

## CHAPTER 8

### **Functional nerve recovery after bridging a 15 mm rat sciatic nerve gap with a biodegradable nerve guide**

MF Meek,<sup>1</sup> F Klok,<sup>2</sup> PH Robinson, FRCS,<sup>1</sup> J-PA Nicolai,<sup>1</sup> JFA van der Werff,<sup>1</sup>  
A Gramsbergen<sup>2</sup>

<sup>1</sup> Department of Plastic Surgery, University Hospital Groningen,  
Groningen, The Netherlands

<sup>2</sup> Department of Medical Physiology, Groningen University,  
Groningen, The Netherlands

*Submitted for publication, 2000*

**Abstract**

*Recovery of nerve function was evaluated after bridging a 15 mm sciatic nerve gap in the rat with a biodegradable poly(DL-lactide- $\epsilon$ -caprolactone) nerve guide. Nerve recovery was investigated by analyzing the foot prints, by analyzing videorecordings of gait, by electrically eliciting the withdrawal reflex, nerve conduction velocity and electromyography (EMG). Sensory nerve function recovered as measured by the electrostimulation. Motor nerve function partly recovered but electromyograms remained highly abnormal throughout the study. It is concluded that functional reinnervation by regenerating axons occurs after bridging a 15 mm nerve gap with a biodegradable poly(DL-lactide- $\epsilon$ -caprolactone) nerve guide, but the walking patterns remain abnormal. The use of video analysis is a useful tool to record and analyze the walking patterns of the rat. Further studies are necessary to investigate the possibility of obtaining selective reinnervation of specific muscles.*

**Introduction**

The most commonly used technique to bridge a peripheral nerve gap is an autograft. One promising alternative to prevent a donor site is the use of artificial nerve guides. Several conduit materials have been investigated.<sup>1</sup> The use of biodegradable nerve conduits is particularly promising. After functioning as a temporary scaffold for axon outgrowth, they degrade in the course of months or years.

Den Dunnen et al. showed that a biodegradable nerve guide composed of an amorphous copolymer of DL-lactide and  $\epsilon$ -caprolactone [p(DLLA- $\epsilon$ -CL)] is effective with regard to nerve outgrowth.<sup>2</sup> They also concluded that a nerve guide with an internal diameter of approximately 1.6 mm and a wall thickness of 0.3 mm functioned best in a rat model. Previous studies with this type of material demonstrated that tube dimensions and swelling of degrading biomaterials are important parameters for the final outcome of nerve regenera-

tion.<sup>3</sup> Meek et al. showed that thin-walled nerve guides tend to collapse,<sup>4</sup> unless some mechanical support inside the lumen is used,<sup>5</sup> such as muscle tissue.<sup>6</sup> Thus far, p(DLLA- $\epsilon$ -CL) nerve guides were used to bridge 10 mm sciatic nerve gaps, which has been shown to be a critical length to bridge in the rat model.<sup>7,8</sup>

Because the results of microscopical analysis and functional nerve recovery may be conflicting,<sup>9-12</sup> conclusions about the return of function cannot be drawn solely on the basis of an analysis of the light- or electron microscopy at the repair site. To establish sciatic nerve function in the rat, often foot print analysis is used to calculate the sciatic function index (SFI). According to Dellon and Mackinnon, however, the gait pattern cannot be analyzed by foot print analysis when chronic foot deformities or automutilation occur.<sup>10</sup> The use of other evaluation techniques, therefore, is necessary to obtain reprodu-

cible data on the recovery of nerve function in order to understand the events during recovery. In 1979, Hruska et al. introduced footprint analysis using the video analysis technique and assessed the efficacy of measuring swing and stance duration of the normal walking pattern.<sup>13</sup> Westerga and Gramsbergen introduced the use of a mirror to show both the plantar surface and the side-view of the rat's hindpaw.<sup>14</sup> It is important to note that by using the video analysis technique the movements during walking can be objectivated as well.

In this study we bridged a 15 mm gap in the sciatic nerve of the rat by a biodegradable p(DLLA- $\epsilon$ -CL) nerve guide. The recovery of nerve functions was studied by using a combination of evaluation methods (walking track analysis, video analysis, withdrawal reflex, nerve conduction velocity and electromyography). The results were compared with the non-operated contralateral side as well as a non-operated control group.

