ELSEVIER

Journal of Neuroscience Methods 166 (2007) 266–277

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Electrophysiologic assessment of sciatic nerve regeneration in the rat: Surrounding limb muscles feature strongly in recordings from the gastrocnemius muscle

Angie Rupp[a,1], Ulf Dornseifer[b,1], Andrea Fischer[c], Wolfgang Schmahl[a], Karsten Rodenacker[d], Uta Jütting[d], Peter Gais[e], Edgar Biemer[f], Nikolaos Papadopulos[f,*], Kaspar Matiasek[a,**]

[a] *Institute of Veterinary Pathology, Chair of General Pathology & Neuropathology, Ludwig-Maximilians University, Veterinärstr. 13, 80539 Munich, Germany*

[b] *Department of Plastic, Reconstructive, Hand and Burn Surgery, Academic Hospital Munich-Bogenhausen, Englschalkingerstr. 77, 81925 Munich, Germany*

[c] *Section of Neurology, Department of Small Animal Medicine, Ludwig-Maximilians University, Veterinärstr. 13, 80539 Munich, Germany*

[d] *Institute of Biomathematics & Biometry, GSF National Research Centre for Health & Environment, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany*

[e] *Institute of Pathology, GSF National Research Centre for Health & Environment, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany*

[f] *Department of Plastic & Reconstructive Surgery, Klinikum Rechts der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany*

Received 29 May 2007; received in revised form 4 July 2007; accepted 11 July 2007

Abstract

Striking inconsistencies between the results of morphometric and electrophysiologic examinations of the regenerating nerve were observed in a previous study featuring the bridging of a 14 mm gap in the rat sciatic nerve.

To shed light on this dichotomy, seven further rats were subjected to permanent sciatic nerve transection and assessed electrophysiologically, histologically and by retrograde axonal tracing at various postoperative intervals (1 h to 8 weeks).

The results of the histological examinations and retrograde tracing revealed that in spite of the fact that compound muscle action potentials could be recorded in the gastrocnemius muscle, no reinnervation of the gastrocnemius muscle, either physiological or aberrant, had actually taken place. Furthermore, it was established that the electrical activity recorded in the gastrocnemius muscle after stimulation of the proximal or distal stump is generated by surrounding hind limb muscles unaffected by denervation. These are stimulated either directly, or indirectly due to spreading of the impulse.

It is therefore strongly recommended that caution should be exercised when interpreting recordings from the gastrocnemius muscle after stimulation of a regenerating sciatic nerve in laboratory rodents.

Keywords: Rat; Sciatic nerve; Evaluation; Electroneurography; Cross-talk

1. Introduction

Weak correlations between functional and structural evaluations in sciatic nerve regeneration studies in rats have frequently been mentioned and described (Dellon and Mackinnon, 1989; Hadlock et al., 1999; Howard et al., 2000; Kanaya et al., 1996; Nichols et al., 2005; Shenaq et al., 1989); in this context it has been emphasised that abundant axonal regrowth and adequate impulse conduction do not necessarily trigger a return of function (De Medinaceli, 1995).

Recently, the reverse situation, i.e. almost full return of sensory function without any histological evidence of axonal regeneration in the corresponding nerve segments was observed in a study carried out to assess the ability of biocompatible collagen tubes to sustain neural regeneration across a 14 mm gap in the rat sciatic nerve. Rats experiencing no axonal regeneration and also no return of motor function, as determined by footprint analysis, regained close to full sensation in the foot of the operated hind limb. Retrograde tracers were able to prove the saphenous nerve as the primary source of sensory reinner-

* Corresponding author. Tel.: +49 89 4140 2171; fax: +49 89 4140 4869.

** Corresponding author. Tel.: +49 89 2180 3313; fax: +49 89 2180 99 3313.

E-mail addresses: n.papadopulos@lrz.tum.de (N. Papadopulos), kaspar.matiasek@neuropathologie.de (K. Matiasek).

[1] These authors contributed equally.

0165-0270/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.jneumeth.2007.07.015

vation of these animals (Rupp et al., 2007b). The same study also ascertained striking discrepancies between the morphometric examinations and the electrophysiologic assessments of the regeneration sciatic nerve, the latter of which were carried out to complement the functional investigations (data not shown). After 8 weeks of regeneration the motor nerve conduction velocities (NCVs) recorded in the operated hind limbs of all rats were statistically indistinguishable, even though morphometric and electron microscopic examinations of transverse sections of the regenerating sciatic nerve at mid-lesion level revealed that whilst some rats experienced abundant regeneration, others showed none at all. Further investigation of the distal stump and the regenerating tibial nerve 0.5 cm distal to its submersion into the gastrocnemius muscle showed, that only those animals exhibiting large amounts of countable myelinated fibres at mid-lesion level also possessed easily distinguishable and countable myelinated fibres at the more distal levels of examination (unpublished observations). No correlation could be found between the number of myelinated fibres and the NCVs for the operated hind limb of the individual animals.

Furthermore, the compound muscle action potentials (CMAPs), which were recorded from the gastrocnemius muscle (GM) after direct stimulation of the sciatic nerve proximal and distal to the lesion site, exhibited a similar morphology in all rats. These CMAPs were used to calculate the NCVs, since no CMAPs could be recorded in the interosseus muscles of the operated hind limbs in any of the rats.

Two different aetiologies can be put forward as the most probable reason for the large discrepancy between regained impulse conduction and nerve fibre counts observed.

The first is aberrant innervation of the GM by a different nerve (Gassel, 1964), such as one of the proximal branches of the sciatic nerve. The second possibility implies technical difficulties, such as accidentally recording CMAPs from other muscles of the hind limb, a problem also referred to as "cross-talk" (Kuiken et al., 2003).

To determine which of the two aetiologies was responsible for the inconsistencies observed, seven further rats were subjected to extraction of a 14 mm segment from the sciatic nerve with no repair of the defect. At different time-points they were assessed electrophysiologically, histologically and by using retrograde axonal tracers in order to determine the source of innervation of the GM or, alternatively, the source of electrical activity recorded in the GM.

2. Materials and methods

2.1. Study design

Seven male Lewis rats (Charles River Laboratories, Germany; 310–320 g) were subjected to extraction of a 14 mm segment from the sciatic nerve with no repair.

At different time-points after application of the insult to the sciatic nerve the rats were examined electrophysiologically, histologically and by retrograde axonal tracing (Table 1).

Lewis rats had originally been chosen for the previous study on account of their proven resistance to autotomy after sciatic lesions (Carr et al., 1992; Inbal et al., 1980; Panerai et al., 1987), and on account of their sciatic nerves being particularly suitable from an anatomical point of view (Rupp et al., 2007a).

Table 1
Study design and tracer application

Rat	Denervation time	Tracer application	
		Operated hind limb	Contralateral hind limb
1	1 h	Fast blue	Fluorogold
2	1 h	Fluorogold	Fast blue
3	1 week	Fast blue	Fluorogold
4	3 weeks	Fast blue	Fluorogold
5	5 weeks	Fast blue	Fluorogold
6	8 weeks	Fast blue	Fluorogold
7	8 weeks	Fluorogold	Fast blue

2.2. Surgical procedures

The surgical approach for exposing the sciatic nerve, the length and location of the segment excised from the sciatic nerve, the type and location of closing-up sutures, anaesthesia and perioperative management were identical to those applied in the previous study (Rupp et al., 2007b).

In all rats the left sciatic nerve was exposed under general anaesthesia [2 mg/kg midazolam (Ratiopharm, Germany), 150 μg/kg medetomidine (Pfizer, Germany) and 5 μg/kg fentanyl (Deltaselect, Germany) i.p.] by separating the biceps femoris muscle from the gluteus superficialis muscle in its aponeurosis and also from its lateral insertion at the tibial crest, making it possible to fold the unimpaired biceps femoris muscle towards caudal. After excision of a 14 mm segment from the sciatic nerve the proximal stump was provided with a firm ligature 1 mm proximal to its distal end in order to prevent regeneration. Then the biceps femoris muscle was carefully sutured back into place and the anaesthesia was reversed [0.75 mg/kg atipamezole (Pfizer, Germany), 200 μg/kg flumazenile (Inresa, Germany) and 120 μg/kg naloxone (Deltaselect, Germany) s.c.]. All lesions were set at the same place, with the distal end located 4 mm proximal to the submersion of the tibial branch of the sciatic nerve into the GM.

