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Functional, electrophysiologic and morphometric evaluation of peripheral nerve regeneration after bridging a 14 mm gap in the rat sciatic nerve

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For my parents and my brother

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Please note that the consecutive numbering of the Figures and Tables only applies to the Overview of the literature and the Discussion.
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Abbreviations

ATP	adenosine triphosphate
CMAP	compound muscle action potential
CNAP	compound nerve action potential
CNS	central nervous system
EMG	electromyography
ENG	electroneurography
F-wave	“f” is derived from “foot”, where these waves originally were recorded
g-ratio	quotient of the axon area and the total fibre area
H-reflex	Hoffmann-reflex
M-wave	direct motor response
MRI	magnetic resonance imaging
N	Newton [$kg \cdot m/s^2$]
N.	Nervus …
NCAM	neural cell adhesion molecule
NCV	nerve conduction velocity
SFI	Sciatic Function Index
SSI	Static Sciatic Index

> Since the measuring device has been constructed by the observer… we have to remember that what we observe is not nature itself but nature exposed to our method of questioning.
>
> Werner Karl Heisenberg (*Physics and Philosophy*, 1958)

1. Introduction

Peripheral nerve regeneration studies have been conducted in large numbers in recent years with more than 1,150 papers published on this subject between 2004 and 2007 (pubmed). The reason for this is that the functional outcome of peripheral nerve repair procedures is often still considered unsatisfactory (VAREJÃO et al., 2001a,b; JOHNSON et al., 2005; BELLAMKONDA, 2006; CHALFOUN et al., 2006), especially when large nerve gaps have to be bridged (MEEK et al., 2001b) or when peripheral nerves are severed at some distance from their targets (MOLDOVAN et al., 2006). Moreover, there is a search for a method to replace the gold standard for the bridging of peripheral nerve gaps, the autologous nerve graft (JOHNSON et al., 2005; BELLAMKONDA, 2006; CHALFOUN et al., 2006). New insights in the biochemical and ultrastructural components of neural regeneration, together with the expanding area of tissue engineering promise new approaches for solving these problems (CHALFOUN et al., 2006), and consequently need to be assessed in animal studies (BELLAMKONDA, 2006).

The site most often used in peripheral nerve regeneration studies is the sciatic nerve in the rat (VAREJÃO et al., 2001a, 2004a; NICHOLS et al., 2005). Assessment of regeneration can be conducted in many different ways, the methods mainly being divided into functional, electrophysiologic and structural evaluations. Unfortunately, however, there is no consensus as to which methods of assessment provide the most reliable and conclusive information, which means that results of different studies often lack comparability (GERSHENBAUM & ROISEN, 1980; VAREJÃO et al., 2004a).

In the preliminary study (Paper 1) the sciatic nerve and its major branches were assessed anatomically in a total of 210 rats of five different strains. This paper focuses on the availability of sciatic nerve suitable for peripheral nerve regeneration studies, i.e. the length of sciatic nerve before it branches into its tibial and peroneal successors.

In the following main study (Papers 2, 3, and 4) rats were subjected to the extraction of a 14 mm segment of the sciatic nerve and subsequent repair by one of the three following methods: autograft, empty collagen tube, and collagen tube filled with denatured muscle. All lesions were set at the same place, 4 mm proximal to the point at which the tibial nerve submerges into the gastrocnemius muscle.

The extent of regeneration was assessed in a variety of ways. In functional evaluations all rats underwent testing of ambulatory skills, two different footprint analyses and nociceptive testing twice weekly. These examinations were supplemented by electroneurographic assessments. The structural evaluations, which took place after eight weeks of regeneration, comprised morphometric and ultrastructural assessments of the regenerating and the contralateral sciatic nerve at various levels and determination of volumes of the gastrocnemius muscles by water displacement. Additionally, the restoration of the correct anatomical connections for both sensory and motor pathways was examined with the aid of retrograde tracers.

Once all the tests had been completed, the different methods of evaluation were compared and assessed regarding the amount and reliability of the information they provided and their feasibility. Furthermore the suitability of individual methods for assessing specific aspects of regeneration is discussed.

2. Overview of the literature

2.1 Peripheral nerve injuries and regeneration studies

Injuries to peripheral nerves are very common, with more than 300,000 cases reported in Europe every year (MOHANNA et al., 2003) and over 50,000 surgical peripheral nerve repair procedures performed annually in the United States (CHALFOUN et al., 2006).

2.1.1 Origins of research in peripheral nerve regeneration

Attempts at the medical management of neural lesions are documented to go as far back as the seventh century (BUEHLER et al., 1990; HARRIS & TINDALL, 1991), there are however only very occasional reports of attempted nerve repair prior to the late 1800s (NAFF & ECKLUND, 2001). In the nineteenth century then increasing efforts were undertaken to provide peripheral nerve injuries with the appropriate medical attention. In 1873 Hueter described the repair of nerves by sutures placed in the epineurial sheath, a technique that remained the standard method of nerve repair until very recently (HARRIS & TINDALL, 1991). Ultimately, it was the two world wars with their high number of associated neural injuries which drove research forward in the middle of the twentieth century (WOODHALL, 1949; NAFF & ECKLUND, 2001). The availability of medical records on the treatment of war injuries, together with the high incidence of insults to the peripheral nervous system during wartime and the ease with which these could be followed up, led to many retrospective studies on the outcome of medical attention to peripheral nerve injuries. The fact that the results were mainly judged unsatisfactory, provided the impetus for stepping up the research into surgical techniques in this field (WOODHALL, 1949; NAFF & ECKLUND, 2001). Two valuable lessons, however, that were learnt from wartime experiences, were (1) the importance of resecting damaged nerve ends and (2) the necessity for ensuring that end-to-end repairs are subjected to minimum tension (HARRIS & TINDALL, 1991; MILLESI, 1993). Also,

the idea of interposing a nerve graft, which enables surgeons to bridge a gap between proximal and distal nerve ends and thus to treat even an extensive nerve injury without tension, was rekindled in the 1940s by Sir Herbert Seddon (HARRIS & TINDALL, 1991; MILLESI, 1993; NAFF & ECKLUND, 2001). First experiments with nerve grafting had already been described by Philipeaux in 1810 (HARRIS & TINDALL, 1991) and even though good results were reported with this surgical techique, it was still considered to be a last resort (HARRIS & TINDALL, 1991; MILLESI, 1993).

The introduction of the operating microscope in the 1960s propelled peripheral nerve regeneration studies further forward (HARRIS & TINDALL, 1991; DESOUCHES et al., 2005) and made it possible to perform interfascicular repair, as proposed by Sunderland in the 1950s. One of the main protagonists of the new microsurgery techniques in these years was Hanno Millesi, who strongly advocated the use of nerve grafts with interfascicular sutures in those cases where primary repair would lead to too much tension on the suture line (HARRIS & TINDALL, 1991; MILLESI, 1993).

2.1.2 Advances in peripheral nerve repair

Today, the repair of a nerve gap still remains a problem in microsurgery (JOHNSON et al., 2005), as complete recovery of function is rare even in the best-case scenarios (BELLAMKONDA, 2006; CHALFOUN et al., 2006). The most widely accepted method and gold standard for bridging a defect is the use of an autologous donor nerve (JOHNSON et al., 2005; BELLAMKONDA, 2006; CHALFOUN et al., 2006). As this involves creating a second operation site with the associated donor-site morbidity (MEEK & COERT, 2002), and raises issues regarding the increased surgery time and the limited availability of donor nerves, efforts have been increased to find alternative bridging materials (JOHNSON et al., 2005; BELLAMKONDA, 2006; CHALFOUN et al., 2006). Various studies have proposed and tried a variety of autologous tissues such as vein grafts (CHIU et al., 1988; TOS et al., 2000; KARACAOĞLU et al., 2001; MEEK & COERT, 2002; DESOUCHES et al., 2005; JOHNSON et al., 2005), omental grafts (CASTAÑEDA & KINNE, 2002), epineurial sheaths (KARACAOĞLU et al., 2001; CHALFOUN et al., 2006), tendons (CHALFOUN et al., 2006), muscle grafts of both fresh and denatured muscle (MEEK & COERT, 2002; BERTELLI et al., 2005; JOHNSON et

al., 2005; CHALFOUN et al., 2006), and alternative surgical methods such as longitudinally oriented suture bridges (SCHERMAN et al., 2000; KEUNE et al., 2006), end-to-side repair (YÜKSEL et al., 1998; BONTIOTI et al., 2005) or interoperative elongation of the nerve stumps (ARNAOUTOGLOU et al., 2006). Allografts and xenografts have not been effective clinically as they induce host response (CHALFOUN et al., 2006) and therefore require immunosuppressive therapy (KEILHOFF et al., 2005).

Advances in modern tissue engineering have made artificially created tubular structures both of biodegradable and non-biodegradable tissue available for the use as scaffolds (DESOUCHES et al., 2005). Examples of biodegradable tissues include polyesters such as polylactic acid and polyglycolic acid (HADLOCK et al., 2000; FRANCEL et al., 2003; CHALFOUN et al., 2006), lactate-polymers (DA SILVA et al., 1985; MEEK et al., 1999b; MEEK et al., 2001a,b; MEEK & COERT, 2002; MEEK et al., 2003; JOHNSON et al., 2005), genipin-cross-linked gelatin (CHEN et al., 2005), collagen type I (ARCHIBALD et al., 1991; MADORSKY et al., 1998; MADISON et al., 1999; CHAMBERLAIN et al., 2000) or a mixture of collagen type I and III (STANG et al., 2005). Non-biodegradable nerve scaffolds have be fabricated out of materials such as silicone (LUNDBORG et al., 1982; TERRIS et al., 1999; MEEK & COERT, 2002; FRANCEL et al., 2003; ODAKA et al., 2003), polyethylene (MEEK & COERT, 2002), polymethacrylate (KATAYAMA et al., 2006) and polyvinyl (MEEK & COERT, 2002). The problem with non-biodegradable scaffolds, however, is that they have been reported to evoke chronic foreign body reactions (MEEK & COERT, 2002).

Neutral scaffolds can be further modified and enriched with cells that promote regeneration, such as Schwann cells (ANSELLIN et al., 1998; RODRÍGUEZ et al., 2000; HADLOCK et al., 2000; UDINA et al., 2004; KEILHOFF et al., 2005; STANG et al., 2005; CHALFOUN et al., 2006) or stem cells of various origins (CHALFOUN et al., 2006). Alternatively or additionally, interponates can incorporate neurotropic and neurotrophic substances, such as nerve growth factor (CHEN & WANG, 1995; FINE et al., 2002; LEE et al., 2003), brain-derived neurotrophic factor, insulin-like growth factor (JOHNSON et al., 2005), ciliary neurotrophic factor (CHALFOUN et al., 2006), FK 506 (CHUNASUWANKUL et al., 2002; UDINA et al., 2004; SNYDER et al., 2006) or the basal lamina from acellular muscle (MEEK et al., 1999a; MEEK et al., 2001a; JOHNSON et al., 2005). If feasible, these can alternatively be applied systemically (CHUNASUWANKUL et al., 2002; UDINA et al., 2004; SNYDER et al., 2006).

2.1.3 Problem formulation

In the following study a recently developed collagen tube (bovine collagen type I) with an internal diameter of 1.5 mm was to be evaluated regarding its ability to promote and sustain neural regeneration across a gap in a peripheral nerve. Filling a scaffold with denaturated autologous muscle tissue has already been shown to successfully promote neural regeneration (MEEK et al., 1999b), as the large basal lamina tubes from the acellular muscle serve as pathways for the regenerating axons (BERTELLI et al., 2005; JOHNSON et al., 2005; KEILHOFF et al., 2005). Therefore, the effectiveness of the empty collagen tube was to be compared with collagen tubes filled with denatured autologous muscle and with the gold standard of nerve grafting (FRANCEL et al., 2003; BELLAMKONDA, 2006), the autograft (Fig.1).

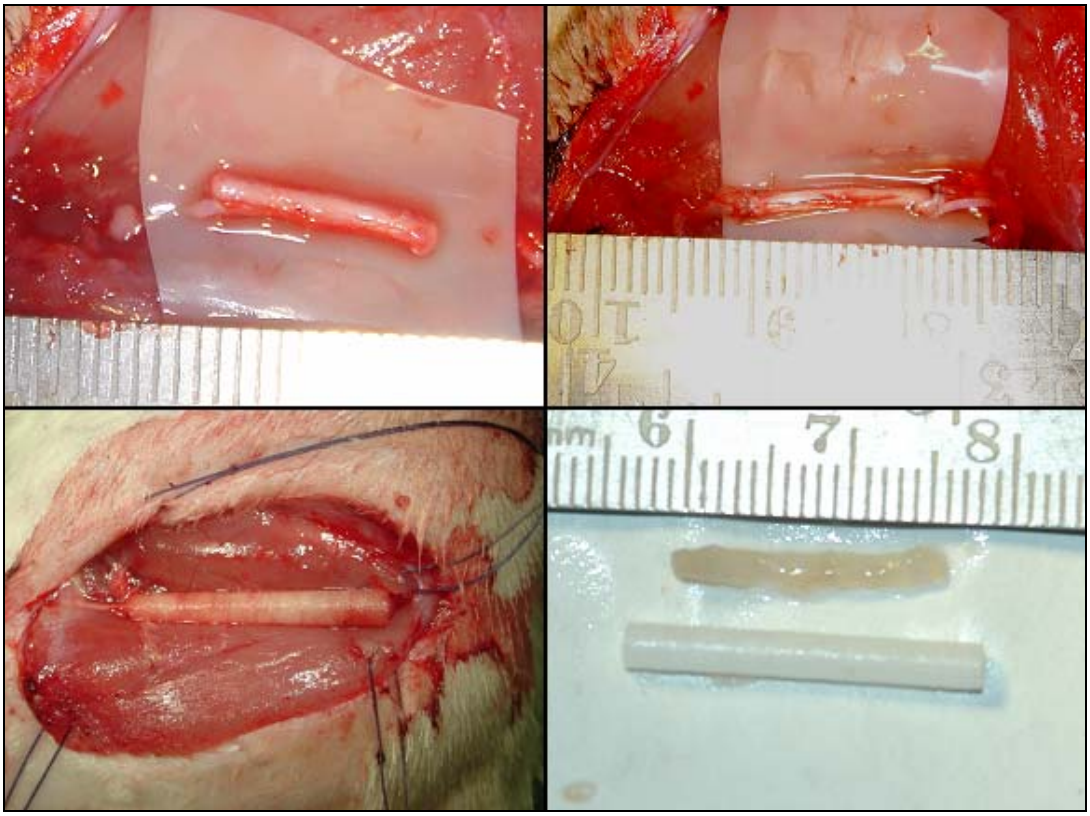

Fig.1 Surgical images (clockwise from top left): excision of a 14 mm segment of the rat sciatic nerve, repair of the defect by autograft, empty collagen tube and autologous muscle, implanted collagen tube.

2.2 Choosing the animal model

Peripheral nerve regeneration studies have been conducted on various species including primates, dogs, cats, rabbits, rats, mice, sheep and goats (KLINE et al., 1964; ARCHIBALD et al., 1991; MADISON et al., 1999; STRASBERG, 1999; VAREJÃO et al., 2004a).

2.2.1 Rate of regeneration

Only minor differences in the rate of regeneration can be observed between the individual species (Table 1), the greatest source of variation being the time the nerve requires to recover from an insult and start regeneration (lag phase).
Generally, the regeneration of peripheral nerves can be divided into three stages (GUTMANN et al., 1944):

(1) the initial lag phase, in which the remaining fibres undergo retrograde degeneration and new axonal growth cones start their relatively slow outgrowth,
(2) axonal elongation, and
(3) remyelination, which follows axonal outgrowth after a five-day lag (SECKEL, 1990) or after the axon has reached and successfully reinnervated the end organ (JOHNSON et al., 2005).

The time required for reestablishing functional connections between nerve and end organ is normally included in calculations regarding the return of motor and sensory function after insult to a specific level of the nerve.

2.2.2 Implications for regeneration studies

In calculations regarding the amount of time expected between providing the insult to the nerve and the return of function, the distance from the point of lesion to the functional organ, the lag phase and the very slightly varying rates of axonal elongation in the different species all play a vital role. If, for example, the sciatic nerve is crushed in both the dog and the rat at midfemur level, the distance the axon has to span in the dog is three to four times greater than that in the rat. As rats normally have a shorter lag phase than dogs, this additionally will lead to an earlier exhibition of regenerative success.

Table 1: Rates of peripheral nerve regeneration

Species	Lag phase	Elongation	Return of function
Human [1,7]	2 - 9 d		1- 2 mm/d
Dog [2,8]	> 8 d	2.3 – 2.5 mm/d	2.8 – 3 mm/d (sensory)
Cat [1,5,7]	~ 8 d	2 – 3.7 mm/d (section) 3 – 4 mm/d (crush)	4 mm/d (sensory)
Rabbit [1,2]	5 – 8 d	3.5 mm/d (section) 4.4 mm/d (crush)	2.0 – 3.0 mm/d (motor) 3.5 – 4.4 mm/d (sensory)
Rat [3,4,8,9]	1 – 4 d	3.0 – 4.6 mm/d (crush)	3.0 – 4.7 mm/d (sensory)
Mouse [6]			1.5 – 1.9 mm/d (motor) 2.0 – 2.9 mm/d (sensory)

[1] GUTH, 1956; [2] GUTMANN et al., 1944; [3] IJKEMA-PAASSEN et al., 2002; [4] PESTRONK et al., 1980; [5] STOLL & MÜLLER, 1999; [6] VERDÚ et al., 2000; [7] MOLDOVAN et al., 2006; [8] FORMAN et al., 1979; [9] FORMAN & BERENBERG, 1978.

Consequently, preference is given to smaller animals when planning peripheral nerve regeneration studies, as the housing and personnel costs can be cut due to shorter regeneration spans. Also the purchase and housing of larger animals like cats, dogs and primates is a lot more expensive than for smaller animals (STRASBERG et al., 1999; NICHOLS et al., 2005), small laboratory animals can be kept in fairly large numbers in a relatively confined space and, because of their size, can usually be handled by one person alone (NICHOLS et al., 2005). Furthermore, reproduction in these small laboratory animals is frequent and plentiful, so that researchers can work with homogenous study groups (VAREJÃO et al., 2001b; NICHOLS et al., 2005) which can easily be kept in standard conditions (STRASBERG et al., 1999).

All these factors have led to the extensive use of rats and mice for research purposes, which also means that scientific information (VAREJÃO et al., 2001a,b) and tools (e.g. molecular probes and cells) are widely available for these species.

2.2.3 Rats versus mice

The reason why rats are preferred to mice in peripheral nerve regeneration studies might be their size. For one thing, handling and intricate evaluations are easier to be carried out with rats, since mice are so much smaller. For another thing, the

sciatic nerve in the rat is the same size as the nerves of the human hand (NICHOLS et al., 2005), and that part of the median nerve located immediately above the wrist is one of the most common sites of peripheral nerve injury in humans (MADISON et al., 1999). Furthermore, at light and electron microscopic level the rat nerve is indistinguishable from the human nerve (STRASBERG et al., 1999).

2.2.4 Choosing the rat featured in the study

When choosing the rat used in peripheral nerve regeneration studies (age, strain, gender) a range of factors have be taken into consideration as outlined below.

2.2.4.1 Age

The age of the animals used in regeneration studies is of great importance. In neonatal animals aged up to four/14 days an insult to motoneurons leads to their death (BELIN et al., 1996; VERDÚ et al., 2000). As soon as the rats reach an age of more than three weeks, however, they exhibit faster neural regrowth after crush (axonotmesis) or section (neurotmesis) when compared to adult animals (BELIN et al., 1996) and this trend continues for the rest of the life of the rats (VERDÚ et al., 2000). In senile individuals (older than 20 months) the majority of the motor nerves regenerate at only an eighth of the rate, and the majority of sensory nerves at about half the rate seen in rats which are only two months old (PESTRONK et al., 1980). The initial lag phase, however, is the same in all age categories and a very small proportion of nerve fibres regenerates at the very high rate of approximately 4.5 mm/d throughout a rat's entire life (PESTRONK et al., 1980).

