Abstract
The rat sciatic nerve is a well-established animal model for the study of recovery from peripheral nerve injuries. Footprint analysis is the most widely used non-invasive method of measuring functional recovery after injury in this model. We describe a new, alternative video analysis of standing (or static footprint video analysis) to assess functional loss following injury to the rat sciatic nerve, during animal standing or periodic rest on a flat transparent surface. We found that this alternative video analysis is technically easier to perform than the corresponding footprint video analysis during walking, but still preserves all advantages of video versus conventional ink track method: i.e. there are few non-measurable footprints, better repeatability, high accuracy and more precise quantification of the degree of functional loss after sciatic nerve injury in the rat.

1. Introduction

Characteristic gait changes occur after unilateral sciatic nerve injury in rats. Gradual disappearance of these changes in time reflects nerve regeneration and functional recovery. The degree of functional loss (or recovery) can be quantified by the method known as footprint analysis (De Medinaceli et al., 1982). The method is simple, non-invasive and has been shown to measure a combination of motor and sensory recovery. It can be used repeatedly to measure functional recovery over time in the same animal.

Since its introduction, it has been modified several times (Carlton and Goldberg, 1986; Bain et al., 1989). The measurements required to make the calculations, which include print length and toe spreads, is limited with up to 25% of the prints unreadable and is unreliable when contractures or injured toes are present (Dellon and Mackinnon, 1989). In addition, the print length demonstrates significant variation with uncontrolled gait velocity, which may incur the functional loss estimation errors (Walker et al., 1994).

The video imaging technique to record the footprints was a considerable advancement in this field (Lin et al., 1992). It allowed digital analysis of the video images and better repeatability. It significantly diminished the number of useless footprints and minimised printing errors in comparison with previous methods. But the problems with changeable gait velocity remained and the special kind of contrasting to increase the visibility of footprints was necessary too.

The self-evident question raised: can we accurately predict the functional
loss by the footprint video analysis in the technically more acceptable static conditions during animal standing?

2. Materials and Method

Twenty-four adult male white Wistar A rats weighing 250 to 350 g were used. Under intraperitoneal Ketamine anesthesia we exposed the sciatic nerve unilaterally and two types of injuries were performed: a crush injury (n=12) and a transection injury with an immediate epineurial repair (n=12). Opposite leg and sciatic nerve were not operated upon and served as a control.

Functional recovery after the sciatic nerve injury was assessed serially using video recording of the plantar aspect of the animal hind feet during occasional rest periods in a glass-bottomed box. For recording we used video camera, set at focal length of 9 mm and placed 20 cm apart from the bottom. No additional contrasting was necessary.

Under such conditions, the positions of the toes and the sole pale skin discoloured areas, caused by spontaneous body weight pressure, were clearly visible in most instances in the footprints during the video tape playback in super-still mode. The distance between the tip of the third toe and the most posterior margin of the sole discoloured area was defined as the print length parameter on the video images.

A single recording of each animal lasted approximately 60 seconds. Video assessment of the hind footprints were obtained twice preoperatively and on post-injury days 1, 7, 14, 17, 21, 24, 28, 35 and 42 for a crush injuries, and on post-injury days 1, 21, 45, 75, 105, 135 and 165 for a transection injuries.

On the same days, assessment of the hind footprints was obtained by applying a quick-drying non-toxic water-soluble ink to both hind feet and allowing the animal to walk freely down the walled walkway, leaving tracks on the underlying paper.

The parameters of print length, 1-5 toe spread, and 2-4 toe spread of four injured and four uninjured hind footprints were measured from both the video recordings and the ink tracks of each animal on each assessment day. The measurements from these four footprints were averaged to determine the parameter values for that day. Ratios of (injured/uninjured)/uninjured hind feet parameters (or factors PLF, TSF and ITF) were determined by both static video and dynamic ink track method. The latter were used for assessment of the functional loss by sciatic functional index SFI as described by Bain et al., (1989).

All data were computerised and statistically analysed with the SPSS program. An alpha value of 0.01 was used as an index of statistical significance.

3. Results

We found good correlation between video recording during standing and dynamic ink track footprint parameter measurements for both 1-5 and injured 2-4 toe spreads only. Reproducibility for these three parameters was also better using the video method. Uninjured 2-4 toe spread by video showed a poor correlation and similar reproducibility as compared with ink. However, both print length parameters measured by video had poorer correlation and reproducibility, particularly the print length factor (PLF) was weakly correlated with that determined by ink.

Contribution of the footprint factors on the estimated functional loss has also changed in conditions during standing. It was most prominent for the 1-5 toe spread factor (TSF), near marginal for the 2-4 or intermediary toe spread factor (ITF) and weak, statistically insignificant for the print length factor (PLF).

Thus, the introduction of a new functional loss index, or so called static sciatic index (SSI), and its estimating formula:

$$SSI = 108.44 \times TSF + 31.85 \times ITF - 5.49$$
was mandatory.

Moreover, using a simple ratio of injured/uninjured 1-5 video toe spread (Walker et al., 1994) as a substitute for the static sciatic index (SSI), we could achieve considerable simplification of the method without any significant loss of accuracy.

Recovery patterns over time determined by the three methods for two types of sciatic nerve injuries in the rat were generally very similar as far as the beginning and the plateau of functional recovery is concerned.

For more details see the reference (Bervar, 2000).

4. Discussion

The toes and the heel position on the video recordings were evaluated during standing and this probably involves different neurophysiological mechanism from those effective during walking. The position during standing is mainly dependent upon postural muscle tone while the toe and the heel position during walking depends upon dynamic changes in muscle activity. This might very well be the cause for the considerable changes of the proposed formula for the functional loss estimation in the static conditions.

On the other hand, our observations confirmed the fact that the 1-5 toe spread is the most useful parameter for measuring functional recovery after a sciatic nerve injury. It is dependent on anatomic contributions from both the peroneal and tibial divisions of the sciatic nerve (Bain et al., 1989) and, therefore, closely follows the sciatic functional recovery as a whole. This parameter also demonstrates the greatest deviation from the normal of all studied parameters allowing measurement of smaller changes after injury and during recovery. As such, this parameter has the greatest “weight” in all proposed formulas and can be used alone in the form of a simple ratio of injured/uninjured hind foot video 1-5 toe spread expressed in % to assess functional loss by the video recording method during standing.

5. Conclusion

Although our static video footprint analysis requires more expensive technical equipment we think it has significant advantages in comparison to conventional ink track method: i.e. there are few non-measurable footprints, it displays better repeatability, and therefore higher accuracy and more precise quantification of the degree of functional loss and recovery after sciatic nerve injury in the rat.

References


