development of methods to improve regeneration following nerve injury is ongoing. Attempts to study the regenerative effect of various neurotrophic substances and manipulations have been complicated by difficulty in the precise quantification of regeneration. Histologic, electrophysiologic, and functional measures of regeneration have all been established (2, 5–7, 11, 12). Of these, functional recovery is the most difficult to measure precisely and does not always correlate well with histologic and electrophysiologic results (13).

The current standard for measuring functional recovery following rat sciatic nerve injury is the sciatic function index (SFI), established in 1982 by DeMedinaceli *et al.* (6) and subsequently modified by Bain *et al.* (1). Calculation of the SFI involves the measurement of various relationships between toes and feet of the hindlimb of recovering animals. These are recorded from inked footprints left by the animals on the floor of a standardized walking track. Development of the formula is based on the observation that following sciatic nerve injury, rats develop characteristic walking patterns that can be reliably reproduced and measured. Their recovery can be followed by this gait analysis, returning to normal when full recovery is achieved.

Walking track evaluations are cumbersome and fraught with technical problems in both performance and analysis, and are therefore subject to error. Additionally, the data obtained from SFI calculations are a reflection of complex integrated function, rather than discrete motor, sensory, or proprioceptive function. Alternative methods for the assessment of functional recovery following sciatic nerve manipulation, which are simpler, more quantitative, and distinguish type of recovery, are therefore desirable. We have previously shown that following crush injury to the sciatic nerve, a new neurobehavioral test battery, developed in the anesthesia literature, predicted functional recovery indistinguishable from walking track analysis (8). In the current study, we sought to expand these observations to a more extensive neural damage model, using a transection injury.

Inbred adult male Fisher rats ($n = 8$), 150–200 g, were used. All animals were housed three per cage with a 12-h light/dark cycle, and were fed rat chow and water *ad libitum*, following Animal Care and Use Committee guidelines. The animals were handled on a daily basis for a 2-week period prior to the study, to acclimate them with the testing area and procedure, and to minimize anxiety-related testing inaccuracies (14).

Following anesthetic induction using inhalational methoxyfluorane, the left hindlimb was shaved and sterilely prepped. The sciatic nerve was exposed at the sciatic notch via a gluteal musculature splitting incision and sharply transected. Immediate microsurgical

[1] To whom correspondence should be addressed at Department of Otolaryngology/Head and Neck Surgery, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114. Fax: (801) 761-7846. E-mail: thadlock@hotmail.com.

0014-4886/01 $35.00

epineurial repair was then performed, using two or three 9-O nylon sutures. The muscles were reapproximated and the wound was closed. During the survival period, autotomy was averted by the administration of Bitter Green taste deterrent (Grannick's, Greenwich, CT) to the toes of the affected hindlimb. Animals underwent periodic behavioral testing using both the standard walking track analysis and the newer extensor postural thrust (EPT) measurements, for a total of 36 weeks.

Functional recovery predicted by each method was assessed, and pair-wise comparisons using Student's *t* tests were made among the two methods for each animal at each time point. Comparisons were also made between baseline function and that following recovery, to identify the time at which statistically significant ($P < 0.05$) recovery from baseline had occurred.

For SFI determinations, animals were allowed to become conditioned to the walking track, a wooden box 8.7 × 43 cm in dimension and darkened at one end. Their hindfeet were dipped in a methylene blue solution, and they were permitted to walk down the track upon a strip of white paper. The prints left by the ink were allowed to dry and then analyzed as described by Bain *et al.* (1). Measurements included print length on both the experimental and the normal sides (EPL, NPL), toe spread between the first and fifth digits on both sides (ETS, NTS), and the distance between the middle of the second and fourth toes on both sides (EIT, NIT). The formula used to calculate SFI was as follows:

$$\mathrm{SFI} = -38.8(\mathrm{EPL} - \mathrm{NPL})/\mathrm{NPL} + 109.5(\mathrm{ETS} - \mathrm{NTS})/\mathrm{NTS} + 13.3(\mathrm{EIT} - \mathrm{NIT})/\mathrm{NIT} - 8.8.$$

All measurements were performed manually in blinded fashion and recorded to the nearest millimeter. Some prints were unmeasurable due to smearing of ink by the tail, or contamination by the forefeet. In these cases, the run was repeated to obtain interpretable results. The maximum distances for each value were recorded for each walking track.