In older animals a decrease in terminal sprouting in the area of the motor endplates can be observed (PESTRONK et al., 1980; VERDÚ et al., 2000) due to disturbed interactions between Schwann cells and axons (KEREZOUDI & THOMAS, 1999; VERDÚ et al., 2000). These disturbed interactions also lead to less proximal sprouting of regenerating motor axons, which generally has the effect of reducing motor recovery in aged animals (VERDÚ et al., 2000), and the occurrence of regenerating axons which are only sparsely myelinated, if at all (KEREZOUDI & THOMAS, 1999).

2.2.4.2 Strain

Strain-specific differences regarding temperament and behaviour have been described in literature (RAY & BARRETT, 1975). These have a great influence on the rats when they are being handled and also affect their affinity to autotomy. Autotomy has been defined as automutilation and self-amputation of the toes after insults to peripheral nerves (KINGERY & VALLIN, 1989; CARR et al., 1992) and varying opinions have been expressed with regard to the background and pathogenesis of this behaviour. Some researchers relate autotomy to return of sensation (KINGERY & VALLIN, 1989; WILLENBRING et al., 1994), which is often associated with 'missensations' (KINGERY & VALLIN, 1989), others are convinced that autotomy can only be observed in animals which regain no sensation at all (DEN DUNNEN & MEEK, 2001); the etiology remains to be clarified (CARR et al., 1992). Nevertheless, differences between strains regarding the occurrence of autotomy have been described. Buffalo, Sprague-Dawley and Brown-Norway rats tend to exhibit this behaviour routinely, whereas Lewis rats do not show it at all (PANERAI et al., 1987; CARR et al., 1992; STRASBERG et al., 1999). Crossing-in of the Lewis strain into other strains is sufficient to have a positive influence on the occurrence of autotomy (INBAL et al., 1980). Furthermore, gender and age also seem to play a role in autotomy. Female rats seem to exhibit it less than male rats (INBAL et al., 1980; WAGNER et al., 1995) and younger animals are less likely to chew their toes than older individuals (INBAL et al., 1980). There seem, however, to be further parameters which influence this kind of behaviour, since there are wide variations between individual animals (INBAL et al., 1980; CARR et al., 1992). In addition to not being affected by autotomy, Lewis rats are reported to be easy to handle (STRASBERG et al., 1999) due to their friendly and trusting nature.

Other temperamental traits exert an influence on the functional outcome of neuronal insults. Two of these are (1) the individual level of activity in an open field, which means how much the rat actually moves of its own accord, and (2) the ability of the rat to cope with chronic stress. It has been observed that very active rats exhibit faster sensory regeneration than rats which choose not to move much (VAN MEETEREN et al., 1997a). In this context is has to be stressed that mobilization and movement have to result from the rats' own motivation (LUNDBORG, 2003). Forced activity leads to increased levels of plasma glucocorticoid levels, which in turn can have a detrimental effect on axonal sprouting and elongation (VAN MEETEREN et al., 1997b; AMAKO & NEMOTO, 1998; VAN MEETEREN et al., 1998). More sedentary animals react to new and stressful situations with strong

reactions on the adrenocortical axis (VAN MEETEREN et al., 1997a). As a result the weights of the adrenal glands increase and higher glucocorticoid levels can be observed (VAN MEETEREN et al., 1997a). Lewis, Holtzmann and Sprague-Dawley rats are regarded as more active strains, Fischer 344 and Buffalo rats as more sedentary strains (RAY & BARRETT, 1975).

There seem to be no differences between the various rat strains as regards the extent of motor regeneration after insult to the sciatic nerve (STRASBERG et al., 1999).

2.2.4.3 Gender

Size and weight of the animals depend not only on the strain, but also on age and gender. Adult male rats of the Fischer 344 strain, for example, usually reach two-thirds of the weight and size of adult male Sprague-Dawley rats (personal observations). The latter can attain body weights of more than 600 g, at the same time exhibiting a femur length of 46 mm or more (personal observations).

Female rats are typically smaller and lighter in all strains.

2.3 Methods for evaluating sciatic nerve regeneration in the rat

In peripheral nerve regeneration studies the sciatic nerve in the rat is the most commonly used of all available sites (NICHOLS et al., 2005). Amongst the reasons for this (see also Section 2.2.2) is the fact that neural regeneration is generally good in rats (CARR et al., 1992; STRASBERG et al., 1999; KEUNE et al., 2006) and this, combined with the relatively short distance to target organs (KEUNE et al., 2006) compared with other animals, makes it possible to demonstrate regenerative success in a reasonable time frame. The sciatic nerve as such is the preferred model for studies on regeneration (VAREJÃO et al., 2001a, 2004a; NICHOLS et al., 2005), as it is easily accessible and represents a mixed nerve, so that motor, sensory and/or autonomic aspects (HADLOCK et al., 1999) can be studied. The last characteristic, however, necessitates performing multimodal tests when evaluating regeneration.

A large variety of tests have been developed and are applied for the purpose of evaluating regeneration (HADLOCK et al., 1999; HOWARD et al., 2000), which makes it increasingly difficult to compare different studies (GERSHENBAUM & ROISEN, 1980; VAREJÃO et al., 2004a). Many of them refer to completely different aspects of regeneration, from axonal sprouting to integrated muscle function.

For simplicity's sake, the methods of evaluation have been divided into four groups according to the anatomical/structural and physiologic/functional level of regeneration (e.g. innervation of the distal stump) rather than according to the techniques of evaluation applied (e.g. electroneurography). At the same time this grouping conveniently corresponds to the chronological sequence of regeneration from the breakdown of existing axons to complete restoration of function (Table 2).

2.3.1 Reinnervation of the distal stump

Reinnervation of the distal stump represents the first step on the long and tortuous road towards regeneration and can be subdivided into

(1) the breakdown of existing axons due to Wallerian degeneration (WD) after placement of the lesion,
(2) sprouting of growth cones from the proximal stump,
(3) elongation of new axons across the lesion site and into the distal stump, and
(4) maturation (myelination and increase in diameter) of the newly formed fibres.

Table 2: Methods of evaluation

	Reinnervation of the distal stump	Reinnervation of the muscle	Restoration of anatomical features	Restoration of function
Observation	–	+	–	+
Motor testing	–	(+)	–	+
Sensory testing	–	–	–	+
Gait analyses	–	(+)	–	+
EMG	–	+	+	+
ENG	+	–	+	–
MRI	+	+	(+)	–
Nerve morphology / morphometry	+	–	+	–
Muscle morphology / morphometry	–	+	+	–
Tracing	Anterograde	(+)	Retrograde	–
EXTRAS	Immuno-histology, Immunohisto-chemistry	Immunohisto-chemistry, muscle weight / volume / circumference	Microangio-grams	Ambulatory testing, sudomotor testing

The earliest sign of WD, the disintegration of the axonal cytoskeleton, is visible in electron microscopic assessments within two to three hours after neural transection. Small myelinated fibres are disembodied faster than larger fibres, unmyelinated fibres degenerate most rapidly of all (GUTH, 1956). The breakdown of myelin takes place from day 2 to day 14, peaking on days 4 to 7 (STOLL et al., 1999). Axonal sprouting in rats begins as from day 2 after axonotmesis (PESTRONK et al., 1980; IJKEMA-PAASSEN et al., 2002) with the axon tips elongating distally at maximum velocity of about 4.5 mm/d (PESTRONK et al., 1980). Stainable myelin sheaths are first noted on the sixth day after outgrowth, and the nodes appear about two weeks later (GUTH, 1956).

2.3.1.1 Nerve morphology and morphometry

The above-mentioned aspects can be quantified by evaluating samples of the regenerating nerve (Figs.2, 3), taken at varying time intervals (GERSHENBAUM & ROISEN, 1980; KEUNE et al., 2006) and anatomical levels (CHAMBERLAIN et al., 2000) under the **light microscope** (DE KONING et al., 1986; DE MEDINACELI,

1995; KOBAYASHI et al., 1997; TERRIS et al., 1999; LEE et al., 2003; ODAKA et al., 2003; KEUNE et al., 2006) or the **electron microscope** (GERSHENBAUM & ROISEN, 1980; CHEN et al., 1995; MEEK et al., 2001a; VAREJÃO et al., 2004b).
More detailed evaluations, especially those relating to the maturation of regenerating axons, are conducted by taking fibre counts and measuring fascicle, axon and fibre diameters and myelin sheath thicknesses in nerve cross sections (KANAYA et al., 1996; KOBAYASHI, 1997; WOLTHERS et al., 2005). These **morphometric** values are then incorporated into ratios (DE MEDINACELI, 1995; CHAMBERLAIN et al., 2000; FRANCEL et al., 2003; LEE et al., 2003; VAREJÃO et al., 2004b; KEUNE et al., 2006) to ensure better and more objective comparison of regenerating nerves (MEEK et al., 2001a).
Good longitudinal evaluation of individual strands is achieved by **teasing** nerves into single fibres under the dissection microscope (GERSHENBAUM & ROISEN, 1980). An excellent portrayal of the occurrence of WD and the degree of the myelin sheath maturation can be obtained in this way (WIECZOREK, 2002).

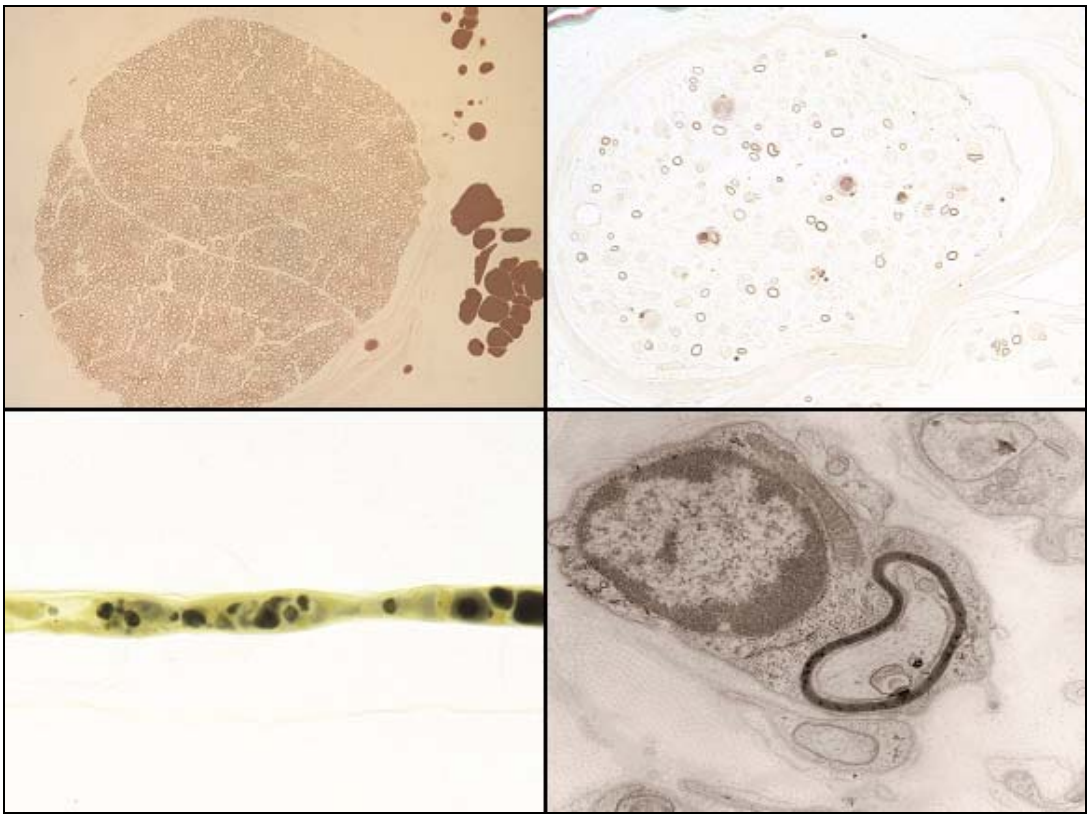

Fig.2: top row: **nerve histology:** physiological appearance (left), under regeneration (right). bottom row: **teasing preparation** of a nerve undergoing Wallerian degeneration (left), **electron microscopic picture** of a regenerating myelinated axon and its Schwann cell (right).

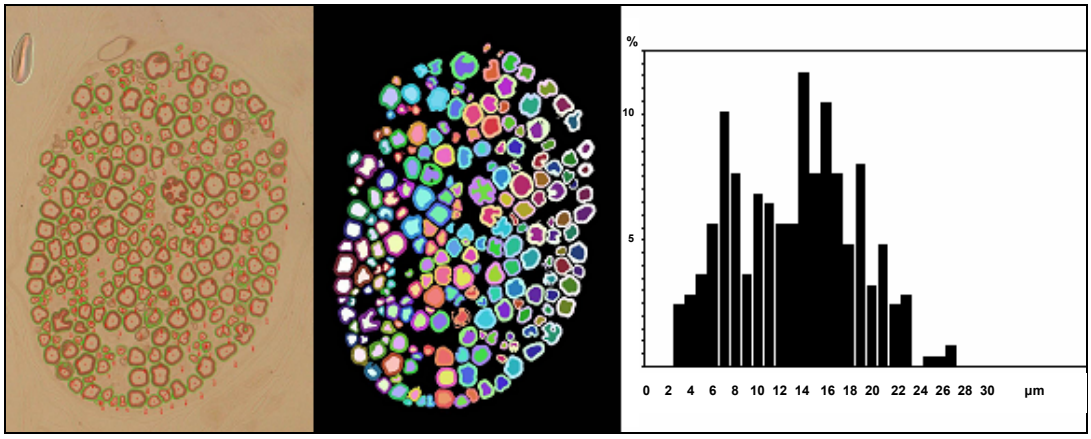

Fig.3 Nerve morphometry: registration of all fibres (left), representation of axons and myelin sheaths with different coloured pixels (middle), histogram of the axon radii (right).

2.3.1.2 Electroneurography

Stimulation of the nerve proximal to the lesion site by either electrical impulses (KOBAYASHI et al., 1997) or magnetic impulses (SMIT et al., 2006) and recording compound nerve action potentials (CNAPs) distal to the site, or vice versa (SMIT et al., 2006), can provide insight into the functional status of nerve regeneration. This method can also be performed in vitro after extracting the nerve and placing it into a recording chamber (CHIU et al., 1988; VLEGGEERT-LANKAMP et al., 2004; SMIT et al., 2006). However, it is important to bear in mind that severed axons retain their electrical excitability for up to four or five days after the insult (DORFMAN, 1990; TERZIS et al., 1976).

The amplitude of the CNAP and in particular its area (CUDDON, 2002) indicate the number of available functioning axons (DELLON et al., 1989; KANAYA et al., 1999; CUDDON, 2002) and their distribution in size. Maturation of the regenerating axons can also be assessed (DELLON et al., 1989; NEGREDO et al., 2004), since the conduction velocity is closely linked to the axon diameters and degree of myelination (TERZIS et al., 1976; WOLTHERS et al., 2005) of the fastest conducting fibres (KANAYA et al., 1996).

2.3.1.3 Nerve immunohistology and immunohistochemistry

It is possible to quantify the expression of protein within the nerve (such as Neurofilament, growth associated protein (GAP-43) or myelin associated growth protein (MAG)), either by denaturing the nerve and analysing its contents via Western blot techniques and monoclonal antibodies (TERRIS et al., 1999), or by staining sections with immunofluorescent antibodies (SHIN et al., 2003).

2.3.1.4 Anterograde tracing

Radioactive (glyco-) proteins are injected near the perikaryon and these are then transported distally at velocities of up to 383 mm/d (GRIFFIN et al., 1976) via fast axonal transport. The radioactive molecules accumulate in the distal regions of the regenerating sprouts, labelling the fastest regrowing axons and also their bulk (FORMAN & BERENBERG, 1978). They are then visualized by scintillation (GRIFFIN et al., 1976; PESTRONK et al., 1980).

2.3.1.5 Magnetic resonance imaging

As soon as 24 h after injury to the nerve an increased signal in T2-weighted MR-images can be observed, which gains in intensity over time and progresses proximo-distally. In the early stages the hyperintensity is caused by degeneration of axons and myelin, in the subacute phase the increased signal results from endoneurial edema. The signals return to isointensity as soon as the physiologic properties of the nerve have been re-established (BENDSZUS et al., 2004).

2.3.2 Reinnervation of muscle tissue

The second step towards regeneration sees the re-establishment of the synaptic connections between the muscle and a reinnervating nerve, which results in a return of muscle function. The muscle contractions, however, often appear incoordinated and wrongly timed if cross-innervation or inappropriate innervation have occurred (GRAMSBERGEN et al., 2000). Denervation for longer than 3 months causes formation of fibrous tissue in the muscle (KOBAYASHI et al., 1997), but a decrease in contraction force can even be observed after shorter intervals of denervation (KALLIANINEN et al., 2002). A delay in the reconstruction of a nerve defect for as little as a month is sufficient to compromise both the return of muscle mass and integrated motor function (KOBAYASHI et al., 1997).

2.3.2.1 Observation of the rat

The easiest way to ascertain whether neuromuscular synapses have been re-established and the muscle has been reinnervated is to watch out for muscle activity during movement or handling of the rat (DE MEDINACELI et al., 1982).

2.3.2.2 Muscle circumference, weight and volume

Denervation-induced muscle atrophy (Fig.4) is quick to appear (MALUSHTE et al., 2004) and the muscle makes a slow recovery “reflecting the amount of reinnervation” (cited in VAREJÃO et al., 2004a).

The **circumference** of muscle groups innervated by the sciatic nerve (CHAMBERLAIN et al., 2000) can easily be determined in the living animal, for example in MR-scans (WESSIG et al., 2004), and compared with control values established prior to the insult. The contralateral side has been shown to compensate with maximal hypertrophy in week 5 after trauma (WESSIG et al., 2004).

After euthanasia both the **wet weight** and **volume** of muscle groups innervated by the sciatic nerve can be determined and – normalized against the body weight – compared with control values (KOBAYASHI et al., 1997; TERRIS et al., 1999) or with the contralateral side (CHUNASUWANKUL et al., 2002; MALUSHTE et al., 2004; VAREJÃO et al., 2004a,b).

However it is important to bear in mind, that fat and fibrous tissue can feign increased muscle circumferences, weights and volumes (KANAYA et al., 1996; VAREJÃO et al., 2004a), just as muscle can be built up through dysfunctional cross-innervation (KANAYA et al., 1996).

2.3.2.3 Electromyography

Within 48 to 72 hours after a crush injury, depending on the distance between the muscle and the site of lesion in the nerve (WESSIG et al., 2004), muscles indicate their denervation by increased insertional activity and abnormal spontaneous activity (RESTREPO et al., 1983). The latter consists mainly of fibrillation potentials (Fig.4) and positive sharp waves, arising from the fact that myofibres are hypersensitized. This is a result of the destabilization of the sarcolemmal membrane due to denervation of the muscle (CUDDON, 2002).

If compound motor action potentials (CMAPs; Fig.4) can be elicited again by electrical stimulation of the innervating nerve, they exhibit decreased amplitudes (RESTREPO et al., 1983; NAVARRO et al., 1994; BENDSZUS et al., 2004; NEGREDO et al., 2004), increased areas and prolonged latencies (RODRÍGUEZ et al., 2000; VALERO-CABRÉ et al., 2001; WOLTHERS et al., 2005). The CMAP amplitude depends on the number of motor nerve fibres responding to the stimulus, the synchronisation of their response and the size of the motor units innervated by the axons (WOLTHERS et al., 2005). A temporal dispersion of the

CMAP (increased area) with or without polyphasia indicates that the regenerating axons conduct at varying speeds due to their different sizes and different stages of myelination (CUDDON, 2002).

