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Side distinct sciatic nerve recovery differences between rats and mice

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Abstract

The Sciatic Functional Index (SFI) is widely used to evaluate functional recovery after sciatic nerve injury, primarily in the rat, and more recently shown useful in the mouse. This quantitative, non-invasive method allows tracking of regeneration capability, visible in the gait of the animal. Using a Martin micro needle holder, carrying a force measured to be 49.2 N, the left sciatic nerve was crushed for 60 s. We accumulated data from walking tracks collected preoperatively and 1, 7, 14, 21, and 28 days after injury. SFI values were first calculated in the traditional manner. Then using the preoperative values as the normal value in the postoperative calculations, SFI was again calculated; this isolated the calculations to either injured or contra lateral leg giving a “split” plot. The traditional SFI calculations resulted in typical shaped graphs for both rats and mice. However, the “split” SFI calculations showed how rats and mice differ in their recovery from sciatic nerve injury. The mouse graph shows the intact leg remaining stable and the injured leg having functional impairment, which then recovers. The rat graph showed functional impairment of the injured leg, however, the intact leg had an increase in SFI values as if to compensate until the injured leg showed recovery.

Keywords: *Sciatic Functional Index, motor function, recovery, peripheral nerve injury, axonal injury*

Introduction

The Sciatic Functional Index (SFI), calculated from measurements of experimental animal footprints, has been shown to be a reproducible and accurate indicator of peripheral nerve recovery. This quantitative, non-invasive method allows tracking of regeneration capabilities shown in the gait of animals. After establishing normal SFI values the animals are subject to an operational procedure on the sciatic nerve creating an injury. The gait is then monitored over time to see the changes in the SFI result. The standard animal used in these types of experiments has been the rat. Initially presented by De Medinaceli et al. (1982), SFI was modified by Carlton and Goldberg (1986) and by Bain et al. (1989). There have also been calculations made to modify the index to be more specific for the mouse by Inserra et al. (1998). In this experiment we

proposed to replicate the SFI experiment using both types of animals, rats and mice, to note the similarities and differences in functional recovery. Then we endeavored to manipulate the footprint data to isolate the performance of each leg individually, again to note the similarities and differences between the species.

Depending on the evaluation method of functional recovery (Nichols et al. 2005), in nerve crush injuries, there is a simultaneous nerve functional recovery in various degrees. Traditional SFI experiments usually show recovery within 3 weeks for crush injury (Yao et al. 1998; De Souza et al. 2004; Vogelaar et al. 2004; Pavić et al. 2007). Dijkstra et al. (2000) observed a quick and complete functional motor nerve recovery in their crush group. The Sciatic Static Index (SSI) has also been used to monitor recovery of sciatic nerve injury. It is much

less time consuming (Bervar 2000; Grasso et al. 2004) and highly correlates with SFI results (Grasso et al. 2004; Baptista et al. 2007). However, in the static state some motor processes may not be taken into account. Paw length is not included in the formula for SSI.

SFI makes the assumption that the contra lateral (uninjured) leg remains a constant; this is also the case with SSI calculation. Assuming this to be true there would be no alterations in the SFI results using the contra lateral leg as normal or using the pre-injury values as normal in the SFI formulae. Meek et al. (2003) noted that injured rats generally have a walking pattern with shorter gait stance duration for the injured leg than for the uninjured leg. In addition, Dellon and Dellon (1991) documented in their paper that the contra lateral hind footprint in a severe nerve injury (sciatic cut) compensates by carrying an increased load and adjusts for this by altering its hind foot walking track. It is unknown how each leg, individually, reacts to a nerve crush of one leg. Therefore, calculating SFI as we did should show the true nature of footprint recovery.