For postoperative analgesia, the rats received metamizol (200 mg/kg p.o.; corresponds to three drops of Novalgin®; Ratiopharm, Germany) upon waking up and buprenorphine (50 μg/kg s.c.; Temgesic®; Essex Pharma, Germany) every 12 h for 3 days.

2.3. Electrophysiologic examinations

Anaesthesia, equipment, approach of the sciatic nerve and placement of electrodes were carried out in the same way as in the previous study. However, five additional measures (A–E) were applied in order to ascertain the source of electrical activity recorded from the GM of the operated hind limb in the previous study. Care was taken to ensure that the rats' core temperature was maintained at between 36 and 38 °C.

2.3.1. Approach

The rats were placed under the same general anaesthesia as for the surgical procedures, and in order to locate the lesion site and the proximal and distal stumps, the reattached biceps femoris muscle was separated from the gluteus superficialis muscle in its aponeurosis (Step 1). The lateral insertion of the biceps femoris muscle on the tibial crest, however, was left untouched. This experimental setup corresponded directly to the one applied in the previous study.

Additional measure A: in Step 2 half of the lateral insertion of the biceps femoris muscle was dissected from the tibial crest. In Step 3 no communication between the biceps femoris muscle and the GM existed any more and the GM was fully exposed on its lateral side. The insertions of the deeper thigh muscles on the medial side of the lower leg, such as of the caudofemoralis muscle, the semimembranosus muscle, the gracilis muscle and the semitendinosus muscle, were not severed.

2.3.2. Stimulation sites and electrodes

In both hind legs the sciatic/tibial nerve was stimulated with two monopolar needle electrodes positioned directly on the nerve (length 12 mm; diameter 0.3 mm; Viasys Healthcare Supplies 2003 Catalogue No.: 019-404700; Nicolet, Germany).

On the operated hind limb the stimulation points lay proximal and distal to the lesion site. On the unoperated, contralateral hind limb they were at mid-thigh level and at the medial malleolus.

Additional measure B: the following four stimulation points (Fig. 1) were added to the two original stimulation sites (distal (1) and proximal (2) stump) when recording from the GM of the operated hind limb: the most proximal visible part of the sciatic nerve (3), two different points on the musculocutaneous branch (4 and 5) of the tibial/sciatic nerve, and the first major branch of the musculocutaneous nerve (6). The musculocutaneous nerve of the hind limb is a small motor and sensory nerve that branches very proximally from the sciatic nerve (Puigdellívol-Sánchez et al., 2000). To allow direct stimulation of the first major branch of this musculocutaneous nerve, the caudofemoralis muscle was transected in its proximal quarter.

2.3.3. Recording sites and electrodes

The recording electrodes (also monopolar needle electrodes) were placed subcutaneously on the plantar side of the foot, with the active (negative) electrode over the interosseus muscles and the reference (positive) electrode on the plantar side of the third toe. The ground electrode was inserted subcutaneously on the lateral side of metatarsus V.

In the operated hind limb the recording electrodes were also placed further proximal to perform a second set of measurements. In this setting the active electrode was positioned intramuscularly within the GM and the reference electrode was placed subcutaneously over the GM. The ground electrode was positioned subcutaneously between the stimulating and recording electrodes.

Additional measure C: both monopolar and bipolar (concentric) needle electrodes (length: 40 mm; diameter: 0.5 mm; recording area: 0.068 mm^2; Viasys Healthcare Supplies 2003 Catalogue No.: 019-721500; Nicolet, Germany) were employed for intramuscular recordings of CMAPs in the GM of the operated hind limb. The reason for this was that concentric needles are preferable to monopolar electrodes for intramuscular recordings (Horning et al., 1972) and narrow down the source of error (Gassel and Trojaborg, 1964) due to the fact that smaller CMAPs are recorded and the background noise is suppressed (Cuddon, 2002; Gassel, 1964; Scholle et al., 2005).

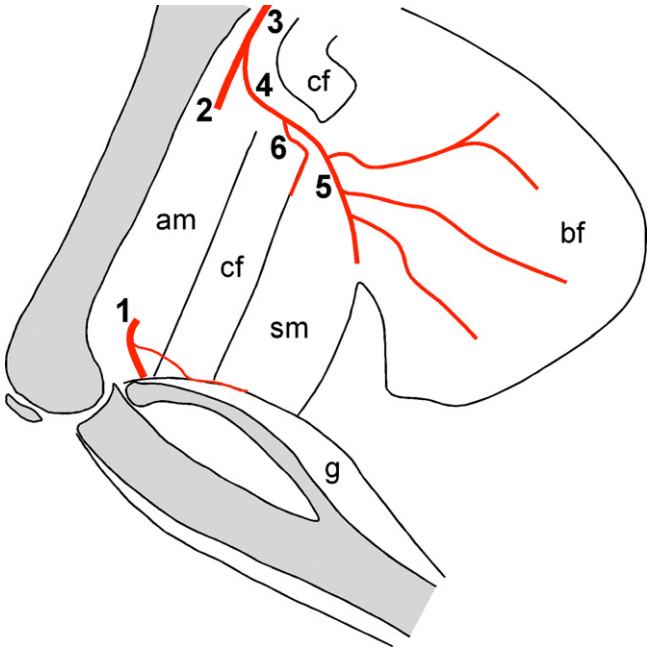

Fig. 1. Stimulation points in electrophysiologic examinations. Distal stump of the sciatic nerve (1), proximal stump (2), most proximal visible part of the sciatic nerve (3), musculocutaneous nerve of the hind limb (4 and 5), first major branch of the musculocutaneous nerve (6); landmarks: biceps femoris muscle (bf), semimembranosus muscle (sm), adductor magnis muscle (am), caudofemoralis muscle (cf), gastrocnemius muscle (g).

2.3.4. Equipment, stimulation intensity and calculation of NCVs

All stimulations and recordings were completed with a Viking Quest electrodiagnostic unit and associated software (Viasys Healthcare; Germany). One hertz single pulse with duration of 0.2 ms were delivered with increasing intensities until a potential could be recorded from the relevant muscles. In the previous study, a stimulation intensity of 1.9 mA had been found to provide supramaximal stimulation.

The CMAP latencies, defined as the lag between stimulus and onset of the first deflection of the action potential from the baseline, were measured at supramaximal stimulation intensities. Then the rats' hind limbs were straightened and the distances between the stimulating active electrodes were ascertained with a tape measure to the nearest millimetre. These values were subsequently used to calculate the individual NCVs.

Additional measure D: in cases where no CMAPs were recorded either from the interosseus muscles or from the GM

after stimulation with an intensity of 1.9 mA, larger intensities were applied in order to determine whether spreading of an impulse could lead to recordable CMAPs.

2.3.5. Transection test

Additional measure E: the musculocutaneous branch of the sciatic nerve was transected at different levels working from distal towards proximal in order to locate the source of electrical activity more precisely.

2.4. Retrograde tracing studies

Eight days before euthanasia the GMs in all rats were injected with retrograde tracers Fast blue (Polysciences) and Fluorogold (Biotium). Rats 1 and 3–6 each received a total of 6 μl of 5% Fast blue inserted into various areas of the GM on the operated hind limb and a total of 4 μl of 1% Fluorogold injected into various areas of the GM on the contralateral side. In Rats 2 and 7 the application sides were swapped (Table 1).