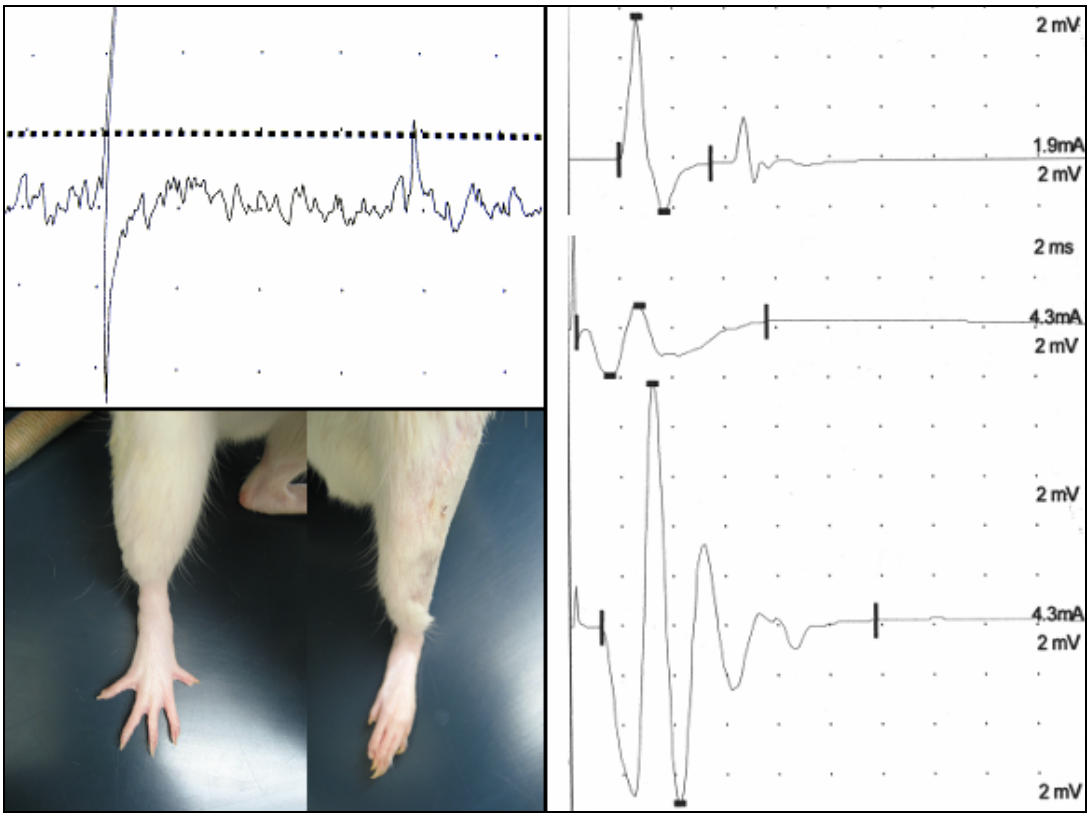

Fig.4: Left hand side: **fibrillation potentials** recorded in the gastrocnemius muscle (top), **denervation atrophy** of calf and shin muscles (bottom).
Right hand side: **compound muscle action potentials** recorded from the gastrocnemius muscle. Physiological image (top), longer duration and lower amplitude (middle), longer duration and polyphasia (bottom).

2.3.2.4 Muscle morphology and morphometry

Muscle samples can be evaluated by **light microscopy** to ascertain the cross-sectional appearance of individual fibres (JAWEED et al., 1955; WESSIG et al., 2004). Denervated muscle fibres are irregular in diameter, become angular or anguloid (ODAKA et al., 2003), and are surrounded by abnormal quantities of fibrous material (Fig.5). In **morphometrical assessments** the fibre diameters are measured and compared with control values (KALLIANINEN et al., 2002; WESSIG et al., 2004).

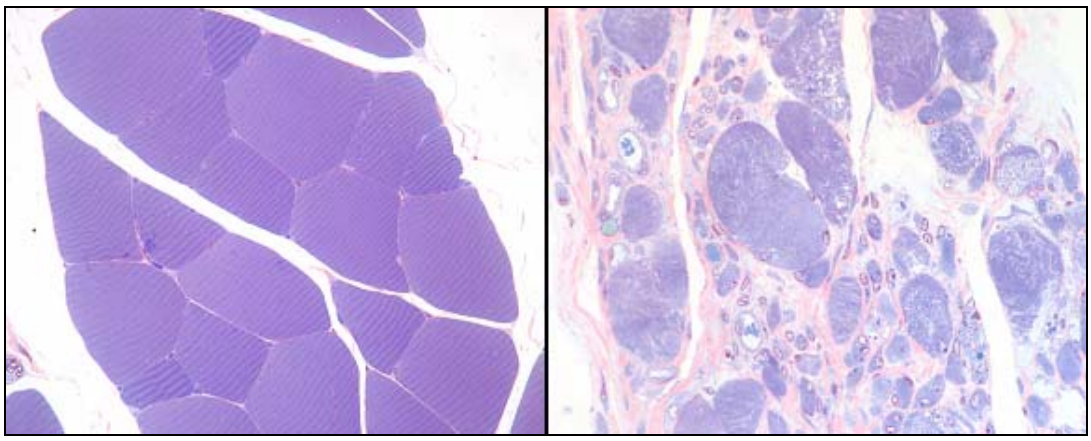

Fig.5 Muscle histology: physiological appearance (left), denervation atrophy (right).

2.3.2.5 Muscle immunohistochemistry

The different **muscle fibre types** can be ascertained using antibodies for myosin heavy chains (BODINE-FOWLER et al., 1997) or the myofibrillar ATPase (JAWEED et al., 1955; HERBISON et al., 1974; PACHTER et al., 1985). During reinnervation, fibre type grouping can be observed (ODAKA et al., 2003). Furthermore, compensatory hypertrophy of fibre type I can be seen, since this fibre type is neurotrophically not as dependent as types IIa and IIx (PACHTER et al., 1985).

The **extent of reinnervation** can be visualized by incubating muscle samples with a marker for the neural cell adhesion molecule (NCAM). This molecule is usually only expressed at neuromuscular junctions in the adult individual, but after denervation it is expressed along the entire sarcolemma (KALLIANINEN et al., 2002).

Investigation of **neuromuscular end plates**, which deteriorate within 2 weeks after denervation, can be undertaken by incubating muscle sections with acetyl-thiocholine-iodide solution to visualize acetylcholine-esterase. The sections are then examined with regard to morphology, size, location and number of nerve endings of the neuromuscular endplates. It has been shown that these exhibit a granular appearance with possible polyneural innervation during reinnervation (IJKEMA-PAASSEN et al., 2002).

2.3.2.6 Isometric contraction measurements

The contraction force of the lower leg muscles can be quantified by attaching either the third toe (after having immobilized the foot in a foot mould; KERNS et al., 1987;

TERZIS & SMITH, 1987) or the distal tendons (KANAYA et al., 1996; KALLIANINEN et al., 2002) to a force displacement transducer (HERBISON et al., 1980; KERNS et al., 1987; TERZIS & SMITH, 1987; ZHAO et al., 1992; BODINE-FOWLER et al., 1997; KALLIANINEN et al., 2002; ODAKA et al., 2003). The sciatic nerve is stimulated proximally.

2.3.2.7 Magnetic resonance imaging

Within two days after insult to the supplying nerve, a prolongation of the T2-relaxation time can be observed in the freshly denervated muscle due to increased blood perfusion. This coincides with the occurrence of spontaneous muscle activity (WESSIG et al., 2004).

2.3.3 Restoration of the neuroanatomical structures and pathways

The goal of regeneration in the peripheral nervous system is to achieve re-establishment of the correct physiological structures and pathways. However, it is interesting to note that these properties do not need to be restored in their entirety as compensating and modulating mechanisms occur (GRAMSBERGEN et al., 2001). These include sprouting, an increase in motor unit sizes (KOBAYASHI et al., 1997; BROWN & IRONTON, 1978) and, above all, plasticity on the cortical (DELLON & MACKINNON, 1989; CUSICK et al., 1990; DONOGHUE et al., 1990; SANES et al., 1990) and subcortical level (CHEN et al., 2002; BRUSHART et al., 2005). The extent of plasticity is difficult to quantify though. The only feasible method is to compare the results of functional assessments (see 3.4) with the results of morphologic assessments of muscles and nerves and of retrograde tracing.

2.3.3.1 Retrograde tracing

Retrograde tracers such as Choleratoxin, Horseradish-Peroxidase, Fluorogold or Fast Blue (Fig.6) are injected either intracutaneously (sensory pathway) (PUIGDELLÍVOL-SÁNCHEZ et al., 2005) or intramuscularly (motor pathway) (BODINE-FOWLER et al., 1997; GRAMSBERGEN et al., 2000; SARIKCIOGLU & OGUZ, 2001) into target regions innervated by the sciatic nerve. After transsynaptic uptake the tracers are transported to the perikaryon by retrograde

axonal transport (KÖBBERT et al., 2000) and are visualized with the aide of immunohistochemical procedures (DA SILVA et al., 1985; BRUSHART et al., 1998; MADORSKY et al., 1998; KÖBBERT et al., 2000) or with appropriate filters (NEGREDO et al., 2004) as some tracers display fluorescence (KÖBBERT et al., 2000; OZTAS, 2003; VALERO-CABRÉ et al., 2004; PUIGDELLÍVOL-SÁNCHEZ et al., 2005, 2006). Alternatively the tracers can be administered directly into the nerve under observation (BRUSHART et al., 1998; VALERO-CABRÉ et al., 2004; NEGREDO et al., 2004; BRUSHART et al., 2005) and then visualized as before.
By comparing the marked neuronal cell bodies with the physiologic distribution of the corresponding motoneurons in the spinal cord or with the corresponding sensory neurons of the dorsal root ganglia which are responsible for the target area, it is possible to determine whether the correct anatomical connections have been re-established. The amount of aberrant innervation of a muscle, for example, can be quantified by examining the motoneuron pools in the operated and unoperated sides of the rat (GRAMSBERGEN et al., 2000). Furthermore, with certain tracers it is possible to determine the extent of neural dentritic arborisation which usually decreases after neural injury (GRAMSBERGEN et al., 2000).

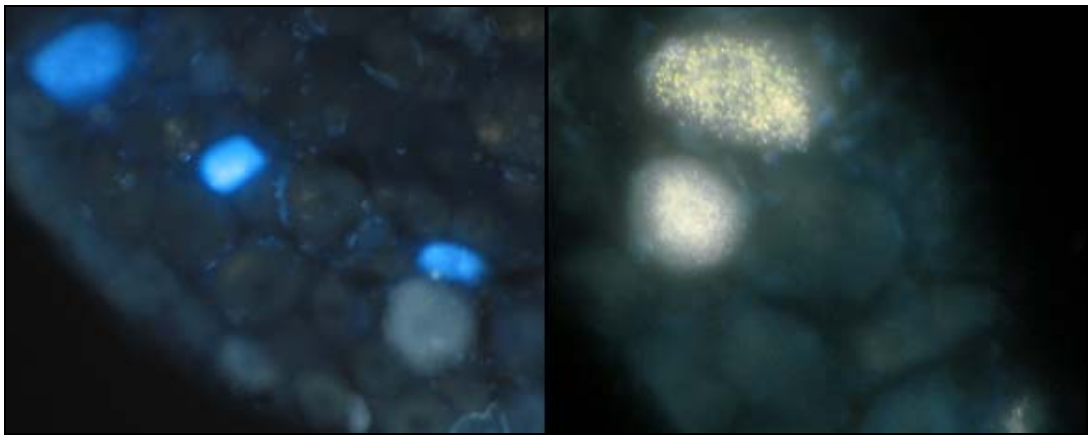

Fig.6: Retrograde tracers: Fast Blue (left) and Fluorogold (right) in dorsal root ganglia.

2.3.3.2 Nerve and muscle morphology

Nerve morphometry (and also muscle morphology and immunohistology) provides the researcher with good insight into the trophic condition of regenerating structures (DE MEDINACELI, 1995; KANAYA et al., 1999). This is especially the case when injured and uninjured sides are compared.

2.3.3.3 Electromyography and electroneurography

The electrophysiologic properties of muscles and nerves can only be restored once the anatomical base values have been re-established. A comparison of conduction velocities, threshold changes, neural late responses (H-reflex and F-wave), CMAPs and CNAPs (RODRÍGUEZ et al., 2000) measured on both the injured and uninjured sides, together with calculations of H-reflex/M-wave ratios (VALERO-CABRÉ & NAVARRO, 2001), are additional ways of enabling the researcher to gain an impression of the degree of restoration. F-wave testing sheds light on the conduction in the proximal stump and the functional condition of the nerve cell body, as the F-wave results from antidromic stimulation of the perikaryon after supramaximal electrical stimulation of the axon of the α-motoneuron, which is then volleyed back along the same pathway and produces a muscle contraction (BISCHOFF, 2002: ESPIRITU et al., 2003). The H-reflex, however, as its suffix indicates, is dependent on the functioning of a spinal reflex, but does not represent a purely monosynaptic reflex (MISIASZEK, 2003). Here the α-motoneuron is activated by afferent Ia fibres and a group of Ib fibres after electrical stimulation of the α-motoneuron with submaximal intensity (BISCHOFF, 2002).

2.3.3.4 Magnetic resonance imaging

In T2-weighted MRI-images the return of hyperintensive signals to isointensity in both nerve and muscle tissues can be viewed as evidence that the physiologic structures have been re-established (BENDSZUS et al., 2004; WESSIG et al., 2004). Furthermore, functional MRI can help to monitor the rate of cortical recovery and the extent of cortical reorganization after regeneration of the peripheral nerve (PELLED et al., 2006).

2.3.3.5 Microangiograms

Since the intrinsic blood vessel system is essential to the survival of the nerve, microangiograms can be made to establish the existence of the endoneurial, perineurial and epineurial blood vessels with their anastomoses. For this examination the rat is heparinized before death and infused intravenously with barium sulfate (RESTREPO et al., 1983).

2.3.4. Restoration of function

Functional regeneration comprises the sum of all types of structural regenerations plus the imponderable factor plasticity, which is why the results of functional assessments and structural assessments correlate with each other very little, if at all (DE MEDINACELI, 1995; KANAYA et al., 1995; WOLTHERS et al., 2005). Nevertheless, functional evaluations play an important role in the assessment of peripheral nerve regeneration (DE MEDINACELI et al., 1982; HADLOCK et al., 1999; HOWARD et al., 2000; NICHOLS et al., 2005) as they denote how well the body (especially the central nervous system) is adapting to or compensating problems arising mainly from aselective reinnervation (DELLON & MACKINNON, 1989). The phenomenon of cross-innervation, which can lead to extensive maladaptive functioning (GRAMSBERGEN et al., 2000) is very common after nerve injury (BODINE-FOWLER et al., 1997), especially after neurotmesis in combination with a gap, and is a thorny problem which requires much further research (BRUSHART et al., 2005).

The period of time usually required for the restoration of function is 3.0 ± 0.2 mm/d for sensory nerves after section (FORMAN et al., 1979). Motor fibres exhibit a similar rate of regeneration (3.0 to 4.4 mm/d; FORMAN & BERENBERG, 1978), the amount of time required to establish functioning synapses however remains unclear. Regardless of the species tested, authors give different rates for the functional regeneration of various fibre types, with the nociceptive and autonomic nerves being slightly faster. This is either because small myelinated and unmyelinated fibres regenerate faster than large myelinated ones (NEGREDO et al., 2004), or because large myelinated fibres need more time to restore effective synaptic connections with target cells (NAVARRO et al., 1994). The latter is probably more likely as a recent study has succeeded in showing that in ideal conditions, the growth rates are similar for both motor and sensory fibres (MOLDOVAN et al., 2006).

2.3.4.1 Observation of gait

Gait requires coordinated function involving sensory input, cortical integration and motor response (DELLON & MACKINNON, 1989; WALKER et al., 1994; VAREJÃO et al., 2001b). The ultimate goal of a sciatic nerve regeneration study is a rat that exhibits a regular, symmetrical and fluent step cycle with no dragging of the operated hind limb. The foot should be placed at the right time in a straight line

with the dorsal side facing upwards and should exhibit adequate toe spread (MEEK et al., 2001b).

The most basic test involves filming walking rats from lateral and ventral simultaneously and scoring the animals as “normal” or “abnormal” with regard to different aspects of their walking pattern; these aspects include the toe spread during the stance phase, foot placement, exorotation of the affected foot and the regularity and fluency of walking (MEEK et al., 2001b). The walking pattern can be scored in finer detail using the **modified Basso, Bresnahan and Beattie (BBB) locomotor scale** (BASSO et al., 1995; SCHIAVETO DE SOUZA et al., 2004). In **ankle kinematics** (Fig.7) (YU et al., 2001; VAREJÃO et al., 2003, 2004a,b) rats are filmed from lateral whilst moving at a constant speed. The angles in the ankle during the different parts of the stance phase are measured in stills from videos taken of the rats as they walk (VAREJÃO et al., 2003, 2004b).

A different parameter of gait, the **stance factor**, is determined by calculating the ratio between the amount of floor contact of operated and contralateral hind limbs (WALKER et al., 1994; MEEK et al., 2003, 2004; VAREJÃO et al., 2004a). And a further option for assessing the extent of functional nerve regeneration is provided by evaluating the **ground reaction forces** on an instrumented walking track (HOWARD et al., 2000). Here the peak forces and impulses in the various directions are measured in both hind limbs.

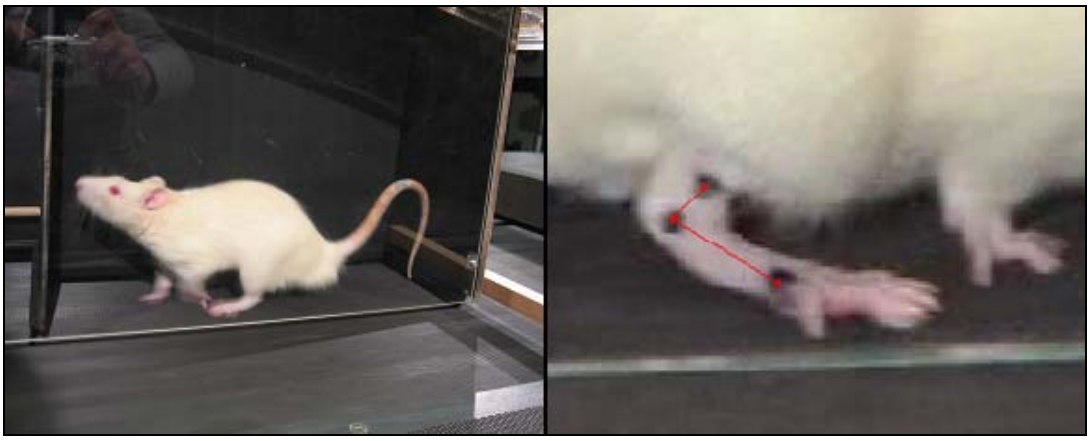

Fig.7 Ankle kinematics: rat walking on a treadmill (left), ankle angle created from a still (right).

2.3.4.2 Ambulatory assessments on beams or parallel bars

In order to gain insight into gross motor function, the time taken for rats to negotiate an elevated flat 8 cm wide “runway” is measured (DING et al., 2001, 2002). More intricate motor function paired with motor learning and hind limb coordination can be investigated by getting the rats to traverse elevated parallel bars (Fig.8). Both the time taken to run along the bars and the number of mistakes made by placing both hind paws on one bar, placing a hind paw beside a bar or swinging under the bars, are recorded and scored (DING et al., 2001, 2002). Similarly, the number of times misplacement of paws occurs can be evaluated by getting the animal to cross a floor consisting of parallel metal rods (MELNICK et al., 2002), a horizontal runway of transverse metal bars (BALLERMANN et al., 2006) or simply a narrow beam (GOLDSTEIN & DAVIS, 1990; KARL et al., 2003).

Fig.8 Ambulatory assessments on parallel bars. Physiological placement of the hind limb (left), unphysiological placement (right).