Materials and methods

Animals

Twenty, 3-month-old male, Wistar rats weighing 250–350 g and 20, 4-month-old male, C3H mice weighing 30–40 g were kept in a temperature controlled room (24°C) with 12 h light/dark cycle (lights on at 8:00 a.m.) with free access to water and food. Experiments were performed between 4 and 7 p.m. All experiments were carried out according to the Ethical Committee guidelines, Medical School, J. J. Strossmayer University in Osijek, Croatia and in accordance with Croatian law regarding the handling and treatment of laboratory animals.

Surgical procedure

Fifteen rats were premedicated with midazolam (Dormicum[®], Roche, Basel, Switzerland), a benzodiazepine to relax the skeletal muscles. Then using a procedure described previously (Pavić et al. 2007), 15 members of both species were anesthetized using isoflurane (Foran[®], Abbott, Queensborough, UK), so that the animal was always awake but slowed motorically, followed by an intraperitoneal injection of ketamine hydrochloride (Ketanest[®], Pfizer, Vienna, Austria). An incision was made in the medial part of the left thigh (operated leg). The muscles were moved without lesion with a blunt instrument to reveal the sciatic nerve. A crush injury was inflicted with microsurgical forceps 1 cm proximal to the tibial and peroneal nerve bifurcation of

the left sciatic nerve. The instrument was closed to the first notch and held for 1 min. The force exerted at the tip of the instrument was measured to be 49.2 N. After each crush, in both species, the nerve was visibly pinched. Following the procedure, muscle and skin were closed. The remaining 10 animals, 5 rats and 5 mice, were sham operated following the above method, except no crush injury was delivered. The contra lateral sciatic nerve was not operated on and served as a control (intact leg) (Bervar 2000; Grasso et al. 2004) for the traditional SFI calculations (the normal value).

Sciatic functional index

Two walking tracks were constructed to analyze the function of the sciatic nerve; one for rats (internal dimensions 10 cm × 80 cm × 39 cm) and one for mice (internal dimensions 5 cm × 90 cm × 29 cm). Millimeter graph paper was cut in strips to fit inside the boxes. The feet of the animals were painted with non-toxic acrylic paint and the animals were allowed to freely walk the length of the track. Functional testing was performed before surgery, and then repeated at the same time of the day 1, 7, 14, 21, and 28 days following surgery. The tracks were marked according to the animal tag and checked to see if the requisite number of prints were present. If not, the walking track was repeated. The clearest consecutive three left and right footprints were measured and averaged. The Sciatic Functional Index (SFI) was calculated using three formulae derived from De Medinaceli et al.'s original calculations (1982).

Carlton and Goldberg (1986):

$$\text{SFI} = \{(NPL - EPL)/EPL + (ETS - NTS)/NTS + (EIT - NIT)/NIT\} \times 73$$

Bain et al. (1989) refined for rats:

$$\begin{aligned} \text{SFI} = & -38.3\{(EPL - NPL)/NPL\} \\ & + 109.5\{(ETS - NTS)/NTS\} \\ & + 13.3\{(EIT - NIT)/NIT\} - 8.8 \end{aligned}$$

and Inserra et al. (1998) refined for mice:

$$\begin{aligned} \text{SFI} = & 118.9\{(ETS - NTS)/NTS\} \\ & - 51.2\{(EPL - NPL)/NPL\} - 7.5 \end{aligned}$$

where E = injured, N = normal, PL = paw length, TS = toe spread (between 1st and 5th toes), IT = intermediate toe spread (between 2nd and 4th toes).

The data was reorganized by using the preoperative value as the normal (N) in the above formulae.

Consequently, the preoperative experimental (E) leg was used for the normal value in the experimental leg calculations. This manipulation forces the preoperative day results to be equal to 0 for Carlton and Goldberg, -7.5 for Inserra et al., and -8.8 for Bain et al. The resulting two plots (operated and not operated) represent the isolated behavior of the injured, left, and contra lateral intact legs of the experimental animals.

Table I. Refers to Figure 1.