Immediately after the electrophysiologic examinations, the rats were perfused transcardially with 2 ml of 2% lidocain-hydrochloride (beta-pharm, Germany), followed by 100 ml of 0.1 M phosphate buffered saline (PBS; pH 7.4) and 250 ml of 4% paraformaldehyde in 0.1 M PBS. The spinal cord segments L1 to S1 were removed according to Gelderd and Chopin (1977), post-fixed in the same fixative for 6 h and then transferred into 15% sucrose in PB for 15 h. After freezing the individual segments in liquid nitrogen, the spinal cord was cut on a cryostat in 30 μm thick transverse sections and thaw-mounted on polylysine-coated glass slides. The spinal cord segments were identified according to Molander et al. (1984) and examined by epifluorescence under a Zeiss Axiophot® microscope equipped with a mercury lamp, a 365 nm excitation filter, a 395 nm dichroic beam splitter, and an LP 420 nm barrier filter.

2.5. Dissection and examination of the lateral thigh area

After perfusion, the area of the lateral thigh was closely inspected under a dissection microscope on both the operated and contralateral side in all rats to establish the courses of any distal extensions of the sciatic nerve.

2.6. Histological examination of the distal stump

In order to determine whether the rats had experienced physiological reinnervation of the GM, the distal stump plus its tibial successor were harvested in Rats 3–7, stretched on a piece of paper and immersed in 2.5% glutaraldehyde in Soerensen's phosphate buffer (pH 7.4) for 1 h. After fixation, samples were rinsed with Soerensen's phosphate buffer and a 2 mm transverse segment was excised from each distal stump, which then underwent post-fixation in 2% OsO_4 for 2 h at room temperature, repeated buffer rinses and a graded alcohol series before being embedded in epoxy resin. For histologic evaluations semithin sections (0.5 μm) were mounted on triethoxysilane-coated slides and stained with azur II-methylenblue-safranin.

2.7. Muscle volumes

After perfusion the GMs of both sides were extracted in Rats 3–7 and the combined volumes of the lateral and medial heads were determined by water displacement. The volumes were then normalised by the body weight of the rats to ensure comparability of results. Additionally, the ratios of the normalised volumes of the GMs on the operated and contralateral sides were calculated.

2.8. Animal health and housing

All rats were weighed weekly and inspected daily as regards grooming, activity levels, signs of autotomy, and infection or inflammation of the foot.

The rats were housed in groups of four on soft bedding in a temperature-controlled room with 12:12 h light cycles, and had free access to standard rat food and water. Additionally, they experienced 4–6 h of 'playtime' daily in a 45 cm × 55 cm × 120 cm cage in groups of 2–7 individuals. Animal studies were approved by the local animal care committee.

3. Results

3.1. Electrophysiologic examinations

In most cases the concentric needle electrode produced smaller CMAPs than the monopolar electrodes, or none at all, when applied in the same recording area and using the same stimulation parameters.

3.1.1. Operated hind limb: recordings from the interosseus muscles

Within 1 h after transection of the sciatic nerve, distinct CMAPs could still be recorded in the interosseus muscles after stimulation of the distal stump. No CMAPs were evident after stimulation of the proximal stump. Within 1 week of denervation, no more CMAPs could be elicited from the interosseus muscles after stimulation of either the proximal or the distal stump, even when stimulation intensities of over 12 mA were applied.

3.1.2. Operated hind limb: recordings from the GM

(a) *Stimulation of the distal stump* (*stimulation point 1*). Immediately after transection of the sciatic nerve and until week 5, virtually biphasic CMAPs were recorded in the GM after stimulation of the distal stump. Gradual dissection of the biceps femoris muscle from its aponeurosis on the tibia (Step 2) decreased the amplitude of the CMAPs. When the aponeurosis had been fully removed (Step 3), clearly recognisable CMAPs were still recorded in Rats 1 and 2. Very faintly recognisable CMAPs could only be recorded in Rats 3 and 4 if stimulation intensities of more than 10 mA were applied.

In week 8 (Rats 6 and 7) no CMAPs could be elicited in the GM any more after stimulation with an intensity of 1.9 mA.

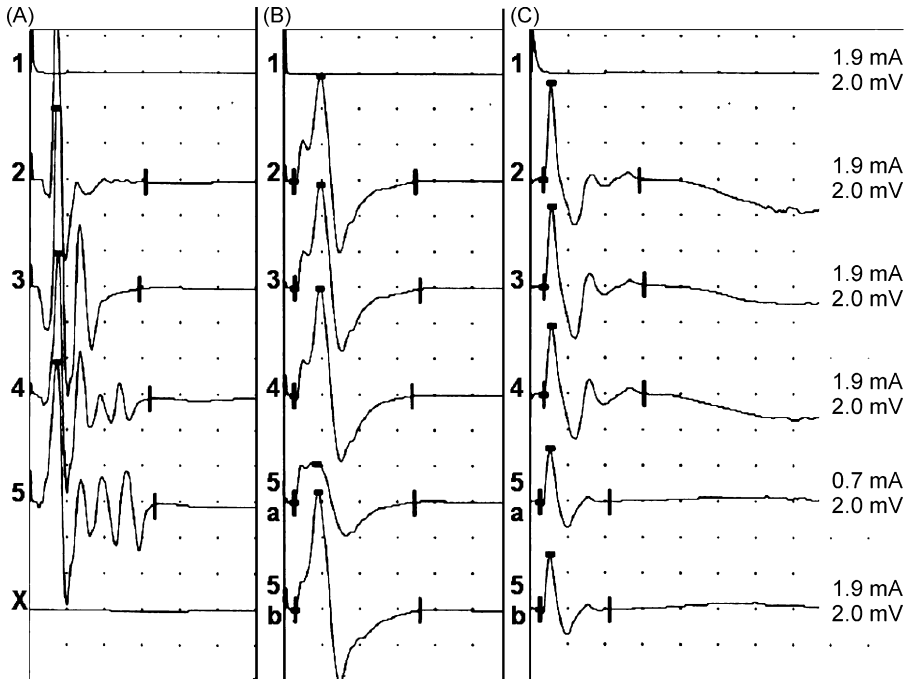

Fig. 2. Stimulation at the proximal stimulation points with gradual dissection of the biceps femoris muscle from its aponeurosis on the tibia (Rat 7). (A) Aponeurosis of the biceps femoris muscle on the tibia still complete (Step 1); (B) half of the aponeurosis of the biceps femoris muscle on the tibia dissected (Step 2); (C) complete dissection of the aponeurosis of the biceps femoris muscle on the tibia (Step 3). Note the decrease in amplitude and polyphasia of the virtually identical CMAPs elicited after stimulation at proximal points 2–5. No CMAPs could be recorded after stimulation of the distal stump (1) with an intensity of 1.9 mA 8 weeks after surgery; CMAPs with decreased amplitudes could be recorded after lowering the stimulation intensity to 0.7 mA at stimulation point 5 (5b). Stimulation point X was the posterior cutaneous nerve of the thigh. Recordings A and B, monopolar electrodes; recording C, concentric electrode; Sensitivity, 2 mV.

This was also the case even when the aponeurosis of the biceps femoris muscle on the tibia had been left untouched (Step 1).

(b) *Stimulation of the proximal stump and the musculocutaneous nerve* (*stimulation points 2–5*). Identical waveforms resembling CMAPs were recorded in the individual animals regardless of which of the proximal points 2–5 was stimulated. A lower stimulation intensity (0.7 mA) also evoked a lower CMAP amplitude in most cases.

Gradual dissection of the biceps femoris muscle (Step 2) as far as full removal of its aponeurosis on the tibia (Step 3) not only decreased the amplitude of CMAPs recorded in all the rats assessed, but in most cases also altered their shape from polyphasic to virtually biphasic (Fig. 2).

(c) *Stimulation of the first major branch of the musculocutaneous nerve* (*stimulation point 6*). After stimulation of the first major branch of the musculocutaneous nerve, the CMAPs recorded in the GM were less polyphasic than after stimulation of points 2–5 (Fig. 3).