The EPT was utilized as a reflection of motor performance and was measured as described by Thalhammer *et al.* (14). Specifically, each animal was placed on the testing surface and the entire upper body was wrapped in a surgical towel. Only the hindlimbs were exposed. The right hindlimb was supported with the examiner's fifth finger while the entire hand grasped the torso of the animal. With the left hindlimb suspended, the animal was held upright and the hindlimb was placed upon a digital scale (Ohaus LS2000, Florham Park, NJ) (see Ref. 8, Fig. 1). Any digital scale with a range of 0 to 500 g is appropriate. The flat surface of the scale is protected with a taped segment of heavy absorbent paper. It is clear when the animal begins to bear weight on the scale. The amount of weight borne by the denervated limb was recorded. The same was done for the unoperated side. The formula for calculating the percentage functional deficit is

$$\text{percent motor deficit} = (\mathrm{NEPT} - \mathrm{EEPT})/\mathrm{NEPT},$$

where NEPT and EEPT represent extensor postural thrust on the normal and experimental sides, respectively.

There were no procedure-related deaths, no postoperative wound infections, and insignificant autonomy (self-mutilation) during the study period.

Motor recovery was analyzed by both walking track and EPT methods. Results are shown in Fig. 1. The SFI data were plotted directly as calculated. The EPT data, originally measured in grams of weight borne by each hindlimb (Table 1), were expressed as percentage deficit from total bearing weight as determined by the weight borne by the unoperated limb. There was no statistically significant difference in the level of functional recovery predicted by either testing modality at any time point.

There was a smaller average standard deviation around the EPT measurements as compared with SFI determinations. This led to earlier detection of statistically significant levels of recovery using EPT measurements (Week 5 and beyond, with the exception of Week 7, versus Week 12 and beyond for SFI). Overall, while significant recovery was observed over the 36-week study period, a substantial functional deficit remained, as is expected following complete neurotmesis injuries. In our previous work using the crush model, both testing modalities had demonstrated complete recovery over 4 to 6 weeks, as is expected with axonotmesis injuries.

The current standard for functional recovery assessment following sciatic nerve injuries has been walking track analysis, which allows computation of the Sciatic Function Index. It provides a quantitative measure of degree of functional deficit, but can be cumbersome, messy, time consuming, and variable. It also provides only one integrated measure of function.

The EPT measurements also provide a quantitative measurement of functional recovery, but are simple, easy to execute, and give consistently less variation between measurements. On average, the time it takes to perform walking track analysis, from recording the prints, cleaning the animal, and measuring the relationships, to calculating the SFI, totals approximately 20–30 min per animal. The EPT testing is done in under 30 s. Given its simplicity, it is easy to employ on a more frequent basis. It has the additional benefit that it requires minimal data manipulation to calculate the percentage motor deficit. Without the cumbersome measurements and calculation steps, it is easier to immediately identify animals whose recovery differs