## Materials and Methods

### Surgical Procedures

In total, 57 Male Wistar rats weighing approximately 250 g (225 - 275 g) were studied. In group A (n = 51), the rats were premedicated with atropine (0.25 mg/kg body weight) and anaesthetized with 1% isoflurane (Forene<sup>®</sup>) and O<sub>2</sub>/N<sub>2</sub>O. The left sciatic nerve was exposed by splitting the left superficial gluteal muscle. A gap of 15 mm was made and bridged by a 18 mm biodegradable nerve guide (Polyganics BV, Groningen, The Netherlands), composed of a

copolymer of 50% DL-lactide and 50%  $\epsilon$ -caprolactone [85% L-lactide (LLA) and 15% D-lactide (DLA)]. The internal diameter of the nerve guide was approximately 1.6 mm (range 1.57 - 1.67 mm) and the wall thickness 0.3 mm (range 0.27 - 0.37 mm). Both the proximal and distal ends of the sciatic nerve were telescoped into the nerve guide and fixed with a single 10-0 nylon epineural suture. The tube was prefilled with phosphate buffered saline. In group B (n = 6), the rats were not operated, received no anaesthesia and served as a control group.

Surgical procedures were performed under an operation microscope (magnification 25 x) and a sterile procedure was used. Following surgery, the animals were housed in a temperature- and humidity-controlled room with 12 hr light cycles and had access to standard rat food and water ad libitum. Good laboratory practice (GLP) was maintained, according to the National Guidelines for Animal Welfare, comparable with the international rules for animal experimentation (International Guide for Animal Biomedical Research and Ethical Code for Animal Experimentation of the Council for International Organization of Medical Sciences).

### Evaluation Tests

#### Walking Track Analysis

At 2, 4, 7, 15, 21 and 36 weeks, finger paint was applied onto the plantar surface of the hind feet and it was ensured that all anatomical landmarks were covered. The rat was allowed to walk down a track, leaving prints of its feet on the paper. From the footprints,

the sciatic function index (SFI) was calculated using the formula developed by Bain and Mackinnon.<sup>15,16</sup> An SFI of 0 is normal whereas an SFI of -100 means total impairment.

Next, the rats were placed in a Perspex runway.<sup>14</sup> The lateral view of the animal was recorded directly with a video camera, whereas the ventral view of the animal was visualized by means of an adjustable mirror under the cage, positioned at an 45° angle. In this manner, a split screen image was obtained with the lateral view of the rat in the upper half and the ventral view in the lower half (Fig. 1). The runway was illuminated with two 120 W concentric bulbs to improve contrast and to enhance the point of foot-contact. Walking movements of the rat were recorded with a video camera containing a stroboscopic shutter (25 frames per sec), creating blur-free stills for analyzing the footsteps, until at least 4 consecutive and non-hesitant step cycles were collected. The video-tape was then replayed frame by frame, until the maximal contact of the rat's foot to the floor was reached. The SFI was subsequently measured from these images.

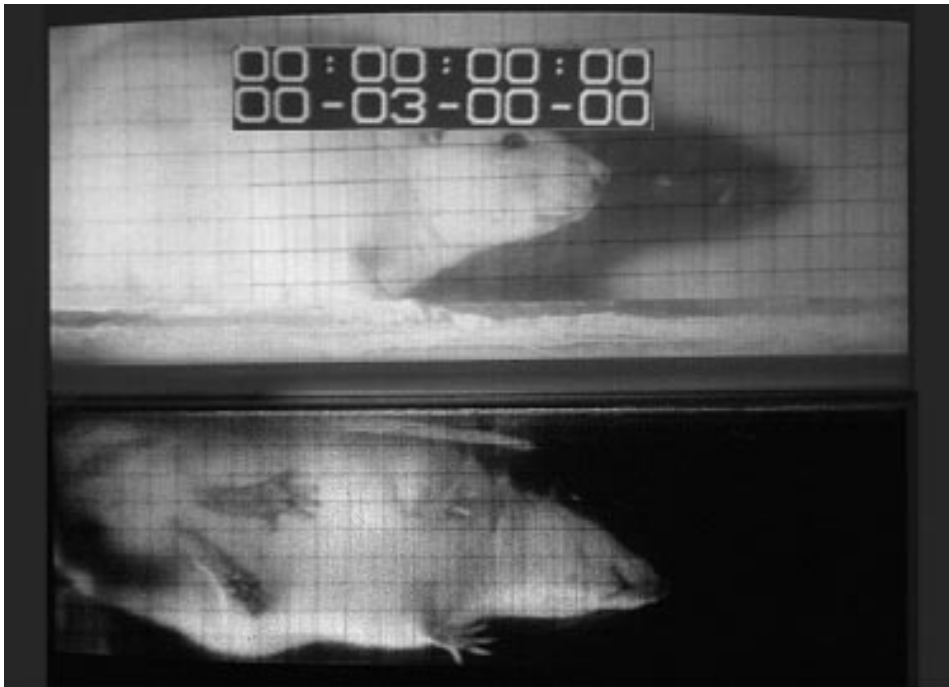
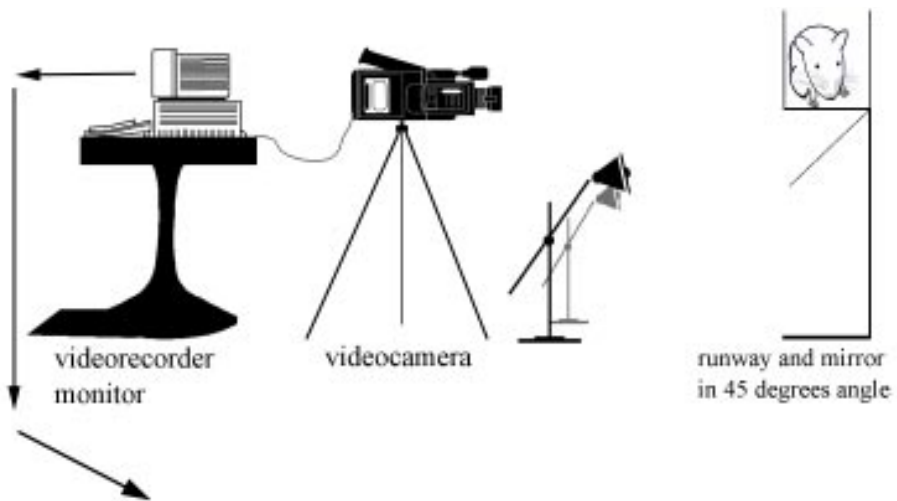
The video recording technique was also used to obtain the stance factor, as described by Walker et al.<sup>17</sup> The stance factor is the ratio between duration of floor contact (gait-stance duration) between the left operated and the right non-operated hind paw. Injured rats generally show a walking pattern with a shorter gait-stance duration of the injured leg than of the non-injured leg.