2.3.4.3 Footprints (motor testing)

Functional evaluation of sciatic nerve regeneration in most research papers features the **Sciatic Function Index** (SFI), a test in which the toe spreads between toes 1 to 5 and toes 2 to 4 and also the print length (tip of the third toe to calcaneus) are measured from footprints of the hind paws of walking rats (Fig.9) (TERZIS & SMITH, 1987; KANAYA et al., 1996; CHAMBERLAIN et al., 2000; VAREJÃO et al., 2001a,b, 2004a,b). These values are then incorporated in a formula to allow comparison of different animals. Although this test, first developed by de Medinaceli and colleagues in 1982 (DE MEDINACELI et al., 1982), is non-invasive, appears to be very objective (VAREJÃO et al., 2004a) and produces reproducible results, especially after self-evaluation by the examiner (BROWN et

al., 1989), it does have its limitations (VAREJÃO et al., 2001b, 2004a), especially when only very little regeneration has taken place (HADLOCK et al., 1999) or the animals develop severe contractures (CHAMBERLAIN et al., 2000) or mutilate their toes in autotomy (HADLOCK et al., 1999). Further points to bear in mind are that print lengths vary with the speed at which the rat walks (Fig.9) (WALKER et al., 1994; VAREJÃO et al., 2001b) and that the contralateral side can serve as a crutch (Fig.9), which means that these footprints are also not physiological (MALUSHTE et al., 2004).

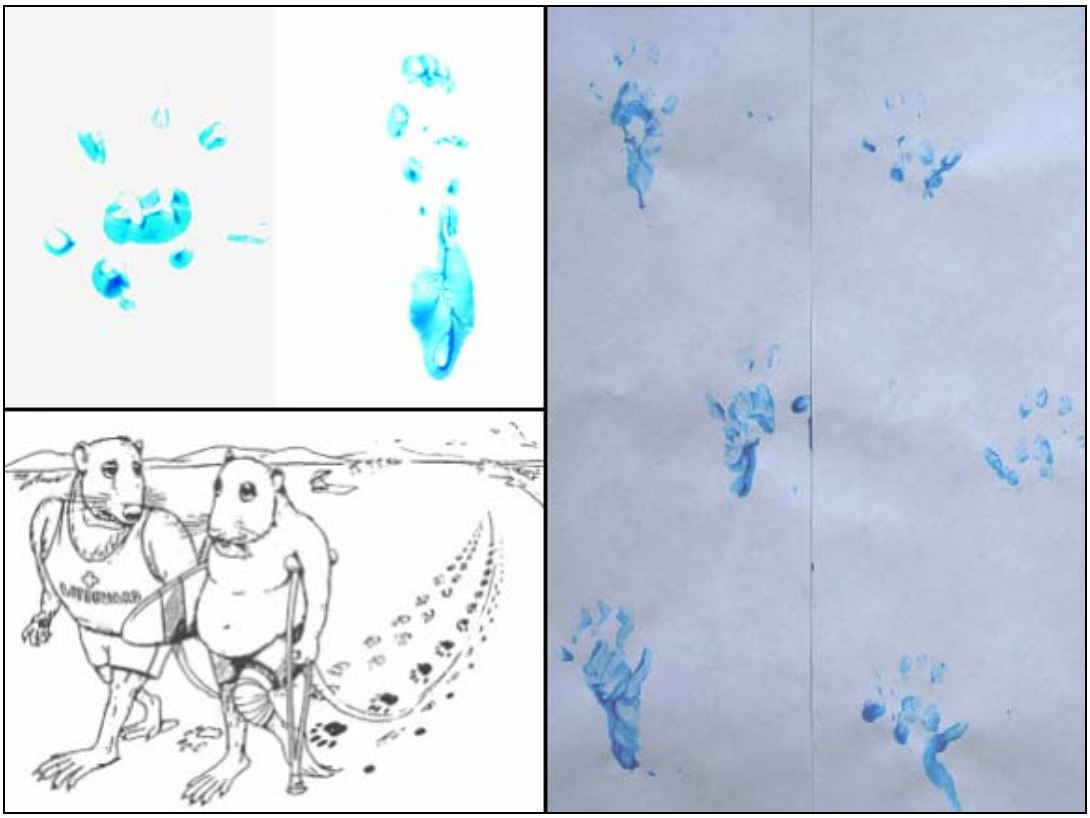

Fig.9 Footprint analysis: left hand side: normal footprint of hind limb (top left), footprint after lesion to the sciatic nerve (top right); rat compensating with the contralateral hind limb after injury to the sciatic nerve (bottom; DELLON & DELLON, 1991).
Right hand side: prints vary with the speed the rat walks: sauntering rat (left), sprinting rat (right).

These drawbacks led to the introduction of the **Static Sciatic Index** (SSI) (BERVAR et al., 2000), which follows the general idea of the SFI. In this case, however, images are obtained by filming the rat at rest from ventral and creating stills of the film. The print length is omitted in the measurements. The **toe-spread factor** described by WALKER et al. in 1994, features a further simplification. Here

measurements are taken only of the distance between toes 1 to 5 on both sides and related to one another. In measurements of the **toe out angle** the degree of exorotation of the foot due to sciatic lesions is determined. Here the angle between the direction in which the rat is progressing and a reference line from the tip of the third toe to the calcaneus is calculated and once again compared to the contralateral side (VAREJÃO et al., 2004a).

Footprint-measuring was and still is considered to be an overall assessment (TERZIS & SMITH, 1987; DELLON et al., 1989; VAREJÃO et al., 2001a,b, 2004b; MARTINS et al., 2006) reflecting the complex combination of sensory input and motor response (KANAYA et al., 1996; BERVAR, 2000; VAREJÃO et al., 2001b; SCHIAVETO DE SOUZA et al., 2004); it has however been demonstrated that of the parameters commonly measured for footprint analysis, only the stride width is dependent on sensory input (WIETHÖLTER et al., 1990). This suggests that footprint analysis primarily measures motor function (WALKER et al., 1994; HADLOCK et al., 1999; YU et al., 2001).

2.3.4.4 Sensory testing

Sensory assessments can basically be divided into exteroceptive and proprioceptive testing.

2.3.4.4 a) Exteroceptive testing

The reaction to a noxious stimulus can take place at two different levels: a subconscious withdrawal reflex or a conscious pain reaction. Both approaches are applied in nociceptive assessments (Fig.10), with the noxious stimuli ranging from pin pricks (NAVARRO et al., 1994; RODRÍGUEZ et al., 2000; NEGREDO et al., 2004), mosquito haemostats (DEVOR et al., 1979), small forceps (KINGERY & VALLIN, 1989; HU et al., 1997; CHAMBERLAIN et al., 2000; PELLED et al., 2006), hotplates (MASTERS et al., 1993; HU et al., 1997; HADLOCK et al., 1999; VAREJÃO et al., 2004b; LIU et al., 2005), electric currents (DE KONING et al., 1986; DEN DUNNEN & MEEK, 2001; MEEK et al., 2003; VOGELAAR et al., 2004), analgesimeters (KINGERY & VALLIN, 1989; KINGERY et al., 1994) and Frey filaments (KINGERY et al., 1994; WILLENBRING et al., 1994; VOGELAAR et al., 2004) to hot and cold baths (ATTAL et al., 1994). The areas recommended for stimulation range from the general area of the sole (MASTERS et al., 1993; ATTAL et al., 1994; HU et al., 1997; HADLOCK et al., 1999; VAREJÃO et al., 2004b), the lateral side of the foot, sometimes including toe 5 (DE KONING et al., 1986;

WILLENBRING et al., 1994; CHAMBERLAIN et al., 2000; DEN DUNNEN & MEEK, 2001; MEEK et al., 2003), to the plantar and sometimes also the dorsal side of the foot including various points on the toes (DEVOR et al. 1979; KINGERY & VALLIN, 1989; KINGERY et al., 1994; NAVARRO et al., 1994; RODRÍGUEZ et al., 2000; NEGREDO et al., 2004).

Note is made of the time to a recognizable withdrawal/pain response or of the existence of any reaction at all.

If the rats are stimulated under a light anaesthetic, muscle contraction potentials in the triceps surae muscle following pinching can be recorded by electromyography. In addition to this, the respiratory and cardiac activities are monitored via an electrode placed in the diaphragm, as both respiratory and heart rate increase during the perception of pain (LIU et al., 2005).

2.3.4.4 b) Proprioceptive testing

These tests derive from examinations for “conscious” proprioception in small animal neurological examinations (DE LAHUNTA, 1977). The afferent aspect of these evaluations requires a functioning branch of the sciatic nerve (or extensive sprouting of the saphenous nerve), whereas efferent pathways can manage without.

The **proprioceptive positioning test** determines the rat´s ability to recognize that it is bearing weight on the dorsal surface of its foot when its paw is ventroflexed by the researcher. The rat should respond to the displacement by prompt repositioning of the foot (HADLOCK et al., 1999). In the **hopping test** the rat is held upright with only the operated limb extended. It is then moved laterally over a flat surface and should respond to this passive movement by active hopping with the hind limb in the same direction (HADLOCK et al., 1999). The same starting position is used with the **placing test** (Fig.10). Here the rat is positioned at the edge of a table or similar surface so that the dorsal surface of the foot makes contact. Physiologically the foot is then flexed and placed onto the table. In the **extensor postural thrust** test (Fig.10) the rat is held in an upright position with only the leg that is to be investigated dangling. It is then lowered onto digital scales and the force applied by the extended limb is read off in grammes and compared with the contralateral side (HADLOCK et al., 1999; VAREJÃO et al., 2004a,b).

A similar, very rodent-specific test, involving motor function dependent on the sciatic/tibial nerve, is the **grasping test**. Here rats are rated in their ability to grasp a grid with both hind limbs (CHUNASUWANKUL et al., 2002).

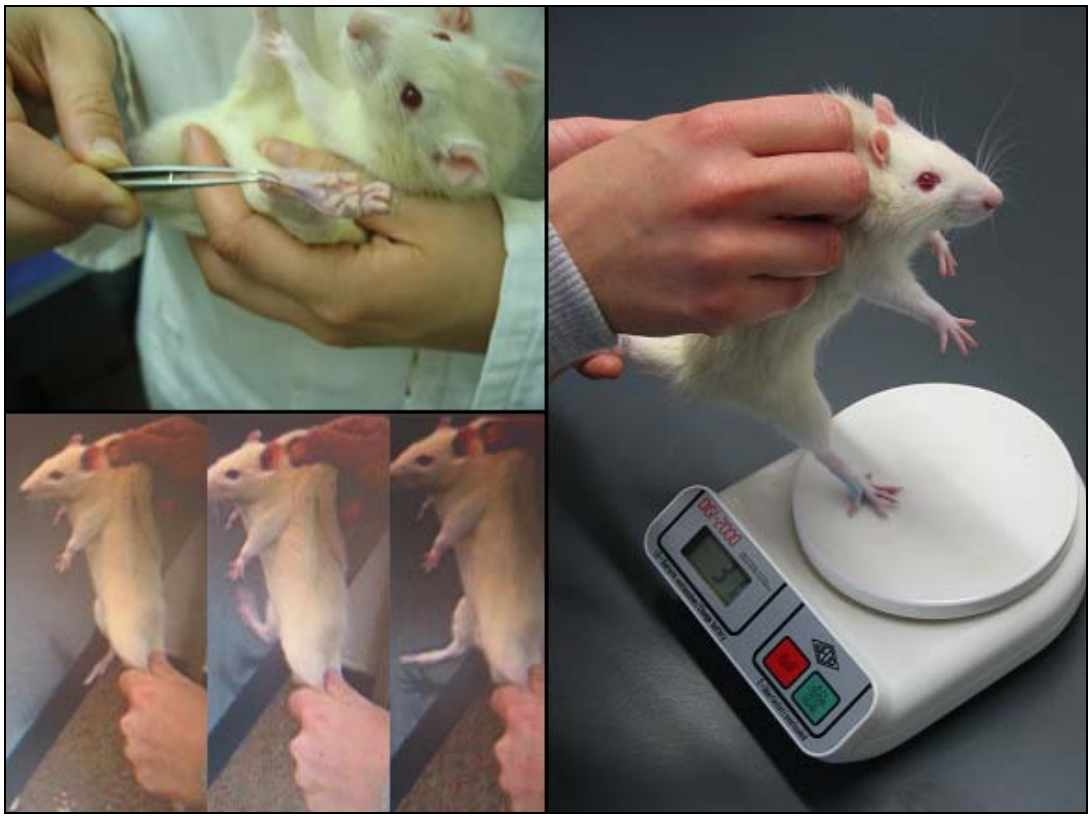

Fig.10 Sensory testing (clockwise from top left): nociceptive testing, extensor postural thrust, placing test.

2.3.4.5 Evaluation of autonomic fibres

In the sudomotor response test, sweating is stimulated by the injection of pilocarpine nitrate. 10 minutes later silicone material is applied to the plantar surface of the rat´s hind feet. As the material hardens it retains impressions made by droplets of sweat emerging from sweat glands; the number of these impressions on the injured side is determined and compared to the contralateral side (RODRÍGUEZ et al., 2000).

2.3.4.6 Electromyography

Electrodes connected to one another and also to an amplifier are implanted into the midbelly regions of the gastrocnemius and cranial tibial muscles and into the lower back region (GRAMSBERGEN et al., 2000; MEEK et al., 2003). Subsequently, the activity pattern of the two antagonistic muscles is explored while the rats are allowed to walk freely. During the reinnervation process both muscles are often continuously active without clear patterns due to inaccurate reinnervation,

sprouting and interrupted proprioceptive feedback, so that coactivation during the stance phase is possible (GRAMSBERGEN et al., 2000; MEEK et al., 2001b; MEEK et al., 2003).

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Strain Differences in the Branching of the Sciatic Nerve in Rats

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Figures: 3

Tables: 5

Summary

The sciatic nerve in the rat is the site most often used for peripheral nerve regeneration studies. The length of sciatic nerve available for research, however, depends on the point at which the sciatic nerve divides into the peroneal and tibial nerves. In the present study, the hind limbs of 150 male adult rats of five different strains (Sprague Dawley, Fischer 344, Wistar Han, Lewis and Nude) were analysed with regard to femur length, the point at which the sciatic nerve divides into the tibial and peroneal nerves, and where these are surrounded by the same epineurium, and the point at which they are encased in individual epineurial sheaths.

The results indicate that the lengths of available sciatic nerve are fairly constant in all strains of rats. In absolute terms, they amount to about one third of the length of the femur for stretches of undivided sciatic nerve, and up to nearly half of the femur length for stretches where the tibial and peroneal nerves are already present, but are still enclosed by the same epineurium. In 61.7% of the hind limbs examined in Fischer rats, however, no sciatic nerve could be seen as such, only in the form of its successors surrounded by separate epineuria. This makes it highly advisable not to use male adult Fischer rats in peripheral nerve regeneration studies with the sciatic nerve as the point of focus.

Keywords: sciatic nerve, division, peripheral nerve regeneration, Fischer rat

Introduction

In peripheral nerve regeneration studies the sciatic nerve in the rat is the most commonly used of all available sites. One of the reasons for this is that neural regeneration is generally good in rats and this, combined with the relatively short distance to target organs compared with other animals, makes it possible to demonstrate regenerative success in a reasonable time frame. The sciatic nerve as such is the preferred model for studies on regeneration, as it is easily accessible and represents a mixed nerve, so that motor, sensory or autonomic aspects can be studied.

Good regeneration (depending on the method of evaluation) over short gaps of up to 10 mm has been demonstrated in a variety of studies (Archibald et al., 1991; Hadlock et al., 2000; Karacaoğlu et al., 2001; Fine et al., 2002; Lee et al., 2003). Recent research therefore aims to cover larger defects (Meek et al., 1999; den Dunnen and Meek, 2001; Castañeda and Kinne, 2002; Meek et al., 2003; Keilhoff et al., 2005), often by incorporating different bridging materials and techniques.

The purpose of the following study was to determine what lengths of sciatic nerve suitable for regeneration studies were available, suitable being defined as the stretch of sciatic nerve where no splitting into tibial and peroneal branches has yet taken place. Five commonly used rat strains (Sprague Dawley, Fischer 344, Wistar Han, Lewis and Nude rats) were investigated, and it was found that apart from the Fischer rats the length of suitable sciatic nerve available was almost identical in the different strains.

Material and Methods

In this study 30 adult males each of Sprague Dawley (SD), Fischer 344 (F), Wistar Han (WH), Lewis (L) and Nude (immunodeficient) (N) rat strains (all Charles River, Germany) were examined. The examinations were conducted post mortem as the rats were donated as carcasses from other studies.

As a first step body weight and foot length (from the heel to the tip of toe 3) of every rat were recorded as basic data. Subsequently the skin of the lateral thigh was incised parallel to the femur and along the lateral side of the tibia in both hind limbs. The sciatic nerve was exposed by separating the femoral biceps muscle from the lateral vastus of the quadriceps muscle in its aponeurosis and from its lateral insertion at the tibial crest, making it possible to fold the femoral biceps

muscle towards caudal. An incision between the medial and lateral heads of the gastrocnemius muscle exposed the course of the tibial nerve towards the foot.
The distance from the middle of the trochanter major of the femur to the patella was noted as another basic value (approximate femur length D0) and then the four following salient points of the sciatic nerve and its branches were determined (Fig.1):

- The point at which the sciatic nerve splits into the tibial and peroneal nerves, but both are still enclosed same epineurial sheath (P1).
- The point from which the tibial and peroneal nerves exhibit separate epineuria (P2).
- The point at which the ramus cutaneous surae caudalis lateralis branches from the tibial nerve (P3).
- The point at which the tibial nerve splits into its two plantar branches (P4).

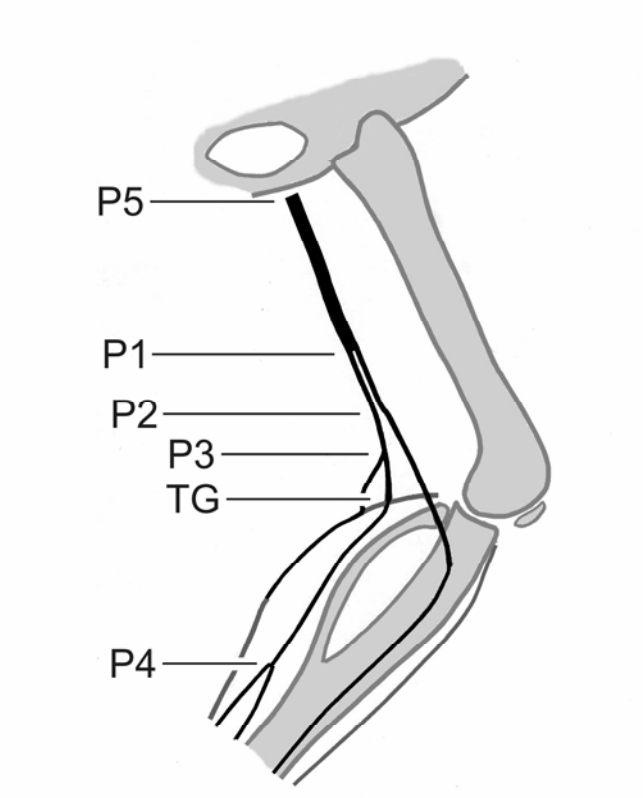

Fig.1: Measuring points
Point at which the sciatic nerve splits into the tibial and peroneal nerves, but both are still surrounded by the same epineurium (P1), point from which the tibial and peroneal nerves have separate epirineuria (P2), point at which the ramus cutaneous surae caudalis lateralis branches from the tibial nerve (P3), point at which the tibial nerve divides into its two plantar branches (P4), most proximal visible point of the sciatic nerve (P5), point at which the tibial nerve enters the gastrocnemius muscle (TG).

