Quantification of Functional Recovery Following Rat Sciatic Nerve Transection

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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.

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 steriley prepped. The sciatic nerve was exposed at the sciatic notch via a gluteal musculature splitting incision and sharply transected. Immediate microsurgical
epineurial repair was then performed, using two or three 9-O nylon sutures. The muscles were reapproxi-
mated 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 un-
derwent periodic behavioral testing using both the
standard walking track analysis and the newer exten-
stor 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 ani-
mal 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 solu-
tion, 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:

\[
SFI = \frac{-38.8(EPL - NPL)/NPL + 109.5(ETS - NTS)/NTS + 13.3(EIT - NIT)/NIT - 8.8}{1}
\]

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 perfor-
mane 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 an-
imal 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

\[
\text{percent motor deficit} = \frac{(\text{NEPT} - \text{EEPT})}{\text{NEPT}}
\]

where NEPT and EEPT represent extensor postural
thrust on the normal and experimental sides, respec-
tively.

There were no procedure-related deaths, no postop-
erative 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 def-
icit from total bearing weight as determined by the
weight borne by the unoperated limb. There was no
statistically significant difference in the level of func-
tional 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 statis-
tically significant levels of recovery using EPT mea-
surements (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 re-
maind, as is expected following complete neurotmesis
injuries. In our previous work using the crush model,
both testing modalities had demonstrated complete re-
covery over 4 to 6 weeks, as is expected with axonot-
mesis injuries.

The current standard for functional recovery assess-
ment 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 rela-
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
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-

![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.](image)

**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.

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**TABLE 1**

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.

REFERENCES