### **Withdrawal Test**

At 2, 4, 7, 15, 21 and 36 weeks, an electrostimulation test was carried out on the sole of the foot using a bipolar electrode consisting of two copper wires. The lateral side of the left (operated) sole was stimulated proximally, distally and in between in all rats, as described by De Koning.<sup>18</sup> A healthy rat immediately withdraws its foot and spreads its toes after stimulation. The threshold, i.e. the lowest current causing this reflex, was recorded. Then, at each evaluation period, the sciatic nerve was cut in 3 rats and again the foot-sole was electrically stimulated in order to verify the role of the sciatic nerve in the withdrawal test at the respective ages.

### **Conduction Velocity (CV)**

At 2, 4, 7, 15, 21, and 36 weeks, the sciatic nerve was stimulated proximally and distally to the nerve guide using a bipolar silver electrode under general anaesthesia in 3 rats. During the recordings, the body temperature of the animals was maintained at 37° C. The muscle action potentials (MAP) from the gastrocnemius muscle (GC) and the anterior tibial muscle (TA) were recorded with micro needle electrodes and the conduction velocity (CV) was measured.<sup>19</sup> The MAP was displayed on an oscilloscope at settings appropriate to measure the latency time from stimulus to the onset of the first negative deflection. A deflection in the form of a compound action potential in the oscilloscopic screen symbolized the neural response. All data were collected on a data recorder. The distance between the electrodes proximally and distally to



*Fig. 1. Sketch and photograph showing the experimental set-up for the video analysis. A lateral view as well as a ventral-view of the animal were simultaneously obtained.*

the nerve guide was measured and the CV was calculated.<sup>20</sup> Finally the animals were killed by an intracardiac overdose of barbiturate.

### **Electromyography (EMG)**

At 15, 21, and 36 weeks of implantation of the nerve guides, EMG electrodes were implanted under anesthesia in the hindlegs of 9 rats (3 at each evaluation period). EMG electrodes were made of multistrand stainless steel wire (diameter 0.003 inch), insulated by a teflon coating except for a bare tip of 1 mm. The electrodes were implanted in pairs and the interelectrode distance was 1-1.5 mm with both bare tips orientated from proximal to distal. A common electrode was placed in the lower back region. Pairs of electrodes were implanted in the midbelly regions of the GC and the TA muscles of both hindlegs. The electrodes were sutured to the fasciae of the muscles and the electrode wires were led subcutaneously to the back and soldered to a miniature connector. EMG recordings were made approximately 8 hr after recovery from the anaesthesia on the day of operation as well as on consecutive days. The animals were allowed to walk freely on a flat surface and they were connected to an amplifier system. The EMG signals were stored and processed off-line on a personal computer. EMG recordings were then rectified and averaged and displayed for visual inspection (For further details see Gramsbergen et al.<sup>21</sup>).