When the first major branch of the musculocutaneous nerve was cut, the CMAPs elicited at proximal stimulation points 2–5 (using the same stimulation intensity) were smaller than when this branch was still intact. If the stimulating electrodes were applied distal to the cut, clearly recognisable CMAPs could still be produced in the GM.

(d) *Transection tests after Steps 2 and 3*. Transection of the musculocutaneous nerve distal to its first major branch resulted in smaller, more biphasic CMAPs after stimulation at points 2–4 than before the nerve was cut. After additional transection of the first major branch of the musculocutaneous nerve proximal to stimulation point 6, no further CMAPs could be recorded at all (even with monopolar electrodes) in the GM after stimulation at points 2–4. On the other hand, stimulation at point 6 resulted in clearly recognisable, almost biphasic CMAPs being recorded in the GM (Fig. 4).

Transection of the caudofemoralis muscle in the mid-belly region resulted in a loss of recordable CMAPs in the GM if stimulation was applied proximal to the point of severance. More distal application of stimulating electrodes still produced a very faint signal.

3.1.3. Contralateral hind limb

After stimulation of the sciatic nerve at mid-thigh level and of the tibial nerve at the medial malleolus, physiological and distinct biphasic CMAPs were recorded from the interosseus muscles. When the active recording electrode was placed within the GM, however, polyphasic CMAPs were also recorded on this side.

The NCVs calculated for the contralateral, healthy hind limbs reached values of between 48 and 56 m/s.

3.2. Retrograde tracing studies

Dissection of the rats made it possible to ascertain the accuracy of tracer application, as in most cases traces of the dyes injected were still visible. In all the hind limbs the tracers had been correctly injected into either the lateral or the medial head or into both heads of the GM. In Rat 7, however, some of the

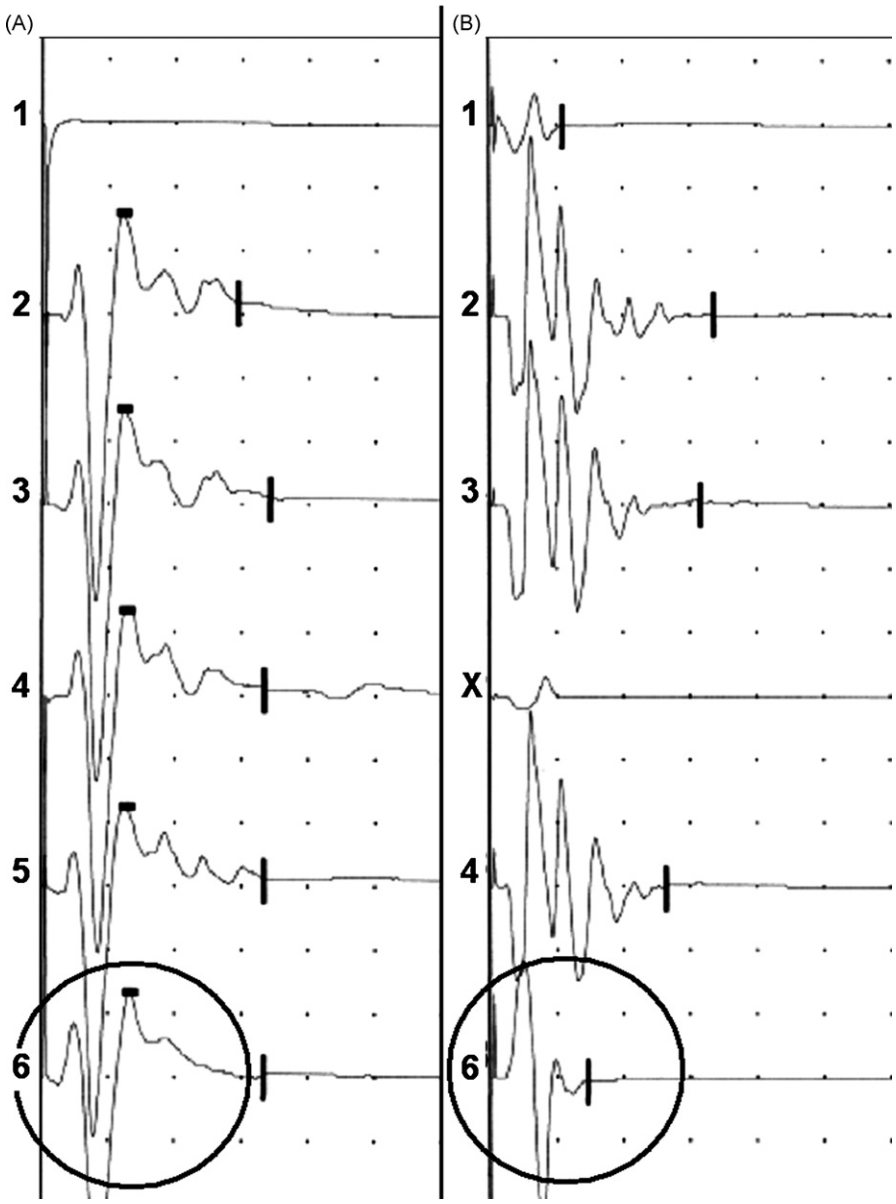

Fig. 3. Stimulation of the first major branch of the musculocutaneous nerve. (A) Rat 4, Step 2. (B) Rat 5, Step 1. Stimulation of the first major branch of the musculocutaneous nerve (6) resulted in less polyphasic CMAP waveforms recorded from the GM than those produced after stimulation of the more proximal points 2–5. Note the small CMAP, which could be elicited after stimulation of the distal stump with an intensity of 1.9 mA 5 weeks after surgery. Stimulation point X was the posterior cutaneous nerve of the thigh. Both recordings conducted with monopolar electrodes; Sensitivity, 2 mV.

injected solution could be found in the biceps femoris muscle of the operated hind limb, the rest in the GM.

In Rats 1 and 2 only the ventral horn cells of spinal cord segment L5 were labelled; the staining was bilateral. In both animals one-half of the grey matter in the spinal cord exhibited only cells with blue fluorescence and the other half only cells with yellow fluorescence.

In Rats 3–6 again only the ventral horn cells of spinal cord segment L5 were labelled. Here, however, the yellow staining was limited to one-half of the spinal cord and corresponded to the tracer injected into the contralateral (healthy) GM.

In Rat 7 the ventral horn cells of spinal cord segments L4 and L6 displayed unilateral fluorescence. In L4 the blue fluorescence corresponded to the tracer injected into the GM of the contralateral healthy side, and in L6 the fluorescence was yellow and corresponded to the tracer injected into the GM of the operated hind limb. Spinal cord segment L5 was bilaterally labelled, one-half blue and the other yellow.

3.3. Dissection and investigation of the lateral thigh area

Close investigation of the lateral thigh area (medial to the original position of the biceps femoris muscle) revealed that the musculocutaneous nerve of the hind limb provides innervation for the caudofemoralis muscle whilst passing medial to it. Very fine neural strands branch towards the proximal end of the caudofemoralis muscle.