Rat traditional	Crush operated rats		Sham operated rats	
	Mean	Standard deviation	Mean	Standard deviation
Preop	-2.39	19.06	0.10	8.52
1 day	-11.50	18.95	-6.64	18.30
7 days	-22.41*	17.84	-2.23	9.18
14 days	-11.87	24.61	-1.31	8.93
21 days	-11.73	17.76	0.69	12.57
28 days	-5.89	13.86	1.26	18.53

Statistically significant: *preoperation compared to 7 days.

Table II. Refers to Figure 2.

Mouse traditional	Crush operated mice		Sham operated mice	
	Mean	Standard deviation	Mean	Standard deviation
Preop	-7.15	13.83	-1.52	3.72
1 day	-27.54	26.54	-11.99	7.88
7 days	-42.97*	31.59	-4.11	4.64
14 days	-12.85	22.64	-4.23	6.13
21 days	-9.35	20.19	-4.33	10.26
28 days	-6.69	14.90	-1.67	5.36

Statistically significant: *preoperation compared to 7 days.

Table III. Refers to Figure 3.

Mouse divided	Crush operated mice	Mean	Standard deviation	Sham operated mice	Mean	Standard deviation
Preop	Operated	0	0	Operated	0	0
	Not operated	0	0	Not operated	0	0
1 day	Operated	-20.82	17.33	Operated	-9.02	15.68
	Not operated	-3.05	22.12	Not operated	-1.18	13.51
7 days	Operated	-38.52*	39.97	Operated	-17.17*	9.17
	Not operated	-1.40	27.28	Not operated	-15.03*	11.20
14 days	Operated	-13.02	25.77	Operated	-12.96	9.04
	Not operated	-6.13	20.60	Not operated	-9.97	17.34
21 days	Operated	-11.27	27.62	Operated	-13.02	24.25
	Not operated	-11.36	18.00	Not operated	-10.92	17.31
28 days	Operated	-10.63	22.63	Operated	-13.93	19.35
	Not operated	-11.92	18.52	Not operated	-13.07	18.33

Statistically significant: *preoperation compared to 7 days.

Statistical analysis

Statistical analyses used in this study are methods of descriptive statistics: average, median, and standard deviation; and nonparametric methods: Wilcoxon Signed Ranks Test, Sign Test, and Friedman Test, for confirming the differences between distributions depending upon the conditions of each separate incident. Although the statistical analysis was completed using formulae put forth by Carlton and Goldberg, Inserra et al., and Bain et al., only the results for Carlton and Goldberg are shown. The results were comparable in regards to significance in the case of each formula. Likewise, with Wilcoxon Signed Ranks Test (Wilcoxon), Sign Test, and Friedman Test; Wilcoxon results are reported in the text. The significance level in this study was <0.05 .

Results

There is an obvious similarity in the SFI values from all three formulae seen in rats and mice. We show the Carlton and Goldberg results because they have not been refined for either species which allows for visual comparison. The main differences observed with the use of Inserra et al.'s or Bain et al.'s formula was the increase in negativity of the results, otherwise the graphs followed the same shape, as expected. Median values are close in value to the mean values, and the standard deviations (Tables I-IV) remain fairly consistent throughout the experiment. Standard deviation reflects variation in individual animals rather than deviation of footprint measurements of any individual.

In rats (Figure 1) a significant drop in SFI values is shown 7 days after injury, followed by functional recovery by 28 days after injury. Wilcoxon in comparing preoperative and 7 days was significantly

Table IV. Refers to Figure 4.