### **Results**

In group A, 20 of the 51 rats sooner or

later showed automutilation of the operated hindpaw. From these rats, the SFI could not be calculated. One of these rats showed automutilation of the complete lateral side of the sole of the foot, and was therefore excluded from the electrostimulation test as well.

### **Sciatic Function Index (SFI)**

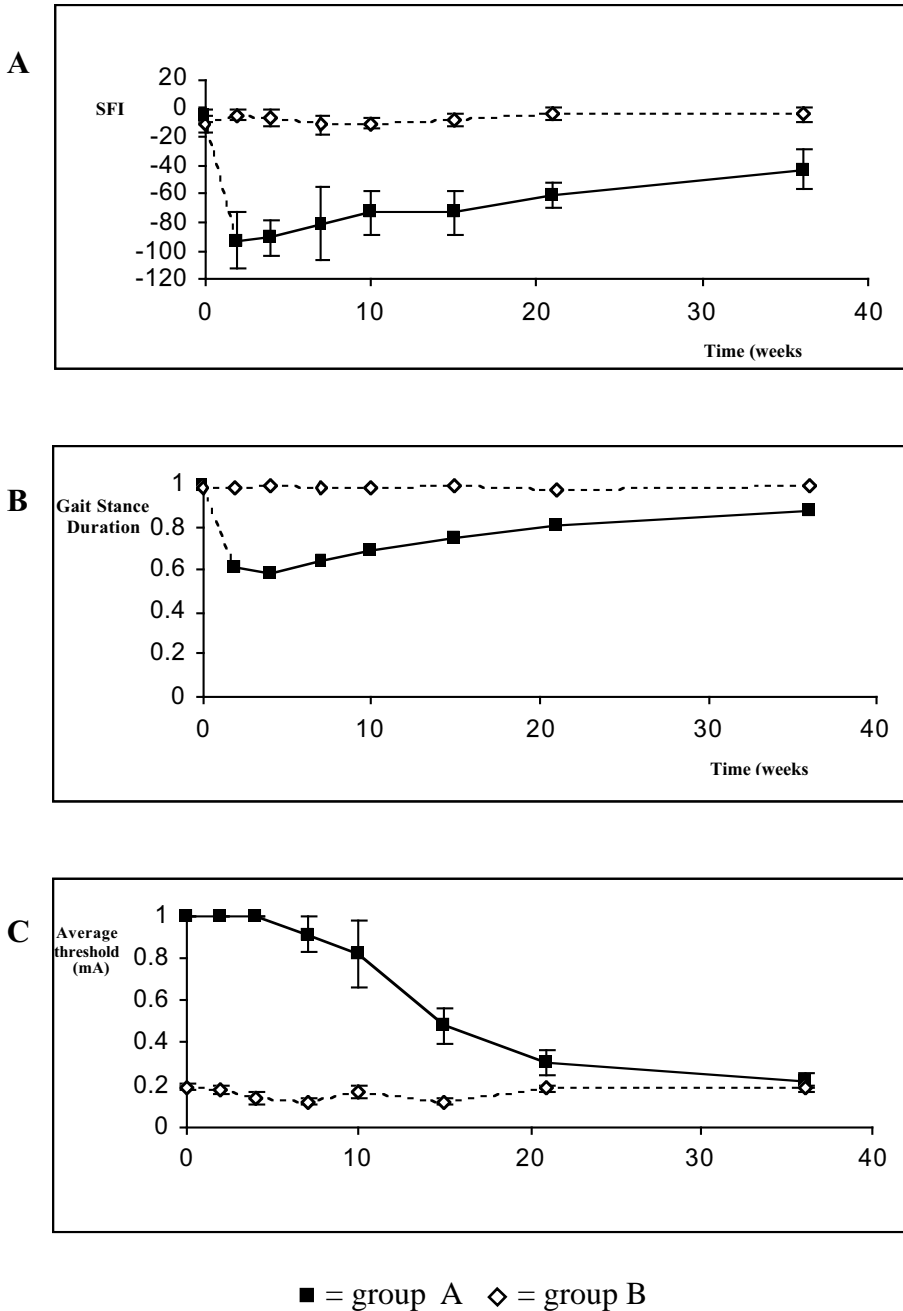
SFI values obtained by video recording did not differ from those on paper in both groups. Preoperative sciatic nerve function indices for group A (nerve guides) did not differ from the control group B (non-operated control rats). In group A, the SFI significantly increased with time from -93 after 2 weeks to -43 after 36 weeks of implantation (Fig. 2A). In group B, reproducible walking track patterns could be measured in all rats and the mean SFI did not change during the study (Fig. 2A).

### **Stance Factor**

In group B no measurable differences were observed in gait-stance durations of the left and right hindpaws throughout the evaluation period (Fig. 2B). In group A, the gait-stance duration of the left injured leg was of a shorter duration than the right non-injured leg. The stance factor increased with time, but did not recover to control values (Fig. 2B).