The following distances were then measured between these points and the point at which the tibial nerve enters the gastrocnemius muscle (TG):
P1 to TG (=D1), P2 to TG (=D2), P3 to TG (=D3) and P4 to TG (=D4).
Furthermore, the most proximal visible point of the sciatic nerve or its two successors (P5), where it/they emerge from between the quadratus femoris and

quadriceps femoris muscles, was determined in all animals where the sciatic nerve had already split into the tibial and peroneal portions at the most proximal point they could be seen, or in at least three hind limbs per strain. On the basis of this the distance P5 to TG (=D5) was then established.

To ensure comparability between rats of different sizes, the values for D1, D2, D3, D4 and D5 were divided by the corresponding femur length (D0) in each animal.

Further calculations were made to ascertain the length of sciatic nerve which can be used for peripheral nerve regeneration studies. Suitable sciatic nerve can fall into two categories: either the sciatic nerve has to be available in one single fascicle (1), or the sciatic nerve can already have split into the tibial and peroneal portions, but these are still enclosed by the same epineurium (2). In order to determine values for the first option the D1/D0 quotient was subtracted from normalised values of D5 (=D5/D0). For the second option the D2/D0 quotient was subtracted from the D5/D0 quotient. As in both cases values normalised by the femur length are deducted from one another, a percentage of femur length is the result. Absolute values (in millimetres) can then be established by multiplying the intermediate values by the average femur length.

An additional 60 female adult rats (15 of these F, the rest L and WH) were examined as regards weight, femur length, D3, and the occurrence of P2 lying proximal to P5.

Statistics

Strains were compared after establishing average values and standard deviations for ascertained weight and distances by Oneway descriptive statistics using the Sigma Stat Software (SPSS). The single standard deviation included 68% of hind limbs evaluated, double standard deviation 95%. Oneway ANOVA was then applied to determine whether differences between the strains examined were statistically significant ($p<0.05$).

In cases where P1 or P2 lay proximal to P5, Chi-Squared-Tests were used to ascertain whether statistically significant differences existed between the strains examined and/or between the right and left hind limbs. Results were then further examined with Fisher`s exact test.

Finally, the t-Test was applied in order to permit comparison of weight, D0, D0/weight and D3/D0 between male and female rats.

Results

Basic data

The average weight of the rat strains differed as follows: F (228.50 g) and N (254.57 g) rats were the lightest, with L (352.73 g) and SD (359.33 g) strains following, WH (410.57 g) rats being the heaviest (Table 1). The two lightest strains (F and N) exhibited relatively long femurs and feet. This became evident when comparing the quotients of femur or foot length and weight (quotient femur length to weight: 14.80% (F), 13.49% (N), 11.24% (L), 10.74% (SD), 9.32% (WH)). The relation between femur and foot length was reasonably constant in all animals, with the F rats (84.53%) having the shortest femurs and L rats (94.33%) the longest femurs in relation to the length of their feet.

Table 1: Basic Data

	WH	SD	L	N	F	TOTAL	p
weight [g]	410.57 ±53.2	359.33 ±52.04	352.73 ±103.37	254.57 ±42.97	228.50 ±59.605	321.14 ±94.75	0.000
D0 [mm]	37.60 ±1.98	37.80 ±2.36	37.17 ±3.56	33.73 ±2.25	32.20 ±2.83	35.70 ±3.50	0.000
foot length [mm]	42.23 ±1.66	41.50 ±1.19	39.40 ±1.39	38.17 ±1.48	38.10 ±1.20	39.88 ±2.20	0.000
D0/weight [%]	9.32 ±1.53	10.74 ±1.76	11.24 ±2.53	13.49 ±1.60	14.80 ±3.07	11.92 ±2.93	0.000
foot length/weight [%]	10.49 ±1.67	11.82 ±1.96	12.19 ±3.66	15.33 ±2.17	17.80 ±4.66	13.52 ±4.03	0.000
D0/foot length [%]	89.15 ±5.87	91.13 ±5.82	94.33 ±8.47	88.41 ±5.26	84.53 ±7.20	89.51 ±7.33	0.000

Approximate femur length (D0); animal groups: Wistar Han (WH), Sprague Dawley (SD), Lewis (L), Nude (N), Fischer 344 (F).
Values are given as mean ± standard deviations. Single deviation depicts the range that includes 68% of examined hind limbs, double deviation includes 95% of examined hind limbs. Values of p smaller than 5% describe significant differences between strains (One-way Anova).

Division of the sciatic nerve into the tibial and peroneal branches (D1, D2)

The distance from the submersion of the tibial nerve into the gastrocnemius muscle to the point where the sciatic nerve splits into its tibial and peroneal branches, but where both still lie within the same epineurial sheath (D1), amounted to close to

one third of the approximate femur length in all strains (31.04% (L), 31.70% (F), 32.56% (WH), 35.11% (SD)) except the N rats (24.04%) (Table 2).

Table 2: Division of the sciatic nerve into tibial and peroneal branches

	WH	SD	L	N	F	TOTAL	p
D1/D0 [%]	32.56 ±10.21	35.11 ±7.81	31.04 ±9.16	24.04 ±11.05	31.70 ±13.15	30.92 ±10.55	0.000
D2/D0 [%]	17.36 ±6.65	21.35 ±5.83	19.11 ±5.43	14.21 ±9.67	26.68 ±14.97	18.90 ±8.72	0.000
D5/D0 [%]	65.44 ±4.79	66.44 ±7.89	67.65 ±2.08	59.21 ±3.20	65.12 ±6.36	64.72 ±5.99	0.102
P1 not visible [% of hind limbs]	8.33	5.00	3.33	15.00	68.33	20.00	0.000
P1 not visible: right hind limbs	5.00	3.33	3.33	8.33	36.67	11.33	
P1 not visible: left hind limbs	3.33	1.67	0.00	6.67	31.67	8.67	
P2 not visible [% of hind limbs]	6.67	0.00	1.67	10.00	61.67	16.00	0.000
P2 not visible: right hind limbs	5.00	0.00	1.67	6.67	31.67	9.00	
P2 not visible: left hind limbs	1.67	0.00	0.00	3.33	30.00	7.00	

Approximate femur length (D0); distance from the point at which the sciatic nerve splits into the tibial and peroneal nerves, but where both still lie within the same sheath (P1) to submersion of the tibial nerve in the gastrocnemius muscle (D1); distance from definitive separation of the tibial and peroneal nerves (P2) to submersion of the tibial nerve in the gastrocnemius muscle (D2); distance from proximal emergence of the sciatic nerve to submersion of the tibial nerve in the gastrocnemius muscle (D5); animal groups Wistar Han (WH), Sprague Dawley (SD), Lewis (L), Nude (N), Fischer 344 (F).
Values are given as mean ± standard deviations. Single deviation depicts the range that includes 68% of examined hind limbs, double deviation includes 95% of examined hind limbs. Values of p smaller than 5% describe significant differences between strains (One-way Anova).

This strain (N) also exhibited a lower value for D2 (distance from TG to where the tibial and peroneal nerves are each surrounded by their own epineuria) than the other strains. Values established for D2 generally revealed a much greater variability than those established for D1, with quotients ranging from 14.2% (N) to 26.7% (F) of femur lengths. Naturally, D1 and D2 could only be ascertained in animals where P1 or P2 could be visualised. In a number of animals P1 and P2 lay proximal to P5 (Figs. 2, 3).

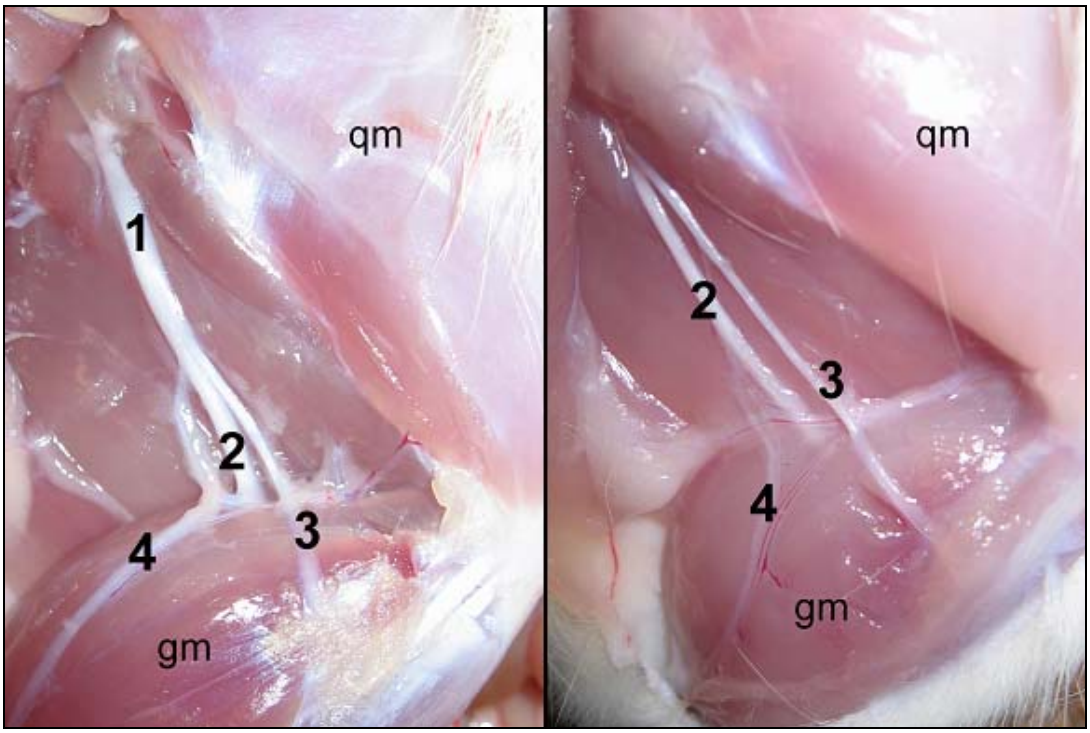

Fig. 2: Lewis Rat. **Fig.3: Fischer 344 Rat.**
Sciatic nerve (1), tibial nerve (2), peroneal nerve (3), ramus cutaneuous surae caudalis lateralis (4), gastrocnemius muscle (gm), quadriceps muscle (qm).

The F strain had the highest percentage of rats in which P1 and P2 were not visible. In these rats this was the case for P2 in more than half the hind limbs examined (61.67%), for P1 in as many as 68.3%! In the other rat strains the number of hind limbs where P1 could only be surmised was at the most 15% of hind limbs examined (N). For P2 this figure lay between 0% (SD) and 10% (N). A significant difference between strains could be proven for both the situations where either P1 ($p=0.000$) or P2 ($p=0.000$) could not be visualised. However, when examining this unusual feature with the F rats left out of account, no significant difference could be seen between the rest of the strains for a very proximal primary splitting of the sciatic nerve ($p=0.09$). In rats where both the tibial and peroneal nerves were each surrounded by their own epineurium, a significant difference ($p=0.038$) could still be noted between the SD, N, L and WH strains, even after excluding the F rats. This result is most probably due to the fact that there were only very few rats whose hind limbs displayed this particular anatomical trait.

The observation that very proximal division of the sciatic nerve and also ultimate separation of the tibial and peroneal nerves seemed to occur slightly more in hind

limbs on the right hand side had no statistical significance (p=0.312 (P1 not visible), p=0.431 (P2 not visible)).

"Usable" parts of the sciatic nerve

Calculations revealed that if the sciatic nerve used for research has to be encased in one single fascicle, all rat strains contained strips of usable sciatic nerve measuring about one third of the femur (31.33% (SD), 32.89% (WH), 33.42% (F), 35.17% (N), 36.61% (L); in absolute terms this amounted to between 10.8 mm (F) and 13.6 mm (L) in the present study (Table 3). If it is sufficient for research purposes that the sciatic nerve has already split into the tibial and peroneal nerves, though with both still surrounded by the same epineurial sheath, it is possible to obtain larger strips between 12.4 mm (F) and 18.1 mm (WH) in length.

Table 3: Usable parts of the sciatic nerve

	WH	SD	L	N	F	TOTAL
sciatic nerve undivided [% of D0]	32.89	31.33	36.61	35.17	33.42	33.81
sciatic nerve undivided [mm]	12.37	11.84	13.61	11.86	10.76	12.07
tibial and peroneal nerves in same epineurium [% of D0]	48.08	45.09	48.54	44.99	38.44	45.83
tibial and peroneal nerves in same epineurium [mm]	18.08	17.04	18.04	15.18	12.38	13.14
tibial and peroneal nerves in same epineurium (including rats with proximal division) [% of D0]	44.76	45.09	47.68	40.20	15.88	38.67
tibial and peroneal nerves in same epineurium (including rats with proximal division) [mm]	16.83	17.04	17.72	13.56	5.11	13.81

Approximate femur length (D0); animal groups Wistar Han (WH), Sprague Dawley (SD), Lewis (L), Nude (N), Fischer 344 (F).

Averages for individual strains differ greatly from the above figures when substituting D5 for D2 in animals where P2 lay beyond P5. In this case the quantity of usable sciatic nerve, where the tibial and peroneal nerves are separate but still

surrounded by a shared epineurial sheath, amounted to only 15.9% of the femur length in Fischer rats as opposed to between 40.2% (N) and 47.7% (L) of the femur lengths in the other strains.

Further branching and division of the tibial nerve (D3, D4)

The branching of the ramus cutaneous surea caudalis lateralis with values of between 18.11% (N) and 20.64% (SD) of femur lengths was very constant in all strains (p=0.132) (Table 4). No homogeneity could be ascertained as regards the splitting of the tibial nerve into its two plantar parts in the calf.

Table 4: Further branching and division of the tibial nerve

	WH	SD	L	N	F	TOTAL	p
D3/D0 [%]	19.79 ±5.40	20.64 ±5.84	19.12 ±5.35	18.11 ±5.28	19.68 ±5.18	19.47 ±5.44	0.132
D4/D0 [%]	62.81 ±7.56	48.32 ±13.56	58.50 ±11.98	35.32 ±13.82	52.92 ±7.75	51.57 ±14.69	0.000

Distance from branching of the ramus cutaneous surae caudalis lateral from the tibial nerve to submersion of the tibial nerve in the gastrocnemius muscle (D3); distance from the division of the tibial nerve into its distal successors from the submersion of the tibial nerve in the gastrocnemius muscle (D4); approximate femur length (D0); animal groups Wistar Han (WH), Sprague Dawley (SD), Lewis (L), Nude (N), Fischer 344 (F).
Standard deviations for every value are given in smaller print beneath the calculated average. Single deviation depicts the range that includes 68% of examined hind limbs, double deviation includes 95% of examined hind limbs. Values of p smaller than 5% describe significant differences between strains (One-way Anova)

Data for the female rats

The 60 females examined exhibited relatively uniform weights with WH rats being the lightest (233.86 g), closely followed by F rats (237.87 g). Rats of the L strain were the heaviest (254.38 g) (Table 5). As no great variation in femur lengths (D0) could be seen, the D0/weight ratio was consequently similar in all strains, amounting to between 12.87% (L) and 14.34% (F).

In 50% of the hind limbs examined in female F rats P2 lay beyond P5, which means that in these hind limbs no sciatic nerve could be seen per se, but only in the form of its distal successors, the tibial and peroneal nerves, which were both already surrounded by their own epineuria. This anatomical trait was not detectable in any of the female L rats examined and in only just over 4% of the hind limbs of

WH rats. Statistical evaluations of this special anatomical trait revealed significant differences in the number of affected hind limbs for the different strains (p=0.000). When F rats were excluded from the calculations, however, no significant difference could be noted between the two remaining strains WH and L (p=1.000). There were also no obvious side preferences for this very proximal splitting of the sciatic nerve.

The branching of the ramus cutaneous surae caudalis lateralis amounted to between 19.76% (WH) and 24.56% (F) of the femur lengths, with an average of 21.04%.

Table 5: Data for female rats

	F	WH	L	TOTAL
N	15	37	8	60
weight [g]	237.87	233.86	254.38	237.60 ±29.88
D0 [mm]	34.10	32.05	32.75	32.66 ±1.85
D0/weight [%]	14.34	13.71	12.87	13.90 ±1.41
P2 not visible [% of hind limbs]	50.00	4.05	0.00	15.00
P2 not visible: right hind limbs	23.33	2.70	0.00	7.5
P2 not visible: left hind limbs	26.67	1.35	0.00	7.5
D3/D0 [%]	24.56	19.76	20.37	21.04 ±6.07

Approximate femur length (D0); distance from branching of the ramus cutaneous surae caudalis lateral from the tibial nerve (P3) to submersion of the tibial nerve into the gastrocnemius muscle (D3); point of definite separation of the tibial and peroneal nerves from where both are surrounded by their own epineurium (P2); animal groups: Fischer 344 (F), Wistar Han (WH), Lewis (L).
Values are given as mean ± standard deviations. Single deviation depicts the range that includes 68% of examined hind limbs, double deviation includes 95% of examined hind limbs.

Discussion

Comparison of data for male and female rats

Examination of 60 relevant papers on peripheral nerve regeneration listed in PubMed, revealed that 47 of these (78.3%) chose male adult rats for their investigations. The reason for this large imbalance presumably lies in the fact that male rats are cheaper to acquire than female ones. Nevertheless an additional 60 adult females were examined in this study regarding the basic data and obvious traits. As mentioned before, a quarter of these animals were Fischer rats, the rest Wistar and Lewis rats. The females exhibited a slightly higher femur length/weight quotient (13.90%) than their male counterparts (11.92%), but had lower absolute values for femur lengths (32.66 mm; males: 35.70 mm) and body weights (237.60 g; males: 321.14 g). Interestingly, the very proximal division of the sciatic nerve also featured in a high percentage of hind limbs in the female Fischer rats (50.0%; males 61.7%), this value also correlating pretty closely in the Wistar Han (females: 4.05%; males: 6.7%) and Lewis strains (females: 0.0%; males: 1.7%). The single really constant trait in the males, the average figure for the branching of the ramus cutaneous surae caudalis lateralis from the tibial nerve, lay with the females within the same range as with the males (females: 21.0% of femur length; males: 19.5%). Statistical evaluation of these values, however, revealed a significant difference for this anatomical trait between male and female rats ($p=0.01$).

Choice of rat strain

As regards the choice of strain, Wistar rats were the subjects of choice in almost half (29 out of 60) of the nerve regeneration studies investigated. The runners-up were the Sprague Dawley rats (31.7%), with Lewis (10.0%), Fischer (6.7%) and Holtzmann (3.3%) strains tailing behind. Average weights of the Wistar rats ranged from 215 to 260 g, which means that usable strips of the sciatic nerve of approximately 6.5 to 7.9 mm (not split) and 9.5 to 11.4 mm (split, but with the tibial and peroneal nerves still surrounded by the same epineurium) should be available. Four out of five of the rat strains subjected to anatomical investigation in the present study were chosen because these were the strains most commonly used in regeneration studies. A further factor influencing the choice of strain was the aim to examine both outbred (SD, WH, N) and inbred (F, L) strains in order to determine via standard deviations whether certain anatomical traits are “strain

specific" or "rat specific". Disappointingly, a comparison of standard deviations with regard to the splitting of the sciatic nerve and the branching of the tibial nerve, did not permit any assumptions in this respect. Examination of the points at which the sciatic nerve divides into its two successors and at which the tibial and peroneal nerves ultimately separate showed Fischer 344 rats (inbred) to exhibit the highest standard deviations for their range of values (31.70% ± 13.51 (D1/D0), 26.68% ± 14.97 (D2/D0)). Nude (outbred) and Wistar Han (outbred) rats (D1/D0: 24.04% ± 11.05 (N), 32.56% ± 10.21 (WH); D2/D0: 14.21% ± 9.67 (N), 17.36% ± 6.65 (WH)) both had lower standard deviations for these traits, but still exhibited more variations than Sprague Dawley and Lewis rats. A comparison of measurements for the branching of the tibial nerve also did not give any indication that anatomical traits were less varied in inbred strains.

Nude T-cell deficient rats were added to the strains examined, as these rats are frequently used in brain research and might provide an alternative option to immunosuppression in future peripheral nerve regeneration studies working with allografts or allogenic Schwann cells.