Rat divided	Crush operated rats	Mean	Standard deviation	Sham operated rats	Mean	Standard deviation
Preop	Operated	0	0	Operated	0	0
	Not operated	0	0	Not operated	0	0
1 day	Operated	4.66	17.72	Operated	3.15	14.36
	Not operated	11.10	14.71	Not operated	9.62	13.18
7 days	Operated	-4.80	11.27	Operated	-1.39	7.86
	Not operated	16.11*	22.11	Not operated	2.24	20.18
14 days	Operated	0.15	24.94	Operated	2.14	7.76
	Not operated	8.54	13.36	Not operated	3.86	14.93
21 days	Operated	5.49	13.96	Operated	8.55	12.12
	Not operated	14.55	20.25	Not operated	10.14	7.33
28 days	Operated	13.21**	17.28	Operated	9.01	9.17
	Not operated	15.02**	18.12	Not operated	9.10	15.14

Statistically significant: *preoperation compared to 7 days; **preoperation compared to 28 days.

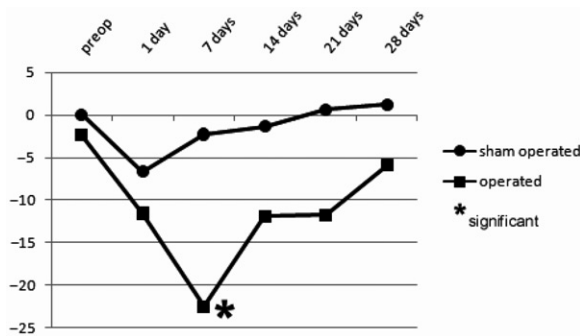


Figure 1. Results of the average SFI values, calculated using the formula of Carlton and Goldberg (1986), and using results from both hind footprints (from operated leg and contra lateral leg) for rats, and the same for sham operated rats.

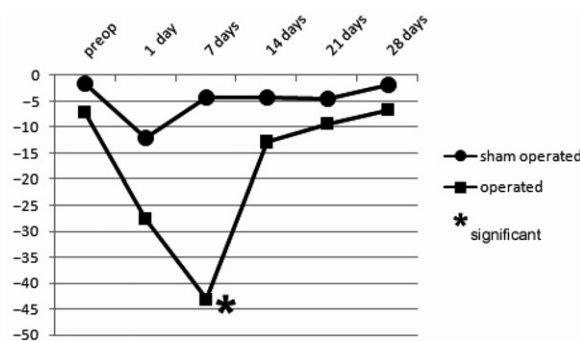


Figure 2. Results of the average SFI values, calculated using the formula of Carlton and Goldberg (1986), and using results from both hind footprints (from operated leg and contra lateral leg) for mice, and the same for sham operated mice.

different ($p=0.007$). Recovery is visible from this graph if we compare the preoperative day with 4 weeks ($p=0.75$). This same pattern is seen in mice (Figure 2), Wilcoxon showed significance at 7 days ($p=0.006$) and no significance at 28 days ($p=0.65$).

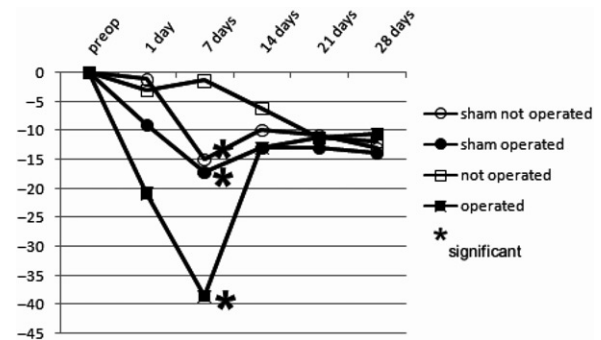


Figure 3. Results of the average split SFI values: operated leg and not operated leg, for operated and sham operated mouse groups.

The sham groups of rats and mice show no significance; at 7 or 28 days (rat p 7 days = 0.50; p 28 days = 0.69; mouse p 7 days = 0.69; p 28 days = 0.90).