### **Withdrawal Test**

In group A, a withdrawal reflex could not be elicited in the first weeks after implantation. From 7 weeks onwards, the first positive reflexes were obtained but with high thresholds. This recovery continued and after 36 weeks, the threshold had decreased to almost control values (Fig. 2C). The values at the



**Fig. 2.** Graphs showing changes in Sciatic Function Index (SFI) (A), the gait-stance duration - ratio injured : non-injured hindfeet (B), and the average current intensity necessary to elicit a withdrawal reflex (C) with time. Filled squares, rats with nerve guides; open diamonds, control rats.

contralateral side were not significantly different from the non-operated control rats.

To verify the role of the saphenous nerve in the reflex, the sciatic nerve was again cut (proximal to the implantation side) in 3 rats at 2, 4, 7, 15, 21, and 36 weeks. Until 7 weeks after implantation of the nerve guides the withdrawal reflex did not disappear after the sciatic was cut. However, from 15 weeks and beyond, the reflex could not be elicited after secondarily cutting the sciatic nerve.

### **Conduction Velocity (CV)**

The mean CV in the right non-operated paw was  $53 \pm 6$  m/s. In the first weeks after implantation of the nerve guides, MAP's could not be obtained. After 7 weeks, MAP's could be obtained in one out of three rats, and the CV was 9 m/s. Thereafter, the mean CV gradually increased to  $27 \pm 8$  m/s after 36 weeks.

### **Electromyography (EMG)**

In control rats, the swing phase started with a shortlasting, brisk burst in the TA. During the stance phase this muscle was electrically silent (Fig. 3; asterisks indicate onsets of swing phases). EMG amplitudes in the GC muscle dropped during the swing phase, whereas the stance phase was accompanied by a tonic burst in the GC muscle.

In the right non-operated side of the animals in group A sometimes extra bursts (Fig. 4; see arrows) and irregular phasing of stance and swing phase could be observed (Fig. 4).

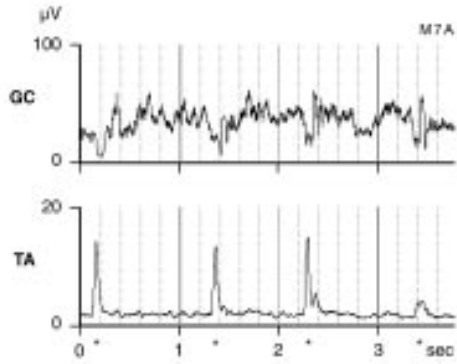
At the left operated side, the EMG patterns of the GC and TA muscles were highly abnormal in all animals. Instead of a brisk burst at the onset of the swing

phase in the TA, the muscle often was continuously active (Fig. 4 and 5), and sometimes even without a clear increase in activity during the swing phase (Fig. 4). The bursts in the GC muscle were irregular and activation during the swing phase often continued (Fig. 4 and 5). Often simultaneous activation of the antagonist (coactivation) was observed. These abnormalities persisted until the end of the study.

### **Discussion**

Despite several reports on excellent morphometrical data on regrowth of peripheral nerves after transection and reconstruction with nerve conduits, full restoration of sensory and motor nerve function after peripheral nerve reconstruction often fails.<sup>9-10</sup> Also clinical results after nerve repair often remain disappointing. Reestablishment of optimal function following peripheral nerve lesions, therefore, continues to be a considerable (clinical) challenge. The aim of the surgeon is not only to repair a damaged nerve so that the maximum number of axons cross the suture line, but also to ensure optimal return of function. The latter, however, is often disappointing. A better understanding of recovery of nerve function and adaptation may help to achieve better function after repair in the future.