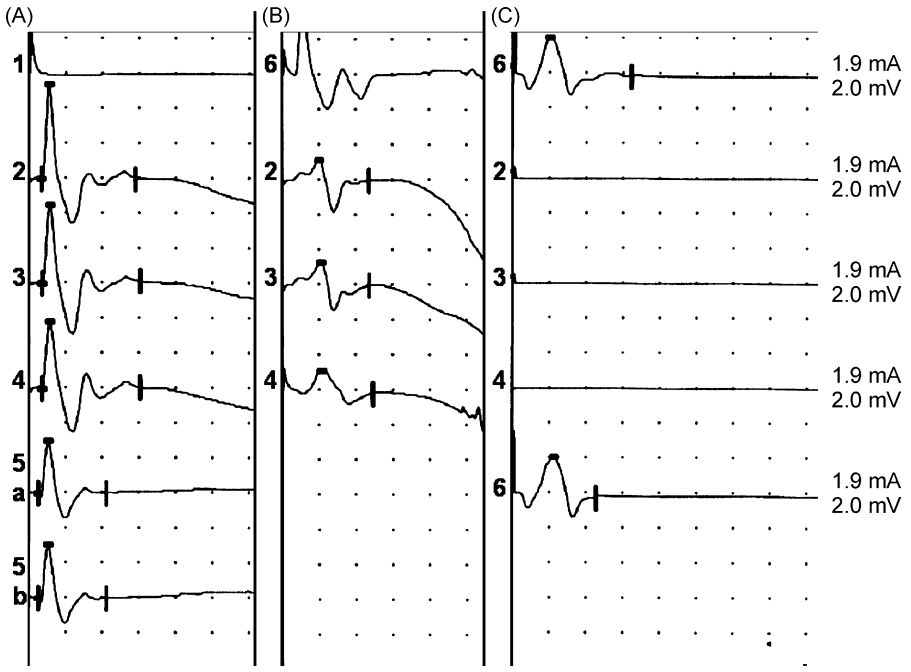

Fig. 4. Transection tests after Step 3 (Rat 7). (A) Musculocutaneous nerve still intact; (B) transection of the musculocutaneous nerve near stimulation point 5; (C) transection of the first major branch of the musculocutaneous nerve proximal to stimulation point 6. Transection of the musculocutaneous nerve distal to its first major branch resulted in smaller CMAPs after stimulation at points 2–4 than before the nerve was severed. After additional transection proximal to stimulation point 6 (but still on the first major branch of the musculocutaneous nerve) no further CMAPs could be recorded at all. Application of stimulation to point 6 still resulted in clearly recognisable CMAPs. Recordings A and B, concentric electrode; recording; C, monopolar electrodes; Sensitivity, 2 mV.

Directly on the border between the caudofemoralis and semimembranosus muscles the musculocutaneous nerve gives rise to a fairly distinct branch extending towards distal. It runs between the caudofemoralis and the semimembranosus muscle and disappears under the semimembranosus muscle at about mid-thigh level. Upon dissection it can be followed towards the distal quarter of the semimembranosus muscle, where it disappears within the muscle (Fig. 5), maybe also providing innervation to the semitendinosus muscle at the same time.

All the more distal branches of the musculocutaneous nerve seem to provide innervation to the biceps femoris muscle, whereas the trunk cannot be followed further than the adipose tissue in the popliteal fossa.

In Rats 5–7 (5 and 8 weeks of chronic injury to the sciatic nerve) very fine strands of neural tissue extending from the proximal stump towards the distal stump could be observed in the operated hind limb.

3.4. Examination of the distal stumps

Microscopic examination of the distal stump of Rat 3 revealed that within 1 week after denervation most of the fibres were already undergoing Wallerian degeneration. Approximately 1% of the fibres still retained their physiological structure. In the distal stump of Rat 4, 3 weeks after transection of the sciatic nerve, no more intact fibres could be seen. This finding remained the same for the rest of the study (Rats 5–7).

3.5. Gastrocnemius volumes (Table 2)

After normalisation of the gastrocnemius volumes with the body weights, denervation atrophy of the GMs was clearly discernible in the operated hind limb, accompanied by compensatory hypertrophy of the contralateral side. The gastrocnemius/body weight ratio after 8 weeks of denervation reached

Table 2
Gastrocnemius volumes

Rat	Gastrocnemius volumes (ml)		Normalised gastrocnemius volumes		Ratio of normalised volumes operated/contralateral hind limb
	Operated hind limb	Contralateral hind limb	Operated hind limb	Contralateral hind limb	
3	1.2	1.6	3.75	5.00	0.75
4	1.0	2.2	2.80	6.16	0.45
5	0.6	2.1	1.58	5.53	0.29
6	0.45	2.6	1.13	6.55	0.17
7	0.6	2.4	1.46	5.83	0.25

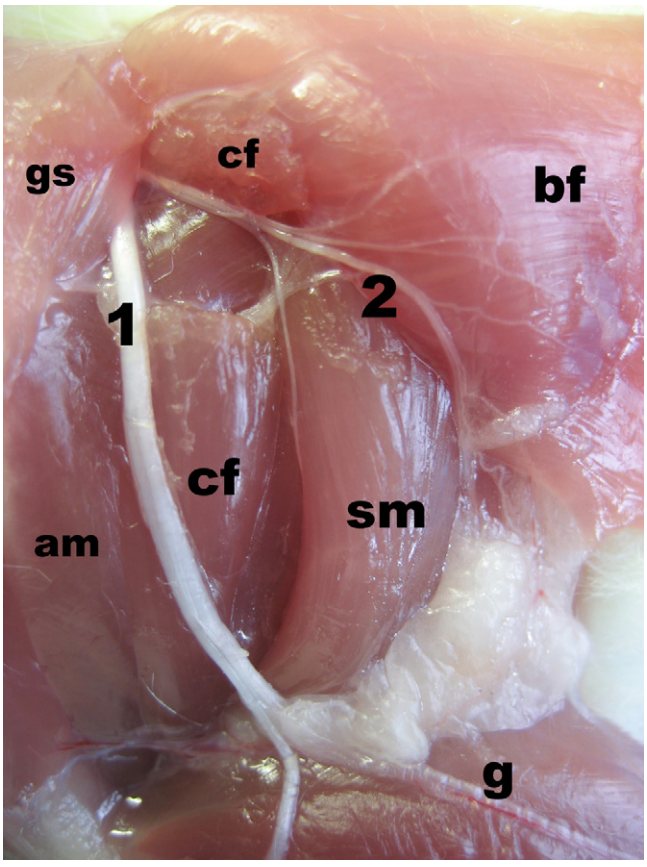

Fig. 5. Anatomical overview of the lateral aspect of the left thigh. The biceps femoralis muscle has been detached in both its insertions and folded toward caudal (Step 3). The caudofemoralis muscle has been transected and the border between caudofemoralis and semimembranosus muscle has been slightly enlarged to reveal the course of the first major branch of the musculocutaneous nerve. Sciatic nerve (1), musculocutaneous nerve (2), gluteus superficialis muscle (gs), caudofemoralis muscle (cf), biceps femoris muscle (bf), adductor magnis muscle (am), semimembranosus muscle (sm) and gastrocnemius muscle (g).

only approximately 30 (Rat 6) to 38% (Rat 7) of the ratio ascertained for 1 week of denervation, and the ratio for operated to contralateral side decreased to 25% (Rat 7) or even less (Rat 6).

3.6. General health condition

Health and behaviour of the rats was normal throughout the study. All animals exhibited a slight gain in weight, groomed themselves well and had moderate to high levels of activity. None of the rats displayed any signs of autotomy or muscle contractures.

4. Discussion

4.1. Source of electrical activity

Three results obtained in the present study strongly suggest that the electrical activity recorded in the chronically denervated GM after stimulation of either the proximal or the distal stump of the sciatic nerve must be generated by surrounding muscles unaffected by denervation. These are stimulated either directly, or indirectly as a result of spreading of the impulse.

Firstly, CMAPs recorded in the GM after stimulation of the proximal stump and adjoining stretches of sciatic nerve diminished continuously and exhibited less polyphasia with gradual removal of the surrounding muscles (Steps 1–3), principally the biceps femoris muscle (Fig. 2).

The pronounced polyphasia observed in the initial examinations of the GM (Step 1) after stimulation of either the proximal stump or other proximally located areas and branches of the sciatic nerve (stimulation points 2–5) would suggest one of two possibilities; either that the nerve providing innervation to the GM is affected by demyelination or by immature axons conducting at different speeds (Cuddon, 2002), or that multiple muscles with equal influence and in different locations in relation to the stimulating and/or recording electrodes are instrumental in creating the electrical activity observed. The latter seems more plausible since histological examinations, retrograde tracing studies and evaluation of the GM volumes did not in any way suggest that the GMs of the operated hind limb experienced reinnervation.