Anatomical variations concerning the sciatic nerve in different species

The very proximal division of the sciatic nerve, with the tibial and peroneal nerves emerging from beneath the medial gluteal muscle, - sometimes still surrounded by the same epineurium, but often already ensheathed by separate epineuria - is not a special anatomical feature solely restricted to the Fischer rat. In horses, for example, the physiological point of division of the sciatic nerve into its distal successors is near the hip joint (Nickel et al., 1984). The tibial and peroneal nerves are still connected, however, until they reach their final separation in the popliteal fossa. In other domestic animals the final point of separation for the tibial and peroneal nerves also lies in the popliteal fossa, the primary separation point, however, being at about mid-thigh level (Nickel et al., 1984). Other researchers have also confirmed the distal part of the thigh as the point of final divergence for the tibial and peroneal nerves (Goshal, 1972; Bennett, 1976). Bennett, however, describes in his study that the tibial and peroneal components of the sciatic nerve could be separated almost to the level of the L6 and L7 anastomosis in the cat, and to the level immediately below the S1 anastomosis with the L6/L7 bundle in the dog. In rats the physiological point for the division of the sciatic nerve is stated to be in the proximal third of the thigh (Hebel and Stromberg, 1976). It is not

explained, however, whether the tibial and peroneal nerves still lie within the same epineurium at this point or whether they are enclosed in separate sheaths. Greene's "Anatomy of the Rat" (1955) gives a more detailed and rather different description: the tibial and peroneal nerves are separable up to their origin in the plexus, however stay contained in the same sheath until they separate in the popliteal fossa. In humans, the physiological division of the sciatic nerve lies before the nerve emerges from the sacrosciatic foramen. The tibial and peroneal nerves stay connected (physically, but not functionally) by multiple layers of connective tissue until they reach the popliteal fossa. Vlodka et al. (2001) undertook measurements of the sciatic nerve similar to those in the present study. They dissected the lower extremities of 15 adult human cadavers to try and determine the point at which the tibial and peroneal nerves had disconnected and were each ensheathed by their own epineurium. In line with the findings of the present study they measured a wide variety of distances from the popliteal fossa to the separation point, making it difficult to find a uniform and acceptable average value for all specimens. These distances, however, did not differ between the left and right sides in humans, where both legs could be measured. This finding lies in contrast to the present study (data not shown), where most rats exhibited different values for their right and left hind limbs. Babinski et al. (2003) describe a case of a human being where the sciatic nerve is completely divided into its individual successors as high up as the pelvis. Investigation of the course towards distal revealed that the two nerves followed two different paths, one passing superior to, the other inferior to the superior gemellus muscle. Only recently (2005), Ugrenovic and coworkers showed that variations in sciatic anatomy are not particularly unusual in human beings. In their study 100 human foetuses were examined. In 27.5% of the legs scrutinised the tibial and peroneal nerves could be seen to lie in separate sheaths as proximal as the posterior femoral or the gluteal region, as opposed to the upper angle of the popliteal fossa. This condition, and also the aforementioned variations in the ultimate separation of the sciatic successors, can cause incomplete blocks of the sciatic nerve during popliteal block anaesthesia, a problem which is described occasionally in literature (Vlodka et al., 2001).

Use of the tibial nerve in research

One of the supplementary results of this study might prove to be useful information for scientists focusing on the tibial nerve as the site for investigations on peripheral

nerve regeneration (Strasberg et al., 1999; Odaka et al., 2003; Malushte et al., 2004). As the point at which the ramus cutaneous surae caudalis lateralis branches from the tibial nerve is the most constant trait in all rats, it is easy to determine the length of tibial nerve available for research. Fischer rats would obviously be ideal in this respect for the reasons discussed at length above.

Limitations

One possible drawback to the present study is that all the rats examined stemmed from the same laboratory. As Fischer 344 rats are a particularly inbred strain, further investigations of this strain bred in other laboratories might reveal whether the very proximal splitting of the sciatic nerve in this strain is a speciality of this lab or of Fischer 344 rats per se. The other limitation of the present study is that D5 was not determined in every animal, which means that calculations of available sciatic nerve were made with average values for every strain. However, it is important to note that a statistical comparison of the quotient of D5 and D0 revealed no significant difference between groups ($p=0.102$), and that the standard deviations only produced values of between 3.07% (L) and 11.87% (SD) of the quotients D5/D0.

Conclusion

This study clearly indicates that certain anatomical traits of the thigh, the foot and the sciatic nerve and its extensions are relatively constant in male adult rats of different strains. Femur length and foot length can roughly be estimated by weighing the animal and multiplying its weight by 9 to 15%, depending on which strain this animal belongs to. More importantly, however, scientists can also approximately determine the length of sciatic nerve available for sciatic nerve regeneration studies. Both the quantity of sciatic nerve undivided into tibial and peroneal branches and the quantity of sciatic nerve already split, but with the branches still enclosed by the same epineurium, can roughly be calculated. The former reaches values of about one third of the femur length, the latter up to nearly half the femur length. This information could usefully complement other factors such as susceptibility to autotomy, price and available molecular probes, which

need to be taken into account when choosing the rat to be used for peripheral nerve regeneration studies.

The use of Fischer rats, especially small male specimens, is not advisable for peripheral nerve regeneration studies using the sciatic nerve as the point of focus. The reason for this is that in many of these animals the sciatic nerve cannot be visualised as such, but has unilaterally or even bilaterally already split into its tibial and peroneal successors before emerging from the hip musculature. This unusual trait does not seem to feature in critical numbers in other strains.

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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

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Abstract

Sensory testing, by providing stimuli for nociceptors of the foot, is a popular method of evaluating sensory regeneration after damage to the sciatic nerve in the rat.

In the following study 20 rats were submitted to double transection of the sciatic nerve. The subsequent 14 mm gap was repaired through guidance interponation. In order to evaluate nerve regeneration, sensory testing was performed additionally to other methods, which included motor testing, morphometry and electronmicroscopic assessments of nerves.

Somatosensory testing revealed that all animals exhibited next to the same amount of sensory reinnervation on their foot regardless of their experimental group. In motor tests, however, two out of the three experimental groups did not improve at all. These groups also failed to show neural regrowth in morphometric and electron microscopic assessments of the associated nerve. Retrograde tracing was able to prove the saphenous nerve as an alternative source of sensory reinnervation in animals with failed sciatic regeneration.

This means that results of sensory testing in the rat should be treated with caution, taking into account the areas tested and the likelihood that in these areas saphenous sprouting could have taken place. Furthermore, it is strongly advised that somatosensory testing should be conducted only on toe five.

Key words: Sciatic nerve regeneration, saphenous sprouting, pain response, withdrawal reflex, rat

Introduction

In sciatic nerve regeneration studies the extent of reinnervation is evaluated by both motor and sensory testing. Generally, sensory assessments can be divided into proprioceptive and exteroceptive testing. To achieve a reliable reaction in the latter, nociceptors have to be stimulated; these are receptors which have a particularly high threshold (Serpell 2005) and which only react to noxes at a level potentially destructive to tissue. The subsequent reaction can take place at two different levels: a subconscious withdrawal reflex or a conscious pain reaction.

The withdrawal reflex is a true reflex which involves the central nervous system on the spinal level. The animal responds to the stimulation of nociceptors simply by withdrawing its leg. For a conscious reaction to pain, however, afferent signals have to reach the thalamus via spinothalamic tracts and the medial lemniscus, and are further projected to the somesthetic cortex via the internal capsule. Conscious pain perception occurs both on the thalamic and on the cortical level, and causes the animal to react with an accurate pain response such as licking its foot or vocalising its discomfort in addition to withdrawing its foot from the noxious stimulus (Navarro et al. 1994).

Given that, after experimental nerve trauma, the afferents have been re-established, the question that now arises is whether a rat with residual sciatic dysfunction is still capable of performing a recognisable withdrawal of the foot. This could be a problem if the effector muscles needed for this action are still denervated or incompletely or inappropriately reinnervated. Additionally, the common technique of wrapping the rat in a towel for immobilisation and restriction of vision during evaluation (Masters et al. 1993, Hu et al. 1997, Varejão et al. 2004a) might not only hinder the rat from performing a withdrawal reflex, but might also make it more difficult for the examiner to recognise weak responses.

In addition to this aspect, there appears to be little consensus regarding the method adopted to evoke a pain response or withdrawal reflex in the rat. In turn, a number of different noxes have been applied, to date, in different ways and on different areas of the foot (Vogelaar et al. 2004, Nichols et al. 2005). The only generally accepted rule is that the medial aspect of the foot should be avoided, as the saphenous nerve caters for this region (de Lahunta 1977, Devor et al. 1979, de Koning et al. 1986, Varejão et al. 2004b).

Various descriptions of extensive saphenous sprouting following permanent sciatic denervation can be found in literature (Devor et al. 1979, Markus et al. 1984,

Kingery and Vallin 1989). This annexation of what was originally sciatic territory on the foot has been described as occurring as early as within the first four days after acute sciatic transection (Devor et al. 1979). Neglecting these observations, recommendations regarding the area to be stimulated for assessment of sciatic function still range from the general area of the sole (Masters et al. 1993, Attal et al. 1994, Hu et al. 1997, Hadlock et al. 1999, Varejão et al. 2004a), the lateral side of the foot, sometimes including toe five (de Koning et al. 1986, Chamberlain et al. 2000, den Dunnen and Meek 2001, Meek et al. 2003), or the plantar and sometimes also the dorsal side of the foot including the toes in various places (Devor et al. 1979, Navarro et al. 1994, Rodríguez et al. 2000, Negredo et al. 2004).

The aim of the present study was to evaluate sciatic nerve regeneration in three different surgical groups after double transection and repair of the subsequent 14 mm gap by guidance interponation. Recently developed collagen type IV tubes, both empty and filled with denaturated autologous muscle tissue, were to be assessed with regard to their ability to sustain neural regeneration across a relatively large gap in a peripheral nerve and benchmarked against the gold standard for the bridging of larger gaps, the autologous nerve graft (Bellamkonda 2006, Chalfoun et al. 2006, Keune et al. 2006). The collagen tubes were filled with denaturated muscle as this modification had proved to be successful in promoting neural regeneration in the past (den Dunnen and Meek 2001, Meek et al. 2001).

Multiple test methods covering every aspect of regeneration were applied, ranging from assessments concerned with gait (Sciatic Function Index, Static Sciatic Index, toe spread factor, ankle angles, balancing), nociceptive and proprioceptive testing, and electrophysiologic examinations to histological and morphological assessments of the muscle and nerve. In view of the very diverse information available on the exact procedure to be followed for somatosensory testing, it was decided to test the rats in a greater number of small fields on the foot than previously suggested in literature to obtain more detailed information about the return of sensation. Only a conscious pain response would be counted as a positive reaction.

Unlike primarily motor-focussed evaluations such as the SFI (Walker et al. 1994, Hadlock et al. 1999) and morphometric assessments, the results of nociceptive testing did not differ significantly between the three surgical groups. The nociceptive assessments established a characteristic pattern common to all the groups for the return of sensation in the foot in more detail than previously

described in literature. The primary source of the sensory reinnervation, however, was revealed to be the saphenous nerve.

This study demonstrates how easily somatosensory assessments can be misleading in the evaluation of sciatic nerve regeneration, especially if they are not backed up by morphometric assessments of the associated structures. Largely undetected compensatory abilities of the nervous system, such as extensive saphenous sprouting in this case, are the cause of this phenomenon and can give the researcher the erroneous impression of successful sensory reinnervation of the foot by the sciatic nerve. Morphometric assessments and retrograde tracing studies tell a different story.

As a consequence caution is advised both in interpreting and executing somatosensory investigations in sciatic nerve regeneration studies.

Material and Methods

Surgical procedures

Twenty male Lewis rats (Charles River, Sulzfeld, Germany; 320 to 440 g) had a 14 mm segment of the sciatic nerve extracted and the defect subsequently repaired by one of the following three methods: group A (n=8) autograft repair, group B (n=6) repair with empty collagen tubes (length 2 cm) and group C (n=6) repair with collagen tubes filled with denaturated autologous muscle (Meek et al., 1999). 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 gastrocnemius muscle. Lewis rats were chosen on account of their proven resistance to autotomy after sciatic lesions (Inbal et al. 1980, Panerai et al. 1987, Carr et al. 1992, Chamberlain et al. 2000), their suitable anatomy of sciatic nerve (Rupp et al. 2007), and their friendly nature (Strasberg et al. 1999), as many evaluations would require frequent handling.

Animal health and housing

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

All rats were housed in groups of four on soft bedding in a temperature-controlled room with twelve-hour light cycles, and had free access to standard rat food and

water. Additionally they experienced four to six hours of ‘playtime’ daily on weekdays in a 45 cm x 55 cm x 120 cm cage in groups of eight to twelve. Animal studies were approved by the local animal care committee.

Intra vitam evaluations

The rats were accustomed to being handled twice daily for five days as from nine days before the operation (D-9 to D-5). The operation itself took place on D0; two days before the operation (D-2) the integrity of sensation on their feet was checked and all reference values were obtained. The first post-op evaluation was on day (D)5, further examinations taking place twice weekly until D56. For nociceptive testing the animals were held firmly in one hand whilst being pinched with a pair of atraumatic forceps at defined points on the lateral, plantar, dorsal and medial aspects of their right hind feet and then of their left hind feet (Figures 1, 2A). Definite vocalisation of protest at the moment of being pinched - with or without retraction of the foot - was rated as a sign that pain had been consciously registered. Application of pressure was stopped as soon as a reaction in the rat could be provoked; the maximum pressure applied was 0.8 N per mm².

Figure 1: Rat being pinched.

Results were noted in a diagram and also expressed as a score denoting the number of remaining denervated areas of the foot. For the latter, the scores of the biweekly examinations were averaged to minimise possible errors in reading the signs of the rat’s pain response. All tests were carried out by the same investigator to avoid interrater variabilities. The investigator was blinded to group assignment of the different rats.

In addition to nociceptive testing, motor return was evaluated by measuring traits of footprints and determining the Sciatic Function Index (SFI). To make the prints the soles of the rats’ hind paws were covered from the tip of their toes to the heel with non-toxic children’s paint.

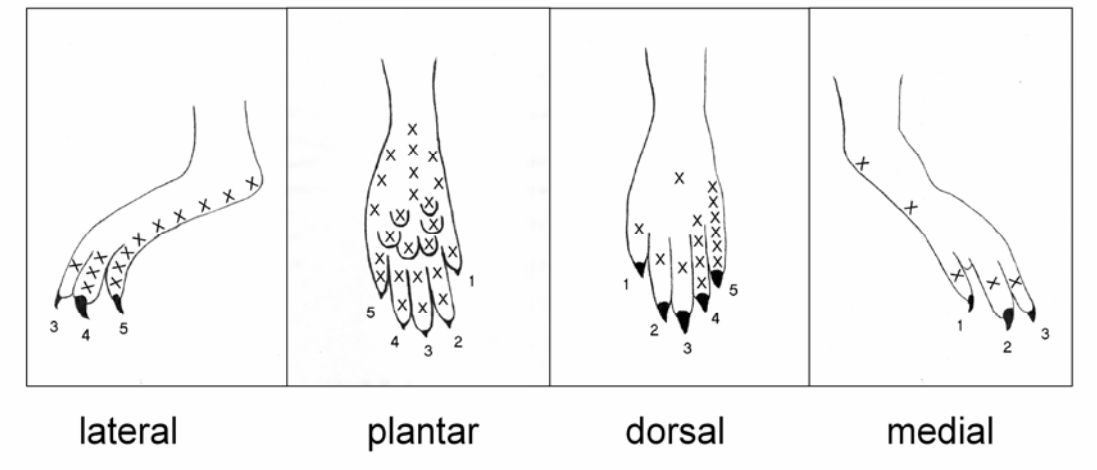


Figure 2A: Pinching points on different aspects of the foot.
On the dorsal aspect the animals were pinched at 14 spots, on the lateral aspect at 13 spots, on the plantar aspect at 24 spots and on the medial aspect at 5 spots.

The rats were then placed on an elevated 8 cm wide balancing beam, which ended in a darkened shelter and was covered with a strip of paper. This technique produces more usable footprints - especially on the operated side – than those obtained when letting the animals run in a corridor (unpublished observations). As there is no wall, the rats have nothing to lean on, and there is no chance of exorotated foot swings hitting the wall and thus producing no footprints. Also, whilst traversing the beam the rats subconsciously trust their unoperated (right) hind limb and keep to the right, enabling clear footprints to be obtained on the left (operated) side. Having experienced extensive prior training, the rats made their way towards the shelter quickly and confidently. Runs were repeated until at least three well recognisable and distinct prints for each side had been acquired; at the most a rat had to undergo five runs. Three footprints for each hind limb were selected for evaluation and the distances between toes 1 and 5 (toe spread (TS)) and toes 2 and 4 (intermediate toe spread (ITS)) and the print length (PL) were measured as described by Varejão et al. (2004a,b). The average measurements for each animal were determined by two independent researchers, who then compared their values blindly. If the difference did not exceed 3 mm, the average between the two values of the examiners was incorporated into the following formula: SFI= − 38.3 x PLF + 109.5 x TSF + 13.3 x ITF − 8.8 (Varejão et al. 2004a,b). The PLF, TSF and ITF were calculated by subtracting measurements taken from the physiological hind limb from those taken from the operated hind limb. The difference was then divided by measurements for the physiological side. SFI scores of approximately −100

indicate total impairment, whereas scores around 0 can only be achieved in rats with complete function of the sciatic nerve (Varejão et al. 2004a,b).

Retrograde tracing

On D47, retrograde tracers were applied to the operated hind limb. One intracutaneous injection, consisting of 1.5 µl of 5% Fast Blue (Polysciences), was performed on the lateral side of the foot at the level of the fifth metatarsal bone. On the medial side of the foot three injections amounting to 2.5 µl of 1% Fluorogold (Biotium) were conducted at the level of the first metatarsal bone.

Eight weeks after the operation (D56) the rats were euthanised with a lethal dose of intraperitoneal pentobarbitone. The dorsal root ganglia (DRG) of the first to sixth lumbar segments (L) were extracted and immersed in 4% paraformaldehyde and 10% sucrose in 0.1 M phosphate buffered saline (PBS) at pH 7.4 for 5 hours. After fixation the DRG were transferred into 15% sucrose in PBS for at least 15 hours and then frozen in liquid nitrogen. Cryostat sections were cut at 16 µm and thaw-mounted on polylysine-coated glass slides. The DRG were then 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 a LP 420 nm barrier filter.

The numbers of blue and yellow labelled cells were semiquantitatively assessed by counting the fluorescent cells on what appeared to be the largest cross section.

Morphometric and electron microscopic assessments

After extraction of the DRG, the sciatic nerve plus its tibial successor were harvested from their most proximal accessible point (near the major trochanter) right down to the middle of the plantar side of the foot in both hind limbs. The nerves were immediately cut into three segments, stretched on a piece of paper, and immersed in 2.5% glutaraldehyde in Soerensen's phosphate buffer (pH 7.4) for 1 hour. After fixation, samples were rinsed with Soerensen's phosphate buffer and cut into 2 mm transverse segments, which then underwent post-fixation in 2% OsO_4 for 2 hours at room temperature, repeated buffer rinses and a graded alcohol series before being embedded in epoxy resin. For morphometric evaluations semithin sections (0.5 µm) were mounted on triethoxysilane-coated slides and stained with p-phenylene diamine.

Morphometry was performed on cross-sections of the tibial nerve 0.5 cm distal to the calcaneus. Photographs were taken of these sections with a Zeiss Axiovert 100® light microscope equipped with a PLANAPO oil immersion objective (100x, n.A. 1.25), a CCD camera and a motorised stage. Pictures were assembled semi-automatically and picture processing was performed by the MT_O_P (Research System Inc., Boulder, CO, USA) software programme.
Electron microscopic assessments were performed on the segments of the tibial nerve taken from the left, operated, hind limb (0.5 cm distal to the calcaneus). Slices with a thickness of 80 nm were contrasted with uranyl acetate and lead citrate, and then examined under a Zeiss-EM10 (Germany).