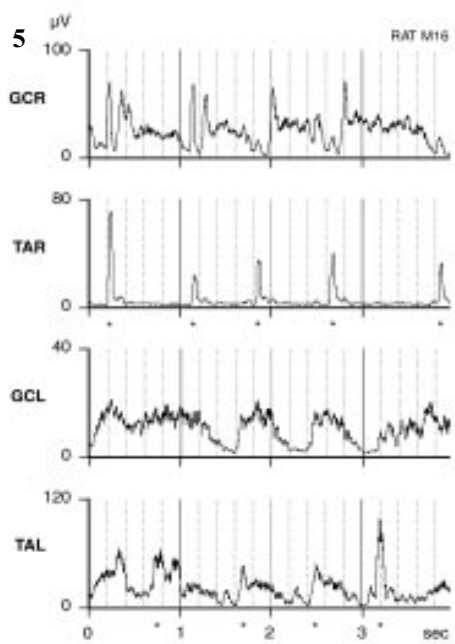
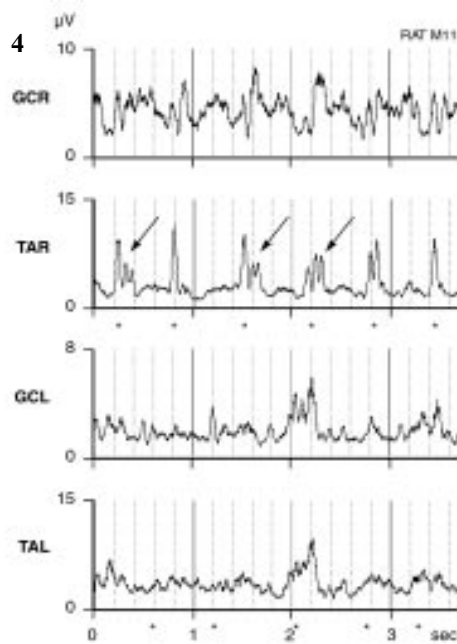
Only a few articles described the use of the video analysis technique for the evaluation of functional nerve recovery in the rat after sciatic nerve lesion and repair.<sup>17,23-25</sup> The use of video recordings of gait in the rat model is therefore an underestimated method. Recently, Dijkstra et al. evaluated the SFI in the



**Fig. 3.** Averaged EMG records of a normal rat. Records from the gastrocnemius (GC) and the tibialis anterior muscles (TA) of the right side. Time in seconds, amplitudes in  $\mu\text{V}$ ; asterisks indicate the onset of swing phases.

rat after crush lesion, after autologous nerve grafting and in a non-operated control group; it was shown that no significant differences were found between the SFI's measured by the finger paint and video analysis technique.<sup>25</sup> They concluded that the use of video analysis proved to be a valuable, non-invasive method for the evaluation of nerve function because the walking pattern and the stance factor can be studied simultaneously. Similar to the results in that previous study, the SFI values obtained with video analysis in the present study did not differ from the finger paint technique.

Chronic foot deformities may result in



**Fig. 4 and 5.** Averaged EMG records after 21 (Fig. 4) and 36 (Fig. 5) weeks of implantation. Records from the gastrocnemius (GC) and the tibialis anterior muscles (TA) of the right side (above) and of the left side (below). Time in secs, amplitudes in  $\mu\text{V}$ ; asterisks indicate onsets of swing phases in the right and left hindpaws; arrows in Fig. 4 indicate abnormal burst activity at the right non-operated side.

gait patterns that render walking track assessment invalid.<sup>10</sup> When auto-mutilation of the hindpaw occurs, the SFI can not be calculated either. Despite this disadvantage, the SFI is a widely used parameter because of its reliability. One recently published study of Shen and Zhu showed that the SFI had a positive correlation with muscle strength,<sup>26</sup> muscle induced action potentials, nerve compound action potential, and motor nerve conduction velocity. Nerve CV's as obtained by the MAP's are indicative for nerve regeneration and target reinnervation.<sup>20,27</sup> Recovery in CV was found in the present study but control values were never achieved. Good recovery of motor nerve function depends on the reinnervation of the new target by the original axons. Correct innervation of the motor endplates is also necessary. Furthermore, changes in the distribution of fibre types of the different muscles innervated by the sciatic nerve may play a role in the functional outcome.

De Koning et al. showed that the local application of electrical stimuli to the sole of the rat's foot is a non-invasive, rapid, easy and very precise method to evaluate the return of sensory nerve function after nerve injury.<sup>18</sup> They also described that there is no conditioning during the test, and it is therefore a widely used technique.<sup>28-30</sup> As in previous studies it was found that the return of sensory nerve innervation of the skin is faster than the return of motor nerve function. The return of sensory function, as measured by the electrostimulation test, may not only be explained by the outgrowth of regenerating axons from the sectioned proximal

sciatic nerve stump but also by collateral sprouts from intact fibers in the skin surrounding the denervated zone.<sup>31-32</sup>

Devor et al. showed that cutaneous reinnervation starts with collateral expansion of afferents from intact neighboring saphenous nerve fibers.<sup>33</sup> Thereafter, with the return of sciatic nerve fibers, the expanded distribution of the saphenous nerve retracts to its original boundaries. They found that approximately 3 weeks after crushing, the regenerating sciatic nerve began to regain its function. This was concluded by the return of sensation to zones not invaded by the saphenous nerve and by the onset of sensation in rats in which the saphenous nerve had previously been ligated. In the present study, the withdrawal reflex could be elicited after 7 weeks of implantation and also after the sciatic nerve was cut. From 15 weeks and beyond the withdrawal reflex disappeared after secondarily cutting the sciatic nerve. We conclude therefore, that recovery of sensory nerve function starts with the expansion of the territory of intact neighboring fibers. After 15 weeks, fibers from the original nerve again started to make functional contact. This is in accordance with the results of Devor et al.,<sup>33</sup> although only after a longer time period.