In the split mouse, shown in Figure 3, the operated leg's footprints showed a deficit in function 7 days postoperatively, while the intact leg footprints remained fairly constant. Wilcoxon showed significance in the operated group, preoperative to 7 days postoperatively ($p=0.008$ for operated, $p=0.69$ for not operated), while preoperative to 28 days ($p=0.09$ for operated, 0.03 for not operated). It can be seen that at week 3 there is equalization of function in each of the formulae. The mouse sham group followed a similar pattern of the not operated leg, however, the SFI values drop in both legs more than those of the operated mice which results in significance shown at 7 days ($p=0.04$ operated, $p=0.04$ not operated) and no significance at 28 days ($p=0.14$ operated, $p=0.23$ not operated). Sign Test and Friedman Test on all these groups (sham operated: 7 and 28 days, sham not operated: 7 and 28 days) were not significant.

In the split rat, shown in Figure 4, values for the injured side fell 7 days after operation, and then

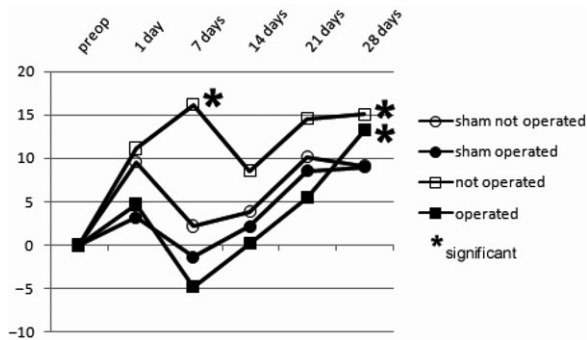


Figure 4. Results of the average split SFI values: operated leg and not operated leg, for operated and sham operated rat groups.

recovered function after this to reach equalized function with the intact leg. Interestingly, the intact leg had a remarkable increase in SFI value, which shows a decrease when the injured leg showed recovery. Wilcoxon showed in the comparison of preoperative day and 7 days not significant ($p=0.13$) for operated and significant ($p=0.03$) for not operated. In the comparison of preoperative and 28 days both are found to be significant ($p=0.02$ for operated, $p=0.03$ for not operated). Sham results show no significance for the operated or not operated leg, at 7 days ($p=0.50$ operated, $p=0.89$ not operated) or at 28 days ($p=0.08$ operated, $p=0.35$ not operated).

Discussion

The crush force which was applied in this experiment had a considerable pressure but of short duration. Compared to most papers we held the pressure for a relatively short time, some held for 10 min (De Souza et al. 2004) others 2 min (Grasso et al. 2004), however, some were held for “seconds” (Dash et al. 1996; Tuma et al. 1999). As a result of the short duration the SFI values are perhaps not as negative but all the features of the SFI graph are present. In crush injury lesions the recovery period is shorter than that of nerve grafts, as described by Carlton and Goldberg (1986), Dijkstra et al. (2000), and De Souza et al. (2004).

In the present experiment species-specific boxes were constructed, and non-toxic acrylic paint, slightly diluted, was used to mark the feet. This produced satisfactory footprints. Various different substances have been used such as soap powder (De Souza et al. 2004), glycerol (Brown et al. 1989), or ink (Bervar 2000). However, there has always existed a problem with measurement accuracy, missing or incomplete prints as well as smearing (Brown et al. 1989; Bervar 2000;

Grasso et al. 2004). In respect to the type of animals, Brown et al. (1989) explained how Wistar rats were problematic in experiments accounting SFI because they walked on dorsum experimental (with lesion on sciatic nerve) feet. We did not experience this even though we used Wistar rats. There is some concern that, between rat species, variation in nerve regeneration occurs. Footprints made by one species of rat, for example, Wistar, differ from a ship rat (Yuan et al. 2005). However, as the preoperative values used were specific to the individual rat or mouse this variable should not come into play, however, our normal values should not be used as a standard normal value for all rats or even another group of Wistar rats as we noticed variability between individuals.