Statistical analyses

Scores for denervated areas were subjected to statistical analysis using Sigma Stat Software (SPSS); as the scores originated from counted data, non-parametric tests were chosen for comparing groups. The Kruskal-Wallis-Test was applied for determining significant differences ($p<0.05$) between the three experimental groups in the different areas of the foot and at the various stages during the test period; the same test was also used for assessing the progress made by the different experimental groups between weeks (W) 5 and W8. The Mann-Whitey-Test was then applied for more detailed comparison of score differences between only two groups at a given point in time and area of investigation.
Statistical analysis of the SFI values was also carried out with the SPSS. Here, the Welch-Test was applied for determining significant differences between the three experimental groups at W8, as there was a large disparity between the mean variations in the individual groups. The t-test was then applied in combination with the Levene-Test for more detailed comparison of the SFI scores of only two groups at W8. To determine whether significant progress had been made in groups between W1 and W8 the paired t-test was chosen. Morphometric results on the unoperated side were assessed by the SAS system. The ANOVA was used to determine whether significant differences existed between the mean fibre densities on the unoperated side of the different surgical groups. On the operated side no statistics were applied in view of the disparate results, which were in any case obvious.

Results

Nociceptive testing

As a general rule heightened reactions to pain could be noted on the operated side when the rat's foot was pinched in areas where pain sensation still existed or had been regained. This was so despite the fact that the same amount of pressure was applied on both the operated and unoperated sides. When pinched on the operated side the rats responded with shriller tones compared to the healthy side, or even by biting and trying to attack the source of pain, which they soon identified as the forceps. Often the area of pain was also licked in an attempt to alleviate discomfort.

This finding stood in stark contrast to the unoperated side, which was always evaluated first, and where occasionally withdrawal reactions without any vocalisation could be elicited. However, when quickly pinching the right (unoperated) side as a control whilst actually testing the left foot, some rats also responded on the contralateral side with shrill squeaks.

On D5 sensory innervation was virtually nil in all the groups both on the *lateral* aspect of the foot and on the lateral sides of toes 4 and 5; however, four of the six rats in group C showed innervation of the heel. A few randomly innervated spots were found which cannot be accounted for. On the *plantar* aspect, all areas apart from the medial edge of the sole, the two medial pads, and toes 1, 2 and 3 appeared to be denervated in all the groups. Sometimes a reaction could be elicited from areas in the midline and the heel (mainly group C). On the *dorsal* side of the foot, pinching of the surfaces of toes 4 and 5 only triggered a signal of discomfort in only one animal; about half the rats in groups B and C responded to being pinched at one or more points in a wedge-shaped area extending from these toes towards the ankle. The rest of the dorsal and the *medial* sides of the foot as well as the lateral, medial, plantar and dorsal sides of toes 1 to 3 were fully innervated throughout testing from D5 to D56.

Over the next eight weeks the return of sensation occurred at different rates for individual rats, but following a set pattern (Figure 2B-D).

On the *lateral* and *dorsal* aspects of the foot, innervation reappeared very slowly from proximal to distal, with lateral and dorsal aspects of the toes being reinnervated at the same time or with a slight time lag on the lateral side. By D21

Figures 2B-D: Return of sensation on the dorsal (B), lateral (C) and plantar (D) aspect of the foot.
Numbers show how many rats reacted to being pinched at the spot indicated. The colour code represents the same results: the lighter the shade, the greater the number of rats which reacted to being pinched. In white areas there was always a reaction. (Medial areas are not shown, as here a reaction to being pinched could always be demonstrated.) Note toe 5, where only animals in group A (autograft group) regained sensation.

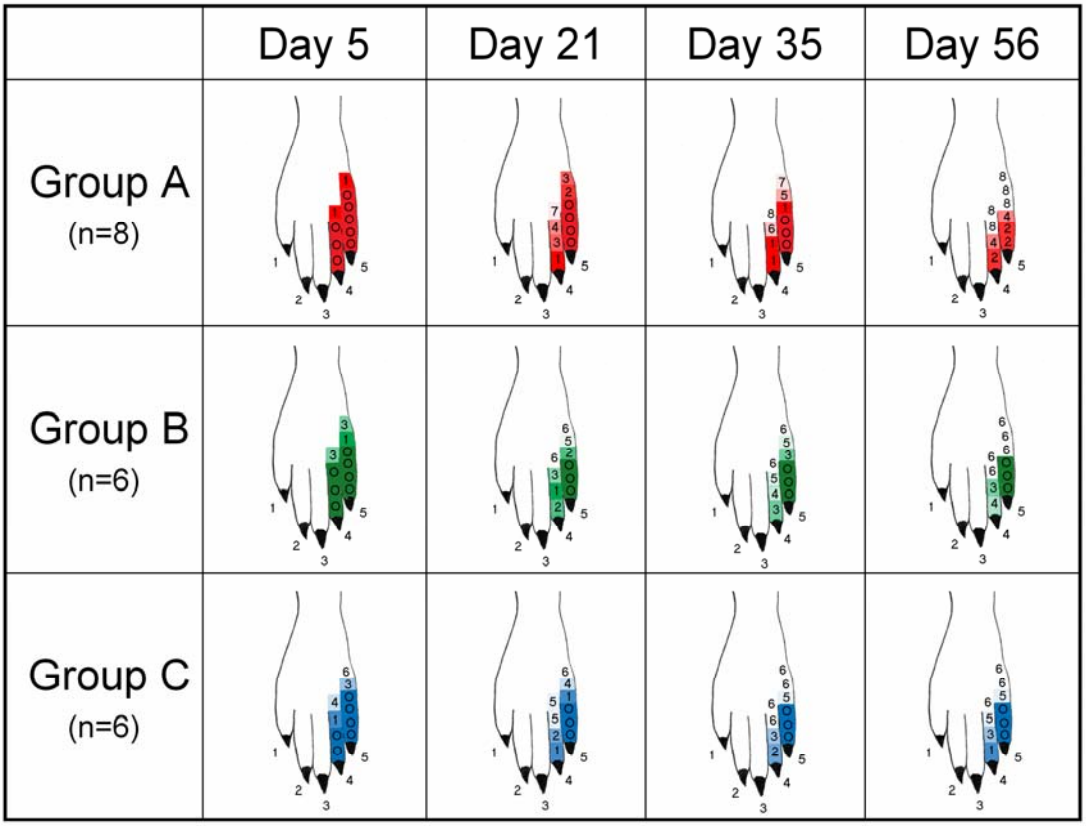


Figure 2B: dorsal aspect

reactions to pinching could be evoked in animals of all groups in areas stretching from the heel to about halfway down the lateral side of the foot and occasionally on the lateral side of toe 4. The lateral and dorsal aspects of toe 5 remained without sensation, whereas frequently the rats showed a pain reaction when pinched on the dorsal aspect of toe 4. The following five weeks then showed distal reinnervation creeping forward at an extremely slow pace. By D56, reinnervation of both the lateral and dorsal aspects had reached the base of toe 4 in all but one animal, and the middle of toe 4 in half the animals of each group. The dorsal side of the tip of toe 4 was reached in just a third of the animals and the lateral side in only one rat of each group. On the dorsal and lateral aspects of toe 5 the situation was even poorer. No animals in groups B and C showed any reaction to being pinched in these areas right up to the end of the study. In the autograft group

(group A) the tip of toe 5 was reinnervated by week eight on the dorsal aspect in one quarter of the animals, on the lateral side, however, in none.

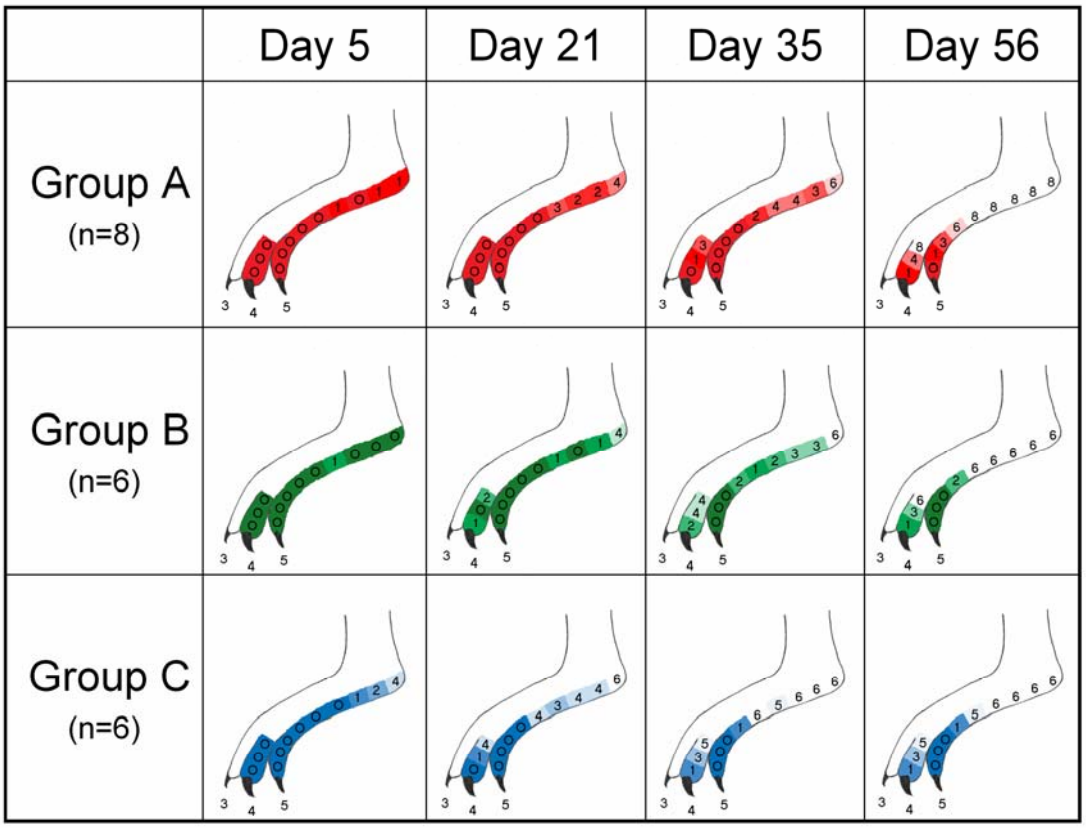


Figure 2C: lateral aspect

The *sole* of the foot – apart from the area of the heel – appeared to be innervated from medial. By D21 almost all animals under observation had pain sensation around the heel and the pad between toes 2 and 3, approximately half the animals along the midline, and only very few (but half the animals in group C) on the lateral side of the plantar aspect of the foot. The underside of toes 4 and 5 with corresponding pads and the pad proximal to those remained more or less without sensation. In the following five weeks reinnervation moved across the sole of the foot to the lateral side – very hesitantly at first in group A, but then with increasing speed – and spread towards and into toes 4 and 5. (In most cases pain sensation in the pads was regained within the same week reinnervation had reached the corresponding toe or in the following 2 weeks.) On D56 almost every rat had achieved complete sensory reinnervation of the soles of its feet and the base of toe 4. The plantar aspect of the tip of toe 4 was reached in nearly half the animals in

every group. As with the reinnervation on the lateral and dorsal sides of toe 5, group A was the only group in which a pain response could be evoked when the rats were pinched on the plantar aspect of toe 5.

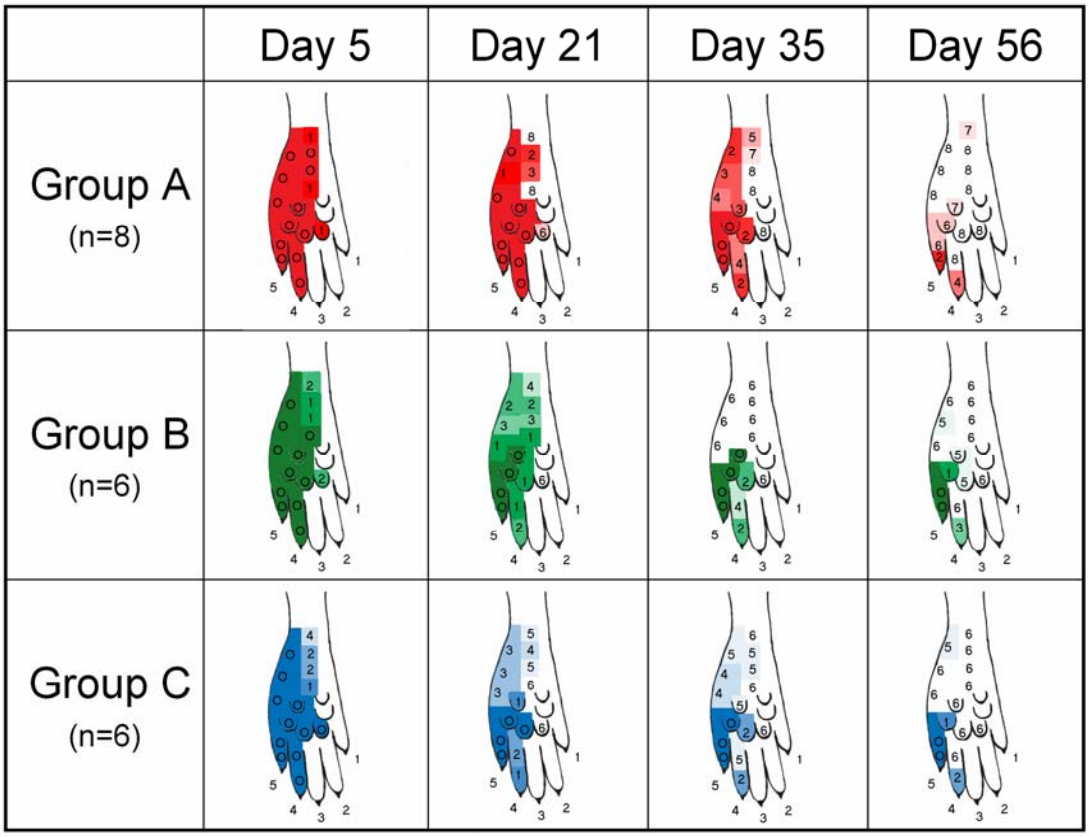


Figure 2D: plantar aspect

In statistical evaluations of the scores for denervated areas on the left hind feet (Table 1) there was no significant difference between any of the three experimental groups for any of the aspects of the foot in week (W) 8 (p=0.942 for the dorsal aspect of the foot, p=0.363 for the lateral aspect, p=0.309 for the plantar aspect and p=0.464 for the sum of the 3 areas). The figures for W5 stand in stark contrast to this, with significant differences being noted between all groups in all areas except the plantar area of the foot. However there were no significant differences at all between groups B and C (p=0.818 (dorsal aspect), p=0.6500 (lateral aspect), p= 0.818 (plantar aspect), p=0.589 (sum of the three aspects)). Analysis of the scores for the return of sensation to the various areas of the foot between W5 and W8, revealed the most striking differences to be in group A (p between 0.001 and 0.005 for all tested aspects of the foot); group B exhibited significant differences for

the scores on the lateral (p=0.005), plantar (p=0.004) and combined (p=0.024) areas of the foot, whereas group C only made significant progress on the plantar aspect (p=0.004).

Table 1: Numerical results of nociceptive assessments

Area of foot	Group	D-2	D5	W3	W5	W8
Dorsal	A	0.00	9.71	7.75	6.31	3.69
	B	0.00	8.83	6.67	4.75	4.17
	C	0.00	7.67	6.08	4.92	4.50
	p value		0.003	0.066	0.039	0.942
Lateral	A	0.00	11.57	10.94	9.38	4.63
	B	0.00	11.83	10.67	8.00	5.58
	C	0.00	10.83	8.50	6.42	5.25
	p value		0.040	0.005	0.003	0.363
Plantar	A	0.00	14.57	11.50	8.19	2.56
	B	0.00	14.00	10.93	6.08	4.17
	C	0.00	13.50	8.67	6.42	4.00
	p value		0.152	0.020	0.084	0.309
Total foot	A	0.00	35.86	30.19	23.86	10.89
	B	0.00	34.67	28.25	18.83	13.91
	C	0.00	32.00	23.25	17.75	13.75
	p value		0.006	0.005	0.010	0.464

The values represent the number of denervated spots in the different areas of the foot.
The maximum score for the dorsal aspect of the foot is 10, for the lateral aspect of the foot 12, and for the plantar aspect of the foot 15, with a grand total of 37 for the entire foot. Significant differences in scores between groups are present when $p<0.05$ (Kruskal-Wallis-Test). Until week (W) 5 group A clearly lags behind the other two groups (B and C), which are almost identical. After this the autograft rats catch up and finish at week 8 with the lowest scores, mostly attributable to the sensory reinnervation of toe 5.

On dissection of one of the rats in group B on D56 no collagen tube could be found, the end of the proximal stump having formed a neuroma. In nociceptive testing this rat had, however, been completely indistinguishable from the other rats in its group.

SFI scores

Analysis of the SFI scores reveals that rats in group A were the only rats to make progress. The return of motor function however did not start until W5 (Figure 4).

At the end of W8, group A, in stark contrast to groups B and C, had made significant progress when scores were compared with W1 (group A: p=0.013, group B: p=0.600, group C: p=0.905).

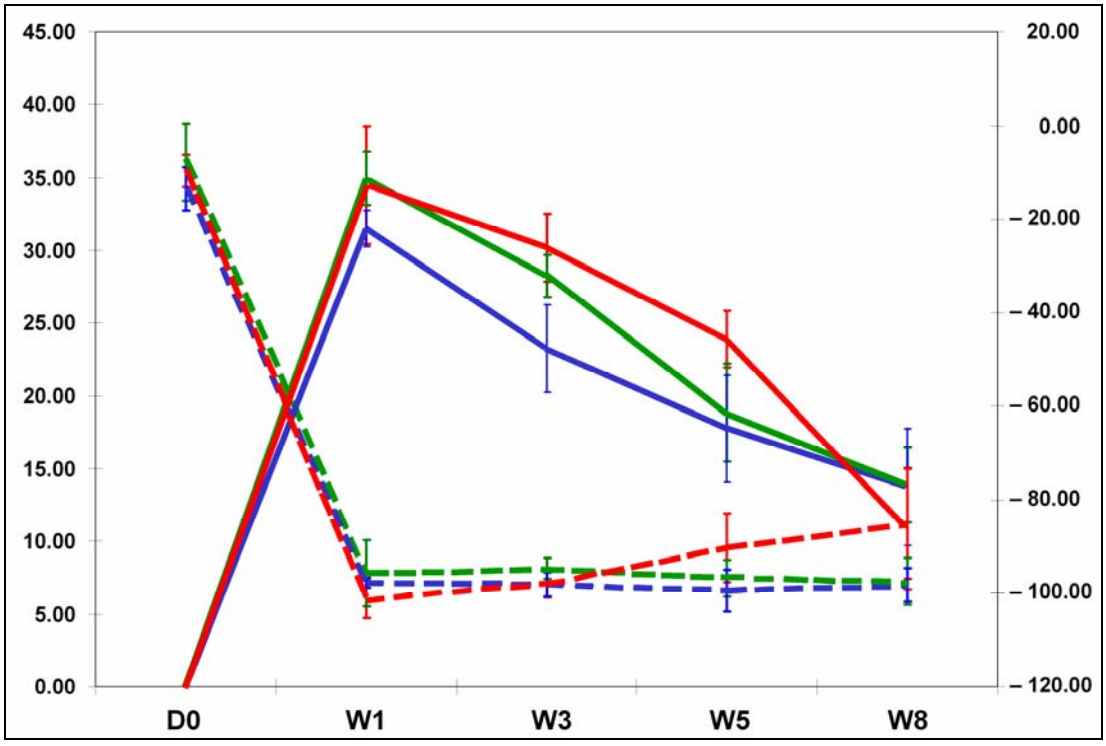
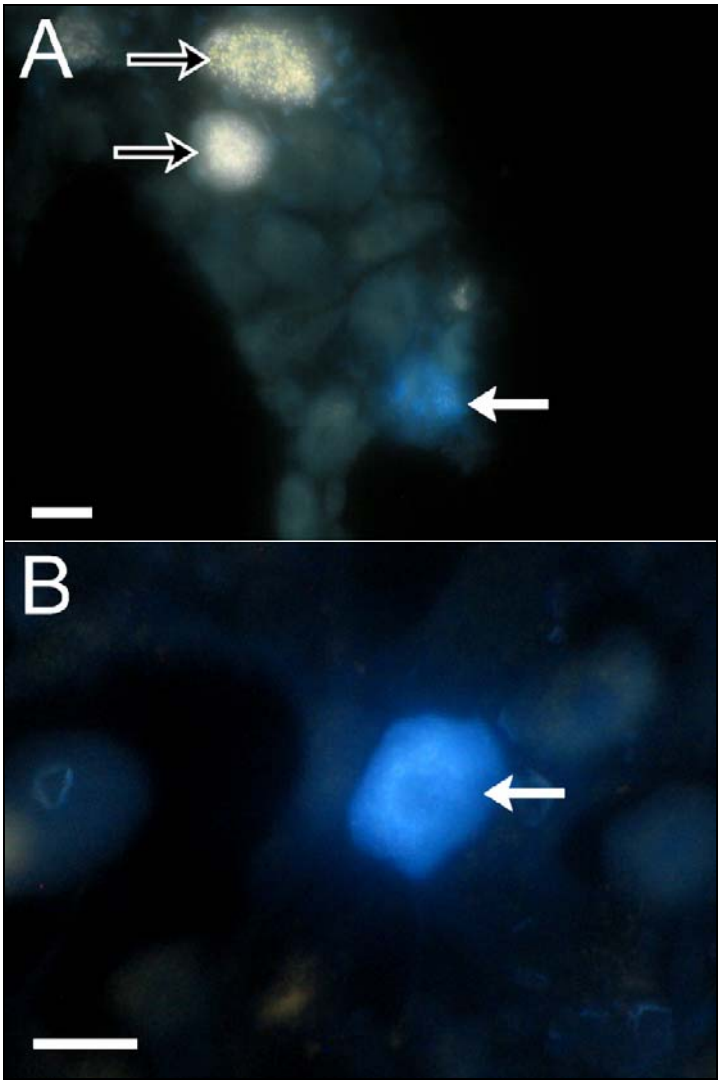


Figure 4: Comparison of results of somatosensory and motor assessments
Somatosensory results (combined scores for nociceptive testing on the whole foot; see table 1 for explanations) are shown by continuous lines, the motor results (SFI) by broken lines. Group A (autograft) is red, group B (empty collagen tube) is green and group C (muscle filled collagen tube) blue. The left ordinate represents the somatosensory scores, the right ordinate the motor (SFI) scores.