EMG's remained abnormal throughout the period of investigation. This can be explained by the fact that muscles received aselective inputs from axons originally connected to other muscles, e.g. cross-innervation. This has been demonstrated to lead to dramatic changes in activation patterns of the muscles.<sup>21</sup> On the right non-operated side, some irregularities could be

observed in the EMG's, such as extra bursts and irregular phasing during stance and swing. This may be explained by a compensation for the rat's inability to bear body weight and adequately propulse with the left hindleg. The increase in quality of the walking pattern with time in conjunction with a severely disturbed EMG activity is confusing. It might be possible that despite aselective reinnervation of the muscles, readjustments in the force recruitment of these muscles occur due to subtle readjustments by supraspinal motor systems.<sup>22</sup> These processes may be situated in the cerebellum. It is well known that the cerebellum is involved in motor learning and modulation of motor output on the basis of ascending spinal cord information. Presently, ongoing research aims at identifying the process of

functional recovery in spite of aberrant reinnervation.

In conclusion, recovery of nerve function over a 15 mm gap in the sciatic nerve of the rat is possible with a p(DLLA- $\epsilon$ -CL) nerve guide without the addition of growth factors or extracellular matrix molecules. Sensory nerve function as measured by the electrostimulation test did recover well but motor nerve function as measured by the SFI, NCV, stance factor only partly recovered. Electromyograms during walking remained highly abnormal throughout the study. Investigation on how to selectively reinnervate antagonist muscles, how to influence the distribution of muscle fibre types, and finding cues for specific axon guidance are the main aims for further research.

## *ACKNOWLEDGEMENTS*

*This research was made possible by the MW-NWO (Dutch Organization for Scientific Research), Den Haag, The Netherlands. This project has been supported by the Foundation "De Drie Lichten", Hilversum, The Netherlands. The assistance of H.L. Bartels with microsurgical techniques is greatly appreciated.*

## References

1. Doolabh VB, Hertl C, Mackinnon SE. The role of conduits in nerve repair: a review. *Rev Neurosci* 1996;7:47-84.
2. Den Dunnen WFA, Van der Lei B, Schakenraad JM, Stokroos I, Blaauw EH, Bartels H, Pennings AJ, Robinson PH. Poly(DL-lactide- $\epsilon$ -caprolactone) nerve guides perform better than autologous nerve grafts. *Microsurgery* 1996;17:348-357.
3. Den Dunnen WFA, Van der Lei B, Robinson PH, Holwerda A, Pennings AJ, Schakenraad JM. Biological performance of a degradable poly(DL-lactic acid- $\epsilon$ -caprolactone) nerve guide: influence of tube dimensions. *J Biomed Mater Res* 1995;29:757-766.
4. Meek MF, Den Dunnen WFA, Bartels HL, Pennings AJ, Robinson PH, Schakenraad JM. Peripheral nerve regeneration and functional nerve recovery after reconstruction with a thin-walled biodegradable poly(DL-lactide- $\epsilon$ -caprolactone) nerve guide. *Cells Mater* 1997;7:53-62.
5. Meek MF, Den Dunnen WFA, Schakenraad JM, Robinson PH. Evaluation of functional nerve recovery after reconstruction with a poly(DL-lactide- $\epsilon$ -caprolactone) nerve guide, filled with modified denatured muscle tissue. *Microsurgery* 1996;17:555-561.
6. Meek MF, Den Dunnen WFA, Schakenraad JM, Robinson PH. Evaluation of several techniques to modify denatured muscle tissue to obtain a scaffold for peripheral nerve regeneration. *Biomaterials* 1999;20:401-408.
7. Lundborg G, Dahlin LB, Danielsen N, Hansson HA, Johannesson A, Longo FM, Varon S. Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. *Exp Neurol* 1982;76:361-375.
8. Francel PC, Francel TJ, Mackinnon SE, Hertl C. Enhancing nerve regeneration across a silicone tube conduit by using interposed short-segment nerve grafts. *J Neurosurg* 1997;87:887-892.
9. Mackinnon SE, Dellon AL. *Surgery of the peripheral nerve*. New York: Thieme, 1988.
10. Dellon AL, Mackinnon SE. Sciatic nerve regeneration in the rat: validity of walking track assessment in the presence of chronic contractures. *Microsurgery* 1989;10:220-222.
11. Buehler MJ, Seaber AV, Urbaniak JR. The relationship of functional return to varying methods of nerve repair. *J Reconstr Microsurg* 1990;6:61-69.
12. Munro CA, Szalai JP, Mackinnon SE, Midha R. Lack of association between outcome measures of nerve regeneration. *Muscle Nerve* 1998;21:1095-1097.
13. Hruska RE, Kennedy S, Bergeld SK. Quantitative aspects of normal locomotion in rats. *Life Sci* 1979;25:171-180.
14. Westerga J, Gramsbergen A. Development of locomotion in the rat. *Dev Brain Res* 1990;57:163-174.
15. Bain JR, Mackinnon SE, Hudson AR, Falk RE, Falk JA, Hunter DA. The peripheral nerve allograft: an assessment of regeneration across nerve allografts in rats immunosuppressed with cyclosporin A. *Plast Reconstr Surg* 1988;82:1052-1066.
16. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg* 1989;83:129-136.
17. Walker JL, Evans JM, Meade P, Resig P, Sicken BF. Gait-stance duration as a measure