Between rats and mice there is a difference in recovery type, as seen in the compensated leg in the rat. SFI experiments have been run using various types of rats, and the standard SFI graph shape has always been achieved, as with mice. The difference in this study is that we show how, using a truly healthy footprint measurement (preoperative), the individual legs react in a nerve crush experiment. Whereas using the traditional calculation, although showing nerve recovery requires the assumption that the contra lateral leg is not effected and therefore normal. It could be argued that the smaller print size of mice, rather than an intrinsic difference between rats and mice cause the differences seen in the split graphs. While a more sensitive measure may pick up this finer variability, we noticed very low deviation of each individual measurement of each animal. While rat measurements are intrinsically larger, the differences between measurements were not frequently larger than 3 mm between footprints of the same walking track. In the mice, the individual measurements of footprints of a walking track usually did not differ more than 2 mm. If this was a case of size then the traditionally calculated SFI would also be affected, but it appears graphically as a variation of that seen in rats. Repeating this experiment with a more “advanced” and sensitive method of tracking, such as video assessment of gait could possibly refine the results in a more comprehensive fashion than traditional SFI tracking.

Another problem that may be experienced in SFI testing is automutilation (Meek et al. 1999, 2003; Dijkstra et al. 2000; Nichols et al. 2005) which seems to afflict animals with nerve graft surgery more frequently than in nerve crush injuries. In our study we had three rats which bit at the plantar surface of the operated leg’s paw. The damage inflicted was superficial; all three recovered spontaneously before testing at 14 days and were not eliminated from the study. Automutilation did not occur in the sham group. SFI cannot be calculated with missing digits.

However, it may be possible for various motion tracking to continue with mutilations (Nichols et al. 2005). Gait cycle and stance phase analysis is also becoming an invaluable technique by some peripheral nerve investigators for the evaluation of function (Varejão et al. 2002). Videotaping allows for the whole motion of the animal to be taken into account when measuring footprints.

Sciatic injury on mice has also previously been described in the literature (Inserra et al. 1998; Yao et al. 1998). It can also be found that mice do not leave good footprints for SFI calculation (Vogelaar et al. 2004) but we again did not find this a problem. We dealt with inadequate footprints with rats and mice in the traditional manner of repeating illegible walking tracks within the same collection time, but this was infrequent.

Most problems dealing with ink or legibility are addressed with computer-based video assessment. The SSI calculation can be assessed with a computer scanning method so that the measurements can be done quickly (Grasso et al. 2004). Bervar (2000) with video assessment confirmed the fact that the 1–5 toe spread is the most useful parameter for measuring functional recovery after sciatic nerve injury (Bain et al. 1989; Bervar 2000), our results also confirm this. However, in SSI the operated and intact legs have the same assumption as SFI that the uninjured leg is normal: $SSI = 108.44TFSF + 31.85ITF - 5.49$; where TSF and ITF are the ratio of (injured – uninjured)/uninjured of either toe spread 1–5 (TS) or intermediate toe spread 2–4 (IT) (Bervar 2000; Grasso et al. 2004). The print length, in these cases, demonstrates variation with uncontrolled gait velocity and is statistically insignificant (Bervar 2000). It has been found in motion of the foot experiments, that the gait velocity highly impacts the results of print length (Varejão et al. 2004). In Belin et al. (1996) only the print length was analyzed (print length ratio = normal print length/experimental print length) and showed there was impairment in adult rats with a 60 s crush injury, however, in this experiment they separated out the tibial nerve, not the sciatic nerve, on which to perform the crush. The Catwalk method (Vogelaar et al. 2004) used similar measurements as in De Medinaceli et al. (1982) but used computer-assisted videotaping, which allows the evaluation of intensity of the footprints. It was found that footprints made with the De Medinaceli et al. (1982) method do not require much pressure to produce a print.