Furthermore, stimulation of the sciatic nerve on the contralateral (healthy) side led to polyphasic CMAPs being recorded from the GM. Recordings from the interosseus muscles after stimulation of the same area, however, exhibited the usual distinct biphasic CMAPs. The NCVs calculated for the sciatic/tibial nerve with recordings from the interosseus muscles of the contralateral hind limb in both the present and the previous study match figures published for physiological values in rats (Arnaoutoglou et al., 2006; Chiu et al., 1988; Ja'afar et al., 2006; Ramerman et al., 1968; Sato et al., 1985; Wolthers et al., 2005). Moreover, the sciatic nerve of the contralateral side examined in the previous study did not itself exhibit any pathological features (data not shown). Taking the NCVs, the condition of the sciatic nerve and the biphasic shape of the CMAPs recorded from the interosseus muscles together, the rats do not seem to be affected in any way on their contralateral sides. Therefore, the polyphasia observed in recordings from the GM must result from the varying distances between the stimulating electrode and the individual muscles receiving stimulation and/or from the varying distances between the areas generating activity and the recording electrode.

Consequently, the removal of the biceps femoris muscle from its proximity to the GM (Steps 2 and 3) means that less muscles remain to create electrical activity, which would then lead to the decrease in polyphasia observed.

Secondly, transection of the musculocutaneous nerve starting distally and working towards proximal, resulted in smaller, less polyphasic CMAPs being recorded in the GM after stimulation of the proximal stump.

The musculocutaneous nerve of the hind limb seems to be identical with an unnamed nerve branching from the tibial portion of the sciatic nerve and providing motor innervation to most of the muscles in closest proximity to the GM, namely the hamstring muscles (semitendinosus muscle, semimembranosus muscle, biceps femoris muscle), the quadratus femoris muscle and the caudofemoralis muscle (Greene, 1955; Hebel and

Stromberg, 1976). Dissection of the lateral thigh revealed that the more distal branches of the musculocutaneous nerve innervate the biceps femoris muscle, whereas the proximal branches supply the semimembranosus muscle, the caudofemoralis muscle and maybe also the semitendinosus muscle.

Gradual denervation of these muscles, especially the biceps femoris muscle, would, therefore, also lead to the decrease and change in shape of CMAPs.

Thirdly, stimulation of the first major branch of the musculocutaneous nerve leading towards distal (stimulation point 6) led to the CMAPs recorded in the GM being less polyphasic than after stimulation at proximal stimulation points 2–5 (Fig. 3); transection of this branch resulted in smaller CMAPs or none at all (again after stimulation of proximal points 2–4). Stimulation applied distal to the point of transection resulted in clearly recognisable, virtually biphasic CMAPs (Fig. 4). This was the case both when the lateral aponeurosis of the biceps femoris muscle had been half detached (Step 2) and fully detached (Step 3) from the tibia.

Close examination of the lateral thigh area revealed that the first major branch of the musculocutaneous nerve runs towards the semimembranosus muscle and disappears within it in its distal quarter (Fig. 5), possibly providing innervation to the semitendinosus muscle at the same time. Severance of the caudofemoralis muscle, whose course is closely followed by this branch of the musculocutaneous nerve, resulted in no CMAPs being recorded at all anymore in the GM after stimulation at point 6. Transection of the caudofemoralis muscle in its midbelly region, therefore, most probably had the effect of injuring this branch of the musculocutaneous nerve, which innervates the semimembranosus muscle.

The results outlined in the last paragraph indicate that the semimembranosus muscle, which receives motor innervation from a major branch of the musculocutaneous nerve, seems to serve as the main source of electrical activity. Other muscles, such as the biceps femoris muscle, and maybe also the semitendinosus and caudofemoralis muscle, play a part, however.

Alternatively, one could conjecture that the first major branch of the musculocutaneous nerve provides aberrant innervation to the GM and is responsible for the CMAPs, which were recorded. One would, however, expect this source of innervation to run distally between the muscles and not within the semimembranosus muscle. It then should have been visible and distinguishable in the investigations of the lateral thigh area, which it was not. Furthermore, assuming that there has been reinnervation of the GM, whether aberrant or physiological, gradual alienation of the GM from the surrounding muscles should not be able to affect the CMAPs to the extent observed in the present study, since intrinsic electrical activity in the GM would be the primary and most influential source. Also, one would not expect the volume of the GM of the operated hind limb to decline to the extent observed. The advanced atrophy of the GM made it increasingly difficult to inject the tracers accurately, which most probably led to the controversial results encountered in the retrograde tracing studies in Rat 7.

Retrograde tracing studies revealed spinal cord segment L5 to be the primary motor pool of the GM. In the control animals (Rats 1 and 2) and on contralateral sides, accumulation of tracer material was seen in the ventral horn neurons of spinal cord segment L5, located in the middle third of bony vertebra L1 (Gelderd and Chopin, 1977). In Rat 7 additional stained motoneurons could be seen in spinal cord segment L4. One explanation for this might be that the exact location of L5 in this rat had been wrongly estimated. Alternatively, it has been reported that the longitudinal locations of motor columns supplying one muscle vary by as much as one segment in individual rats, whereas their transverse location is very consistent (Nicolopoulos-Stournaras and Iles, 1983). This suggests that in Rat 7 the motor column for the GM might have physiologically been further proximal than in other rats, which means that the tracing results of the contralateral side are what one would expect.

In contrast to the unoperated contralateral side and control Rats 1 and 2, injection of the tracer in the chronically denervated GM did not result in any cells exhibiting appropriate fluorescence, even though dissection of the rats confirmed that tracer had been accurately applied. This was the case in Rats 3–6. In Rat 7, however, where Fluorogold had been injected into the GM of the operated hind limb, yellow-labelled ventral horn cells were detected in spinal cord segments L5 and L6. However, in this rat traces of yellow material were found not only in the GM, but also in the femoral biceps muscle of the operated hind limb, which makes it highly probable that Fluorogold reached the spinal cord via motor axons from the femoral biceps muscle. This hypothesis is supported by the fact that the motor columns for the hamstring muscles are further caudal than those of the GM (Nicolopoulos-Stournaras and Iles, 1983).

To summarise, the results of the tracing studies again do not support the hypothesis of reinnervation of the GM, either via the physiological structures or via aberrant pathways. Technical problems, such as decreased dye uptake due to incompatibility of the tracer, can further be excluded since they were successfully applied to the control animals. Additionally, the application sides were swapped in Rat 7 and this did not affect the action of tracers in any way.

4.2. Spreading of impulses

The phenomenon that conduction in the distal stump towards the gastrocnemius and interosseus muscles is not affected immediately after transection of the sciatic nerve is well documented (Dorfman, 1990; English et al., 2007; Terzis et al., 1976) and could also be observed in the present study. However, conduction in the distal stump after the application of strong stimulating impulses, which was ascertained both in the present and the previous study up to at least 8 weeks after denervation, is not compatible with the results of the histological examinations of the regenerating nerve at its various levels in the previous study and the distal stump in the present study. Furthermore, within 3 weeks of chronic denervation no more functional axons could be found anymore in the distal stump, and motor endplates have been reported to deteriorate within 2 weeks of denervation in any case (Ijkema-Paassen et al., 2002). This means that during stimulation of the distal stump the impulse must have

spread to unaffected nerves. A spreading of impulses delivered by the monopolar electrodes, especially after stimulation with high intensities has been described (Cuddon, 2002; Gassel, 1964).