- of injury and recovery in the rat sciatic nerve model. *J Neurosci Methods* 1994;52:47-52.
18. De Koning P, Brakkee JH, Gispens WH. Methods for producing a reproducible crush in the sciatic and tibial nerve of the rat and rapid and precise testing of return of sensory function. *J Neurol Sci* 1986;74:237-246.
  19. Hoppen HJ, Leenslag JW, Pennings AJ, Van der Lei B, Robinson PH. Two-ply biodegradable nerve guide: basic aspects of design, construction and biological performance. *Biomaterials* 1990;11:286-290.
  20. Yu LT, England J, Sumner A, Larossa D, Hickey WF. Electrophysiologic evaluation of peripheral nerve regeneration through allografts immunosuppressed with cyclosporin. *J Reconstr Microsurg* 1990;6:317-323.
  21. Gramsbergen A, IJkema-Paassen J, Meek MF. Sciatic nerve transection in the adult rat: abnormal EMG patterns during locomotion by aberrant innervation of hindleg muscles. *Exp Neurol* 2000;161:183-193.
  22. Gramsbergen A, Van Eykern LA, Meek MF. Sciatic nerve transection at adult and young age: EMG patterns during locomotion in rats. *Equine Vet J* 2000;in press.
  23. Walker JL, Evans JM, Resig P, Guarnieri S, Meade P, Siskin BS. Enhancement of functional recovery following a crush lesion to the rat sciatic nerve by exposure to pulsed electromagnetic fields. *Exp Neurol* 1994;125:302-305.
  24. Lin FM, Pan YC, Hom C, Sabbahi M, Shenaq S. Ankle stance angle: a functional index for the evaluation of sciatic nerve recovery after complete transection. *J Reconstr Microsurg* 1996;12:173-177.
  25. Dijkstra JR, Meek MF, Robinson PH, Gramsbergen A. Methods to evaluate functional nerve recovery in adult rats; walking track analysis, video analysis and the withdrawal reflex. *J Neurosci Methods*, 2000;96:89-96.
  26. Shen N, Zhu J. Application of sciatic function index in nerve functional assessment. *Microsurgery* 1995;16:552-555.
  27. Zelle RT, Miller DW, Kenning JA, Hoenig EM, Buchheit WA. Experimental peripheral nerve repair: environmental control directed at the cellular level. *Microsurgery* 1989;10:290-301.
  28. Van der Zee CE, Nielander HB, Vos JP, Lopes da Silva S, Verhaagen J, Oestreicher AB, Schrama LH, Schotman P, Gispens WH. Expression of growth-associated protein B-50 (GAP43) in dorsal root ganglia and sciatic nerve during regenerative sprouting. *J Neurosci* 1989;9:3505-3512.
  29. Hoogeveen JF, Troost D, Wondergem J, Kracht AHW, Haveman J. Hyperthermic injury versus crush injury in the rat sciatic nerve: a comparative functional, histopathological and morphometrical study. *J Neurol Sci* 1992;108:55-64.
  30. Van Meeteren NLU, Brakkee JH, Hamers FPT, Helders PJM, Gispens WH. Exercise training improves functional recovery and motor nerve conduction velocity after sciatic nerve crush lesion in the rat. *Arch Phys Med Rehabil* 1997;78:70-77.
  31. Lundborg G, Zhao Q, Kanje M, Danielsen N, Kerns JM. Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-end anastomosis? *J Hand Surg [Br]* 1994;19:277-282.
  32. Weddell G, Guttman L, Guttman E. The local extension of nerve fibers into denervated areas of skin. *J Neurol Neurosurg Psychiatry* 1941;4:206.
  33. Devor M, Schonfeld D, Seltzer Z, Wall PD. Two modes of cutaneous reinnervation following peripheral nerve injury. *J Comp Neurol* 1979;185:211-220.