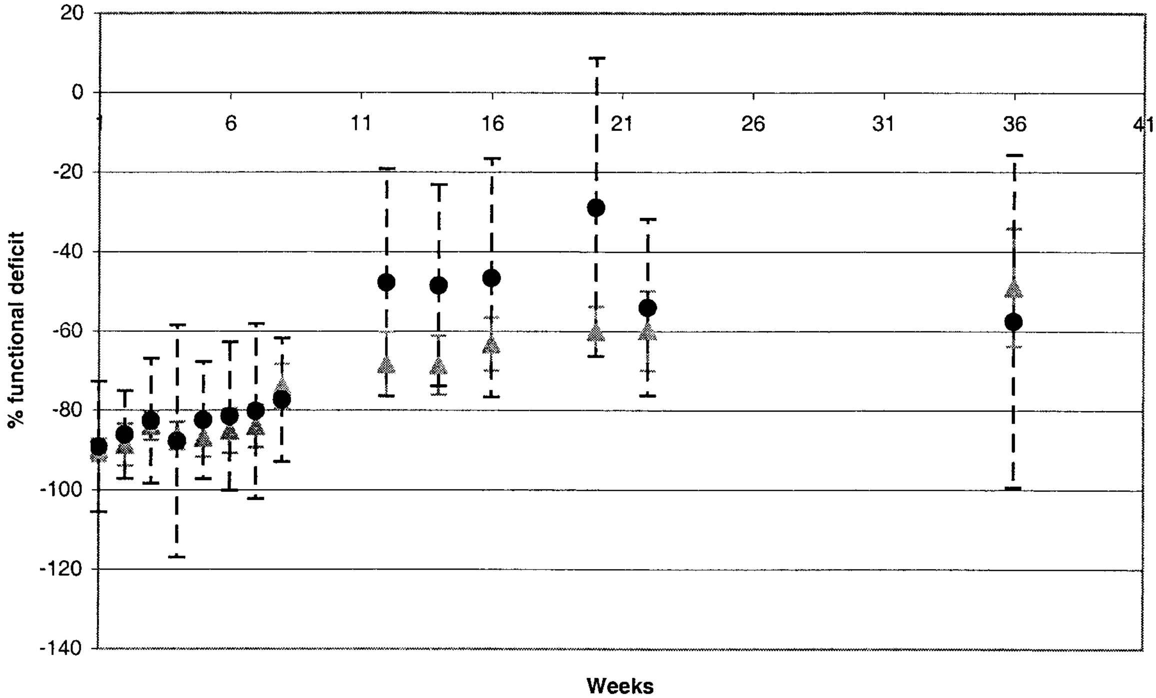

FIG. 1. Graph illustrating percentage functional deficit versus time, determined by both SFI and EPT testing. SFI data are plotted in black circles with dashed error bars. EPT data are plotted in gray triangles with solid error bars.

from that which is expected. It can be accompanied by other neurobehavioral tests when desired, to determine recovery of nociceptive, proprioceptive, and complex integrated function (3, 4, 9, 10).

Potential pitfalls of the EPT do exist. It takes a certain training period for the tester to become comfortable handling the animals, and this comfort level is critical for the animal to behave in a nonfrightened way. There is also a level of recognition of when the animal is bearing its maximum weight, which is critical since the tester is supporting the body of the animal at all times. Once this recognition takes place, through training by an experienced tester, the examination becomes highly reproducible (3, 4, 9, 10, 14).

We had previously shown excellent correlation between SFI and EPT determinations following rat sci-

TABLE 1

Rat No.:	1		2		3		4		5		6		7		8	
Hindlimb:	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Week																
2	11	150	9	130	12	115	15	140	12	116	13	111	13	113	7	99
3	13	192	15	192	16	134	20	133	12	137	15	129	15	137	7	95
4	8	124	11	130	8	113	25	113	12	116	14	102	18	122	8	106
5	15	133	21	115	19	120	26	122	15	110	22	103	15	110	16	120
6	18	119	20	120	12	110	14	110	16	115	20	94	10	118	11	107
7	22	154	14	101	15	102	16	106	7	109	24	118	10	113	11	106
8	13	95	21	110	15	102	15	120	11	110	29	117	15	116	10	104
10	25	106	15	109	28	121	18	117	11	110	20	117	18	121	12	120
12	37	113	33	106	27	110	29	107	14	109	25	97	30	104	30	113
14	53	131	43	120	45	126	44	120	21	129	45	127	33	110	35	154
16	40	135	27	120	55	141	41	118	28	119	32	121	57	131	44	143
18	60	150	56	120	45	110	38	105	30	115	37	118	55	135	47	149
20	73	150	58	131	45	110	42	92	35	110	37	109	57	140	50	150
22	78	166	57	158	47	143	62	122	35	137	47	141	75	138	57	141
36	86	120	72	130	29	116	68	140	45	112	62	116	78	118	54	114

Note. Data represent weight borne (grams).

atic nerve crush injuries. We have now demonstrated that the same holds true for more extensive neural injuries. We conclude that there is a role for EPT measurements as a potential replacement for SFI measurements in the assessment of functional recovery following neural repair.

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