A further clue to this is given by the virtually biphasic shape of the CMAPs recorded in the GM after stimulation of the distal stump. The transection tests revealed that CMAPs only took on this shape after direct stimulation of the first branch of the musculocutaneous nerve, which coincidentally is the one that lies in closest proximity to the distal stump. The close proximity between stimulation point and the nerve actually receiving the stimulation most probably is the cause for the shorter latencies observed when stimulating the distal stump compared to stimulation of the proximal stump. After stimulation of the proximal stump impulses either had to travel first in retrograde direction towards the branching of the musculocutaneous nerve and then ephaptically anterograde, or had to spread over a longer distance due to the anatomical relationship between proximal stump and musculocutanous nerve (Fig. 5).

Impulses were only able to spread, however, when stimulation was performed with high intensities (>1.9 mA) and when the distal aponeurosis of the biceps femoris to the tibia was left untouched. This leads to the assumption that strong impulses may be conducted via the fluid, which collects between the surfaces of the membranes, i.e. the muscles.

4.3. Relevance of the findings in the present study

The number of papers published on sciatic nerve regeneration in rats amount to close on 600 papers in the last 5 years (source PubMed: keywords: sciatic nerve rat regeneration). Examination of the 100 most recently published papers on sciatic nerve regeneration revealed that more than one-third of these studies applied electrophysiologic investigations to evaluate the extent of sciatic regeneration. More than half of these studies in turn featured CMAPs as the primary source of information. Recordings from the gastrocnemius muscle have been, and still are, applied in various studies (Archibald et al., 1991; Chen et al., 2005; English et al., 2006; Hou and Zhu, 1998; Martins et al., 2005; Meek et al., 2003; Negredo et al., 2004; Rodríguez et al., 2000; Udina et al., 2004; Valero-Cabré and Navarro, 2001). Unfortunately, however, one cannot draw any conclusion as to whether these groups encountered the same inconsistencies as we did in the previous study, since in these studies morphometric assessments were either not undertaken at exactly the same time as all the electrophysiologic examinations (Negredo et al., 2004; Rodríguez et al., 2000; Udina et al., 2004), or did not ascertain comparable parameters (Chen et al., 2005; Hou and Zhu, 1998), or were not conducted at all (Archibald et al., 1991; English et al., 2006; Martins et al., 2005; Meek et al., 2003; Valero-Cabré and Navarro, 2001).

4.4. Implications and areas requiring further research

In view of the results ascertained, it is important to note that it is probably only possible to regard morphometric and electrophysiologic assessments in sciatic nerve regeneration studies as redundant (Dellon and Mackinnon, 1989) if the electrophysiologic parameters are derived from direct recordings of the nerve under assessment. This means that the nerve needs to be subjected to the recording of nerve action potentials (NAPs) across the lesion site. More detailed information regarding the electrophysiologic properties of the regenerating nerve can be gained by placing the recording (or stimulating) electrodes distal to the lesion site at various different levels.

Also it is recommended that only CMAPs recorded from the interosseus muscles should be used to assess sciatic function in the rat. This does not only apply to pathological situations, i.e. sciatic nerve regeneration studies, but also to physiological circumstances, since it could be shown in the present study that contributions of surrounding muscles played a large part in the electrical activity observed even on the contralateral healthy side. The interosseus muscles are recommended as there are no large muscles in close proximity to them, which can interfere with the recordings.

Furthermore, in future sciatic nerve regeneration studies, it is advised that some or all of the proximal branches of the sciatic nerve, most importantly the musculocutaneous nerve and the inferior gluteal nerve, should be transected and prevented from regenerating, since the anatomical relations and functions of these nerves have not so far been completely elucidated and might contribute to misinterpretations of results in functional and electrophysiologic investigations. The inferior gluteal nerve has been described to branch from the sciatic nerve just proximal to the musculocutaneous nerve and provide innervation to the superficial gluteal muscle and to the anterior head of the biceps femoris muscle (Greene, 1955; Hebel and Stromberg, 1976). An eye should also be kept on the posterior cutaneous nerve, which seems to be a purely sensory branch of the lumbo-sacral trunk (Greene, 1955), but has been described to anastomose with the musculocutaneous nerve in the popliteal fossa (Puigdellívol-Sánchez et al., 2000).

Moreover, the results obtained in the present study on rats warrant further investigations regarding the size of the individual in which this extensive phenomenon of cross-talk between different muscle groups can be observed.

Generally, cross-talk has been observed after stimulation with high intensities and is said to become particularly evident when high amplifications are used in recordings (Gassel, 1964). Both of these settings are usually required when assessing regenerating or demyelinated nerve fibres (Gassel, 1964; Röder, 1996); high amplifications of the recordings, however, were not the case in the present study. Maybe strong amplification is not necessary for overwhelming neighbouring muscles in small animals, where the limb muscles lie in closer proximity to one another than in larger species.

Examples of situations where cross-talk has been observed in humans, under both physiological and pathological conditions, include electrophysiologic assessments of the small hand and foot muscles (Gassel, 1964; Gassel and Trojaborg, 1964), the knee extensor muscles (Farina et al., 2002, 2004) and the lower limb muscles (Perry et al., 1981). The different compartments of the gastrocnemius muscle in cats also seem to be affected by this phenomenon (English and Weeks, 1989), and cats, together with

dogs, are the animals on which electrophysiologic assessments are most routinely performed.

5. Conclusion

Evaluation of sciatic nerve regeneration by providing stimulation to the sciatic nerve and recording CMAPs from the gastrocnemius muscle in rats should be treated with caution, especially if monopolar needle electrodes are used for recording. In the event of insufficient reinnervation of the gastrocnemius muscle, the electrical activity encountered after stimulation of the regenerating sciatic nerve is most probably generated by surrounding hind limb muscles unaffected by denervation. The two muscles featuring most strongly in recordings from the denervated gastrocnemius muscle are the biceps femoris muscle and the semimembranosus muscle. These are stimulated either directly, or indirectly as a result of the spreading of the impulse, which means that it is also important to set limits on the intensity of the stimulus applied.

Even under physiological circumstances it is very likely that in laboratory rodents the surrounding muscles contribute to CMAPs recorded from the gastrocnemius muscle after stimulation of the sciatic nerve.

Acknowledgements

Many thanks to Andrew Wilson, Dani Thinnes, Stefan Leichtle and Max Fichter for their support during the electrophysiologic evaluations. Furthermore, We would like to express our gratitude to Christine Rupp for proofreading this manuscript more than once and for providing invaluable suggestions.

References

Archibald SJ, Krarup C, Shefner J, Li S-T, Madison RD. A collagen-based nerve guide conduit for peripheral nerve repair: an electrophysiological study of nerve regeneration in rodents and nonhuman primates. J Comp Neurol 1991;306:685–96.

Arnaoutoglou CM, Sakellariou A, Vekris M, Mitsionis G, Korompilias A, Ioakim E, Arhantis A, Beris A. Maximum intraoperative elongation of the rat sciatic nerve with tissue expander: functional, neurophysiological, and histological assessment. Microsurgery 2006;26:253–61.

Carr MM, Best TJ, Mackinnon SE, Evans PJ. Strain differences in autotomy in rats undergoing sciatic nerve transection or repair. Ann Plast Surg 1992;287:538–44.

Chen Y-S, Chang J-Y, Cheng C-Y, Tsai F-J, Yao C-H, Liu B-S. An in vivo evaluation of a biodegradable genipin-cross-linked gelatin peripheral nerve guide conduit material. Biomaterials 2005;26:3911–8.

Chiu DTW, Lovelace RE, Yu LT, Wolff M, Stengel S, Middleton L, Janecka IP, Krizek TJ. Comparative electrophysiologic evaluation of nerve grafts and autogenous vein grafts as nerve conduits: an experimental study. J Reconstr Microsurg 1988;4:303–9.

Cuddon P. Electrophysiology in neuromuscular disease. Vet Clin North Am Small Anim Pract 2002;32:31–62.

De Medinaceli L. Interpreting nerve morphometry data after experimental traumatic lesions. J Neurosci Methods 1995;58:29–37.

Dellon AL, Mackinnon SE. Selection of the appropriate parameter to measure neural regeneration. Ann Plast Surg 1989;23:197–202.