SFI is expressed as the percentage of the difference between injured and intact contra lateral paw (De Souza et al. 2004). As with the video index (Bervar 2000), the not operated leg is used as a control. We calculated the traditional SFI using the

contra lateral (normal) values. We plotted typically shaped SFI graphs for rats and mice. Then using the same data we forced the preoperative values to 0, by using the preoperative values as the normal value; this allowed the left and right legs to be evaluated separately. What we discovered is that rats and mice with a unilateral crush injury to the sciatic nerve both recover, by SFI standards, by 28 days, but it affects their walking in different ways.

In this experiment we see that rats and mice both follow similar recovery patterns to a standardized crush injury. It is shown as expected in Figures 1 and 2. The divided mouse graph (Figure 3) looks like a typical sham and crush injury SFI graph. It also resembles the SSI graph of injured and intact sciatic nerve paw prints. This is what we expect to find in rats and mice. However, the divided rat graph (Figure 4) suggests that the rat shifts weight onto the uninjured side to compensate for the injury. Toe spreads are therefore affected on the uninjured side and hence a graph that doesn't resemble that of a sham. This type of graph was not expected.

We therefore took a closer look at the behavior of the toe spread (TS) values. In the mouse, the TS diminished in the operated leg and in the intact leg the TS value remained constant. In the rat, the TS values were also diminished in the operated leg until 3 weeks when it returned to preoperational measure. But the intact leg showed diminished measure 1 day post operation followed by an increase in TS through the full experimental time of 28 days. The consistency in prints generated by one rat within and between tracks (Brown et al. 1989) was present. This shows there is a side distinct recovery difference between rats and mice.

When motoneurons were measured after a sciatic nerve crush procedure, it was found that there was also a decrease in the intact side (Behnam-Rasouli et al. 2000). This was possibly caused by the crossover interneurons, but it may be the same phenomena that we see with the split SFI graph for the Wistar rats. It has also been found with the Catwalk method that in rats and mice a return to before injury footprint intensity was not achieved until much later than the recovery predicted by De Medinaceli et al. (1982). Even though recovery is shown using SFI formulations, pain persists for some time afterwards, causing both species to reduce the amount of pressure placed on the injured nerve side foot. The mice recovered from this by 28 days while rats persisted through 70 days (Vogelaar et al. 2004). We also saw that the traditional rat SFI did not return to preoperative levels, it is quite possible that this can be explained with the neuropathic pain which Vogelaar et al. (2004) noted. It is also feasible therefore that the rat spreads its toes more on the

intact side to compensate for the decreased pressure placed on the injured side's paw. This seems to only appear when the animal is walking, since a static pose would create a consistent stance for the uninjured leg (Grasso et al. 2004). However, it has been noted that there is contra lateral non-operated paw compensation (Dellon and Dellon 1991), in Sprague–Dawley rats which we confirm here with Wistar rats. This may be an adaptation of the central nervous system (CNS) in the rat. Stance phase research of the rat's gait also points to differences between legs, injured rats generally have a walking pattern with shorter gait stance duration for the injured leg than for the uninjured leg (Meek et al. 2003).

The improvements upon SFI with video assessment and computer-assisted components will only lead to more refinement but with the assumption of contra lateral leg normalcy. We found that using the preoperative values for each leg shows a difference between the species. In footprint analysis and calculation of traditional SFI for rats we can see total functional recovery 4 weeks after lesion of the left sciatic nerve. In mice this same recovery was evident after 2 weeks following injury. When the legs are divided, in rats, the contra lateral leg, which was intact from injury, compensated for the functional impairment of the operated leg. In mice, when the legs were divided, this did not appear to be the case rather the typical SFI plot appeared with the intact leg looking as a typical sham control. The traditionally calculated SFI for rats and mice are quite similar in behavior and recovery research is consequently linked, the results of this study suggest that they should not be. Since results in the rat suggest compensation for its injury and the results in the mouse do not, at least in the same way. With this evidence of the TS differences it can be seen that there is a distinct difference in the recovery process between these two species. The question now is which, rats or mice, better describes man's nerve recovery?

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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