Dorfman LJ. Quantitative clinical electrophysiology in the evaluation of nerve injury and regeneration. Muscle Nerve 1990;13:822–8.

English AW, Weeks O. Electromyographic cross-talk within a compartmentalized muscle of the cat. J Physiol 1989;416:327–36.

English AW, Chen Y, Carp JS, Wolpaw JR, Chen XY. Recovery of electromyographic activity after transection and surgical repair of the rat sciatic nerve. J Neurophysiol 2007;97:1127–34.

Farina D, Merletti R, Indino B, Nazzaro M, Pozzo M. Surface EMG cross-talk between knee extensor muscles: experimental and model results. Muscle Nerve 2002;26:681–95.

Farina D, Merletti R, Indino B, Graven-Nielsen T. Surface EMG crosstalk evaluated from experimental recordings and simulated signals. Methods Inf Med 2004;43:30–5.

Gassel MM. Sources of error in motor nerve conduction studies. Neurology 1964;14:825–35.

Gassel MM, Trojaborg W. Clinical and electrophysiological study of the pattern of conduction times in the distribution of the sciatic nerve. J Neurol Neurosurg Psychiat 1964;27:351–7.

Gelderd JB, Chopin S. The vertebral level of origin of spinal nerves in the rat. Anat Rec 1977;188:45–8.

Greene EC. Nervous System. Transactions of the American Philosophical Society, Anatomy of the rat, vol. 27. New York: Hafner Publishing Co; 1955. pp. 130–131.

Hadlock T, Koka R, Vacanti JP, Cheney ML. A comparison of assessments of functional recovery in the rat. J Peripher Nerv Syst 1999;4:258–64.

Hebel R, Stromberg MW. Anatomy of the Laboratory Rat. Baltimore: The Williams & Wilkins Company; 1976, 35, 36, 39, 133.

Horning ME, Kraft G, Guy A. Latencies recorded by intramuscular needle electrodes in different portions of a muscle: variation and comparison with surface electrodes. Arch Phys Med Rehabil 1972:206–11.

Hou Z, Zhu J. An experimental study about the incorrect electrophysiological evaluation following peripheral nerve injury and repair. Electromyogr clin Neurophysiol 1998;38:301–4.

Howard CS, Blakeney DC, Medige J, Moy OJ, Peimer CA. Functional assessment in the rat by ground reaction forces. J Biomech 2000;33:751–7.

Ijkema-Paassen J, Meek MF, Gramsbergen A. Reinnervation of muscles after transection of the sciatic nerve in adult rats. Muscle Nerve 2002;25:891–7.

Inbal R, Devor M, Tuchendler O, Lieblich I. Autotomy following nerve injury: genetic factors in the development of chronic pain. Pain 1980;9:327–37.

Ja'afar FMH, Hamdan FB, Mohammed FH. Vincristine-induced neuropathy in rat: electrophysiological and histological study. Exp Brain Res 2006;173:334–45.

Kanaya F, Firrell JC, Breidenbach WC. Sciatic function index, nerve conduction tests, muscle contraction, and axon morphometry as indicators of regeneration. Plast Reconstr Surg 1996;98:1264–74.

Kuiken TA, Lowery MM, Stoykov NS. The effect of subcutaneous fat on myeoelectric signal amplitude and cross-talk. Prosthet Orthot Int 2003;27:48–54.

Martins RS, Siqueira MG, da Silva CF, de Godoy BO, Plese JPP. Electrophysiologic assessment of regeneration in rat sciatic nerve repair using suture, fibrin glue or a combination of both techniques. Arq Neuropsiquiatr 2005;63(3-A):601–4.

Meek MF, van der Werff JFA, Klok F, Robinson PH, Nicolai J-PA, Gramsbergen A. Functional nerve recovery after bridging a 15mm gap in rat sciatic nerve with a biodegradable nerve guide. Scand J Plast Reconstr Hand Surg 2003;37:258–65.

Molander C, Xu Q, Grant G. The cytoarchitectonic organization of the spinal cord in the rat. I. the lower thoracic and lumbosacral cord. J Comp Neurol 1984;230:133–41.

Negredo P, Castro J, Lago N, Navarro X, Avedaño C. Differential growth of axons from sensory and motor neurons through a regenerative electrode: a stereological, retrograde tracer, and functional study in the rat. Neuroscience 2004;128:605–15.

Nichols CM, Myckatyn TM, Rickman SR, Fox IK, Hadlock T, Mackinnon SE. Choosing the correct functional assay: a comprehensive assessment of functional tests in the rat. Behav Brain Res 2005;163:143–58.

Nicolopoulos-Stournaras S, Iles JF. Motor neuron columns in the lumbar spinal cord of the rat. J Comp Neurol 1983;217:75–85.

Panerai AE, Sacerdote P, Brini A, Bianchi M, Mantegazza P. Autotomy and central nervous system neuropeptides after section of the sciatic nerve in rats of different strains. Pharmacol Biochem Behav 1987;28:285–8.

Perry J, Schmidt Easterday C, Antonelli DJ. Surface versus intramuscular electrodes for electromyography of superficial and deep muscles. Phys Ther 1981;61:7–15.

Puigdellívol-Sánchez A, Forcada-Calvet P, Prats-Galino Alberto, Molander Carl. Contribution of femoral and proximal sciatic nerve branches to the sensory innervation of hindlimb digits in the rat. Anat Rec 2000;260:180–8.

Ramerman WG, Honet JC, Jebsen RH. Serial determination of nerve conduction velocity in the rat. Arch Phys Med Rehabil 1968;49:205–9.

Röder R. Neurographische Befunde. Fehlermöglichkeiten durch Ableitbedingungen und Messung. In: Hopf HC, Dengler R, Röder R., editors. Electromyographie-Atlas. Praktisches Vorgehen und sichere Befundbewertung. Georg Thieme Verlag: Stuttgart, New York, 1996:88.

Rodríguez FJ, Verdú E, Ceballos D, Navarro X. Nerve guides seeded with autologous schwann cells improve nerve regeneration. Exp Neurol 2000:571–84.

Rupp A, Schmahl W, Lederer W, Matiasek K. Strain differences in the branching of the sciatic nerve in rats. Anat Histol Embryol 2007a;36:202–8.

Rupp A, Dornseifer U, Rodenacker K, Fichter A, Jütting U, Gais P, Papadopulos N, Matiasek K. Temporal progression and extent of the return of sensation in the foot provided by the saphenous nerve after sciatic nerve transection and repair in the rat—implications for nociceptive assessments. Somatosens Mot Res 2007b;24(1/2):1–13.

Sato A, Sato Y, Suzuki H. Aging effects on conduction velocities of myelinated and unmyelinated fibers of peripheral nerves. Neurosci Lett 1985;53:15–20.

Scholle HC, Biedermann F, Arnold D, Jinnah HY, Grassme R, Schumann NP. A surface EMG multi-electrode technique for characterizing muscle activation patterns in mice during treadmill locomotion. J Neurosci Methods 2005;146:174–82.

Shenaq JM, Shenaq SM, Spira M. Reliability of sciatic function index in assessing nerve regeneration across a 1 cm gap. Microsurgery 1989;10:214–9.

Terzis JK, Dykes RW, Hakstian RW. Electrophysiological recordings in peripheral nerve surgery: a review. J Hand Surg 1976;1:52–66.

Udina E, Rodríguez FJ, Verdú E, Espejo M, Gold BG, Navarro X. FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogeneic schwann cells. Glia 2004;47:120–9.

Valero-Cabré A, Navarro X. H reflex restitution and facilitation after different types of peripheral nerve injury and repair. Brain Res 2001;919:302–12.

Wolthers M, Moldovan M, Binderup T, Schmalbruch H, Krarup C. Comparative electrophysiological, functional, and histological studies of nerve lesions in rats. Microsurgery 2005;25:508–19.