A statistically significant difference between scores in W8 could also be noted between the autograft group and the collagen tube groups, regardless of whether they were empty or filled (A and B: p= 0.024; A and C: p= 0.017). No significant difference was found between groups B and C (p=0.773).

Retrograde tracing

Fluorescence microscopy revealed yellow (Fluorogold) and blue (Fast Blue) labelled neuronal cell bodies in DRG belonging to the spinal cord segments L2, L3 and L4. No labelled neurons were evident in the DRG corresponding to the spinal cord segments L1 and L6, and only three rats exhibited one to four cells with blue fluorescence in the DRG of L5.



Figures 3 A,B: Tracing studies
Yellow (open arrows) and blue (white arrow) labelled neuronal cell bodies in the DRG of the spinal cord segment of L2 (A) and L3 (B). Scale bar is 20 µm (A,B).

The numbers of labelled ganglion cells, both yellow and blue, were the lowest in the DRG corresponding to the spinal cord segment L2. Here at the most four labelled cells of each colour could be noted, and this not in every animal. The DRG of L3 showed both yellow and blue fluorescence in all animals examined and at least one ganglion cell was double labelled in every animal (Figures 3 A,B). The maximum of double labelled cells was four. The largest number of cells exhibiting yellow fluorescence in the DRG of L3 amounted to 52 ganglion cells in one cross section, whereas at the most five cells were labelled with Fast Blue (blue). The DRG of L4 exhibited cells with blue fluorescence in all but two of the rats examined and the blue ganglion cells reached cell counts of up to 15 cells per cross section. Fluorogold-labelled cells (yellow) were visible in all DRG of L4 and amounted to

between seven and 27 cells per cross section. In about half the DRG of L4 both blue and yellow (double-labelled) fluorescent cells could be seen.

Morphometric and electron microscopic assessments

Morphometric assessments of the tibial segment 0.5 cm distal to the calcaneus revealed that animals in group A experienced some regeneration in this area. The mean fibre density in this group reached about on third of the reference values which had been calculated from the contralateral unoperated side. Amongst rats in groups B and C, only one animal possessed a total of 23 countable regenerated myelinated fibres on its combined fascicular area of more than 920,000 μm^2. All the others had none (Table 2).

Table 2: Mean fibre densities [myelinated fibres/mm^2] in plantar extensions of the tibial nerves

Group	Right hind limb	Left hind limb
A	1,181.8	386.1
B	1,317.2	0
C	1,319.5	0

The values represent the mean fibre densities of myelinated fibres in the three experimental groups A (autograft), B (empty collagen tubes) und C (collagen tubes filled with denaturated muscle) in plantar extensions of the tibial nerves 0.5 cm distal to the calcaneus.

These findings were confirmed by electron microscopy. Here small myelinated and unmyelinated axons could be seen in all animals in group A. The myelinated axons were either set in regenerative groups or clusters, or could be found individually. In contrast to this, only one out of the twelve animals in groups B and C displayed some (but very few) myelinated axons, another displayed one single small myelinated axon, and the rest showed none at all. Unmyelinated axons with decreased diameters and in low numbers were discernible in eight out of the twelve animals. In all segments examined, ovoids, Buengner bands and collagen pockets could be seen, the rats of groups B and C exhibiting increased signs of myelin destruction and also more denervated Schwann cells when compared to animals of group A.

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 and all of them placed their feet appropriately when moving. In daily adspectory

assessments no evidence of inflammation of the foot could be detected in any rat except for one animal in group B, which developed a chronic ulceration of its heel in week 2. This did not regress throughout testing, but also did not provide any hindrance to activity. The affected rat still placed its foot with the plantar side downwards when walking and also exhibited no decrease in weight-bearing or excessive licking. In somatosensory evaluations this rat had not been distinguishable from the other rats in its group either with regard to progress or with regard to final results for sensory reinnervation. Clinical examinations also revealed that this rat was normal.

In none of the rats could any signs of chronic inflammation of the tissue be found upon post-mortem examinations.

Discussion

Sensory testing

In the end, nociceptive testing revealed very little divergence between the autograft (A) and interponate groups (B and C), the major difference being at toe 5, where only animals from group A were able to regain sensation. Interestingly, groups B and C, which finished the study with more or less the same result as group A, as regards the return of pain sensation, both made faster progress than group A until D35 and then for the most part showed little improvement right up to the end of the study. In contrast, the autograft group maintained reinnervation at a steady pace right up to D56 and, as already mentioned, was the only group to have achieved any pain sensation on the lateral, dorsal or plantar aspects of toe 5.

A similar time frame for rats regaining sensation on the lateral aspect of their feet after various injuries to the sciatic nerve and their repair is described by den Dunnen and Meek (2001). In their study the animals reacted to electro-stimulation at the most proximal stimulation point (lateral to the heel) about three to four weeks after surgery, and at the most distal stimulation point, just proximal to toe 5, approximately two weeks later.

Correlation with other methods of evaluation

Results gained from tests primarily concerned with motor function such as footprint analysis (Walker 1994, Hadlock 1999) follow the same pattern as the return of sensory innervation for animals in groups A (autograft goup). In these rats a steady improvement could be seen, accelerating as from W5. In contrast to this, neither of the interponate groups (groups B and C) improved at all in motor tests (SFI). This finding does not correlate at all with their readily detectable progress in sensory assessments (Figure 4). Morphometric examinations of the plantar tibial extensions (5 mm distal to the calcaneus) of the rats in groups B and C revealed only very few myelinated fibres in one of the animals, in the others none at all. In the same segment of most rats in group A moderate amounts of regenerating myelinated axons could be observed.

Electron microscopic assessments of the tibial plantar extensions distal to the calcaneus were then carried out to rule out the possibility of having overlooked large numbers of regenerating unmyelinating axons which are virtual invisible in light microscope histology. However, only scattered regenerating unmyelinated axons with decreased diameters compared to normal axons could be seen in eight of the twelve animals in groups B and C, and in only one of the animals could myelinated minifascicles be detected. As a consequence of these results the source of sensory reinnervation in groups B and C has to be reconsidered.

Source of sensory reinnervation

Assuming a distance of approximately 53 mm from the proximal end of the lesion to the ankle and a further 38 mm from the heel to the tip of the third toe (Rupp et al. 2007), regenerating axons would have to travel about 91 mm from the proximal stump to completely reinnervate the foot right up to the tip of the toes, if they took the direct route. Given a rate of regeneration of about 1.4 to 2 mm per day (Gutmann et al. 1944, Navarro et al. 1994), or even faster (de Koning et al. 1986, Varejão et al. 2004a), it is possible that sensory reinnervation of the foot could have occurred as a result of regrowth of the tibial and peroneal nerves. However, if the results of footprint evaluations and morphological studies are taken into account, it seems far more plausible that sensory reinnervation of the foot resulted from extensive sprouting of an uninjured neighbouring nerve. This could be either the saphenous nerve or the musculocutaneous nerve of the hind limb, a branch of the sciatic nerve which also seems to innervate digits of the rat's foot (Puigdellívol-

Sánchez et al. 2000). The proximal edge of the operation site, especially in animals with collagen implants, however, lay so close to the point where the sciatic nerve emerges from beneath the medial gluteal muscle, that the chances of the musculocutaneous nerve not being injured during experimental procedures were very slim. Also, the distribution of denervated areas after the insult, together with the distinct reinnervation pattern (from proximal to distal on the lateral and dorsal aspects of the foot, and from medial, then reaching distal, on the sole, with sensory reinnervation always taking place earlier on toe 4 than toe 5), strongly indicate that the source of reinnervation must be located somewhere in the medial region of the ankle, and this is exactly where the saphenous nerve is located (Greene 1955, Hebel and Stromberg 1976). Further substantial support is lent to this theory by the findings of the retrograde tracing studies. Injections of a retrograde tracer into the skin on the medial side of the foot resulted in neuronal cell bodies in the DRG of L2, L3 and L4 being labelled. This observation correlates exactly with expectations, since the saphenous nerve has been reported to be responsible for sensory innervation of this area (Greene 1955, de Lahunta 1977, Devor et al. 1979, de Koning et al. 1986, Varejão et al. 2004b) and the central distribution of the saphenous nerve lies in spinal cord segments L2 to L4 and their respective DRG (Greene 1955, Seltzer and Devor 1984). Carl Molander and colleagues state in their retrograde tracing study (Puigdellívol-Sánchez et al. 2000) that the femoral nerve contributes mainly via DRG of L3 and L4 to the sensory innervation of the foot, and this statement corresponds directly with our findings. Application of a different retrograde tracer to the skin on the lateral aspect of the foot, which physiologically is innervated by a branch of the sciatic nerve (Greene 1955, Hebel and Stromberg 1976) also resulted in labelling of neuronal cell bodies in the DRG of segments L2, L3 and L4. At least one double-labelled cell could be found in the L3 DRG of every rat and this finding, coupled with the fact that the central origin of the sciatic nerve normally lies further caudal than that of the saphenous nerve (Markus et al. 1984), namely in spinal cord segments L4, L5 and L6 (Greene 1955), leads to the conclusion that sensory reinnervation on the lateral side of the foot must primarily be attributable to the saphenous nerve. This is definitely the case for the rats in the interponate groups (groups B and C), which did not exhibit any regrowth of the plantar tibial extensions of the sciatic nerve at all. With group A rats, however, one could argue that some regenerating sciatic/tibial fibres (extending to the DRG of L4) were also traced.

Concerning the three rats (one rat in every group) which exhibited up to four blue labelled cells in the DRG of L5, one could surmise that in these rats the integrity of the very proximal branch of the sciatic nerve, the musculocutaneus nerve, might not have been completely compromised during the operation. Central distribution of this nerve has been reported to lie mainly in the DRG of L5 (Puigdellívol-Sánchez et al. 2000). After a further study, however, on "regenerative and collateral sprouting to the hind limb digits after sciatic nerve injury in the rat" the authors reached the conclusion that this nerve only seems to have limited capacity for compensatory innervation of the foot by collateral sprouting after a sciatic injury (Puigdellívol-Sánchez et al. 2005).

Extensive collateral sprouting of the saphenous nerve

The phenomenon of extensive collateral sprouting of the saphenous nerve after sciatic nerve damage has also been described by Devor and co-workers (1979), who examined distributions of tibial, peroneal and saphenous nerves in the foot after sciatic nerve crush both by behavioural testing (pinching) and electrophysiological recordings of single unit potentials. The distribution of denervated areas after sciatic damage and also the period of time and the consistent pattern in which sprouting of the saphenous nerve takes place (mostly completed by day 35), corresponds with the results recorded in the present study. In Devor´s study, however, the saphenous nerve never progressed into toes 4 and 5, and only into parts of toe 3, even in cases where sciatic regrowth was prevented. This finding lies in direct contrast to the result established for the rats in groups B and C of the present study, where toes 3 and 4 are clearly reinnervated by what must be the saphenous nerve – at least on the sole of the foot, as there are virtually no regenerative myelinated axons of plantar extensions of the tibial nerve to be seen. Kingery and Vallin (1989) support the present findings when they described the average cutaneous nociceptive response to pinch on the dorsum of the toes to take place at eleven weeks after chronic sciatic section in 3.8 ± 0.8 of five test areas. This must mean that toe 3 was reinnervated in all and toe 4 in some animals, assuming the critical areas for reinnervation lie on the lateral side. Pictures in the results indicate that the same seems to apply for the plantar side of the toes. Increase of the saphenous receptive field, however, took longer than in the present study continuing until week eleven. Markus and colleagues (1984) described similar results after sciatic denervation of the skin of the foot. Recordings

in the medial dorsal horn of the spinal cord indicate that after acute sciatic denervation, stimulation of toes 1, 2 and 3 and the corresponding metatarsal areas - on the plantar side only of toe 1, but on the dorsal aspect of the foot in all three toes - causes a reaction in the normal somatotopic areas of the saphenous nerve. Chronic sciatic denervation for 21 days resulted in receptive fields of the saphenous nerve expanding into the area dorsal to the fourth metatarsal bone and also towards lateral on the plantar aspect of the foot. These results correspond exactly with those established for D21 in the present study.

A very plausible reason why in our study toe 3 displayed sensation right from the first day of testing (D5) is given by Puigellivol-Sanchez et al. (2005), who mention in their work that the saphenous nerve has been reported to be physiologically responsible for toes 1 and 2 and the proximal phalanx of toe 3. Since toe 3 was only tested at one spot on the medial, lateral and dorsal sides and at two spots on the plantar side, it is highly possible that areas either originally innervated by the saphenous nerve or affected by vast and rapid sprouting of this same nerve were pinched when the animals where evaluated for pain responses.

A vast and very rapid reorganisation of sensory somatotopic areas after sciatic transection and ligation has also been described for the spinal cord and the S-I area of the cortex, 85% of which is predominately activated by the sciatic nerve and 15% by the saphenous nerve. Cusick and co-workers (1990) showed that within one to three days after injury to the sciatic nerve, the area activated by the saphenous nerve annexes an additional 23% to 26% of the total hind paw cortex. Seven to eight months later the area represented by the saphenous nerve does not differ significantly from the normal total hindpaw representation. On the spinal cord level, the saphenous nerve already seems to have spread into the representational area of the sciatic nerve and even further on the medial side of the dorsal horn by day 21 after sciatic transection and ligation (Markus et al. 1984).

Mechanical hyperalgesia

Mechanical hyperalgesia developed in the present study on the operated side in areas still innervated or reinnervated. However, it should be pointed out that this observation is subjective on the part of the examiner (albeit having conducted more than 320 examinations in all). Increased reactions such as shriller squeaks, increased guarding or attacking the source of pain could be observed when the

rats were pinched with a force not exceeding 0.8 N/mm² on the foot of the operated hind limb. On the contralateral side normal reactions to pinching were registered. This phenomenon of hyperalgesia on the operated hind limb also has also been noted by Kingery and co-workers (1989, 1994) and Markus and colleagues (1984). Kingery and Vallin described in 1989 that the adjacent neuropathic hyperalgesia mediated by the saphenous nerve lasts at least 12 weeks after sciatic transection with excision of the distal segment. In a later study Kingery and co-workers (1994) found that after sciatic crush injuries medial areas innervated by the saphenous nerve exhibited lower thresholds to both pressure and heat until sciatic reinnervation of the foot occurred. Hypersensitivity to pinch is attributed to collateral sprouting of high-threshold mechanoreceptors in the saphenous distribution (Kingery and Vallin 1989, Attal et al. 1994). A reduction in pain-inhibitory control in the central nervous system, together with ectopic excitability, central sensitisation after A-fibre sprouting in the dorsal horn (Vogelaar et al. 2004), primary sensory degeneration and a phenotypic switch are said to contribute to the causation of neuropathic pain. The exact aetiology, however, still remains largely unknown (Abrams and Widenfalk 2005).

In contradiction to all these findings of hyperalgesia, the study by Devor and others (1979) states that “no convincing instance of hyperactivity or hyperresponsiveness” could be noted. On the contrary, some rats showed only sluggish responses. A practical explanation for this large difference in reaction might be the way in which examinations were conducted in the present study. As only “reaction” or “no reaction” were distinguished, and nothing in between, it could be that subconsciously more pressure was applied when pinching the animals on the operated side. Therefore, whilst evaluating the injured side, internal controls were undertaken by intermittently pinching the uninjured (contralateral) side, and then this side also exhibited hyperalgesia or in this case even allodynia. This, however, is most likely the result of focused attention on stimuli, which can lead to an enhanced perception of pain or stimuli in general (Miltner et al. 1989). In future studies this could be remedied by using an analgesimeter or Frey filaments to exert pressure, as these permit the application of quantified stimuli.

Contribution to mechanical hyperalgesia by chronic tissue inflammation due to repeated nociceptive stimuli, however, can be excluded. In no rat, except one, could any evidence for inflammation be noted in daily inspections or post-mortem examinations of the feet. No behavioural changes regarding the use of the affected foot were evident in any of the rats. Both progress and result of sensory

reinnervation of the rat affected with chronic ulceration of the heel were identical with that of the other rats in its group. Therefore one can surmise that the chronic inflammation did not seem to provide any hindrance to the compensatory sprouting of the saphenous nerve even though a certain spatial closeness between both activities was given.

Source of sensory reinnervation on toe 5

The source of sensation on toe 5 of animals in group A can only be surmised. As reinnervation ceased to change after D35 in animals where hardly any sciatic regeneration could be observed (groups B and C), but exhibited a slow and constant progression in animals with proven regrowth of the sciatic nerve, one can presume that the source of sensation in toe 5 must be the plantar or sural extensions of the tibial or peroneal nerve. The results published by Devor and others (1979) and Kingery and Vallin (1989) also point in this direction, making it clear that the saphenous nerve never had the capacity to achieve full innervation of the hind foot in a rat. An elegant solution to this question could be provided by further retrograde tracing studies, with the tracer being injected intracutaneously on toe 5.

Conclusions

As a consequence of the results of the present study and the findings published by Devor et al. (1979) and Kingery and Vallin (1989), it must be concluded that results of both withdrawal reflex testing and pain response testing should be treated with caution. The areas tested, and the likelihood that collateral sprouting of the saphenous nerve could have taken place must be taken into account.

When conducting somatosensory evaluations, care should be taken to stimulate the rat only in areas definitely not affected by sprouting of the saphenous nerve, i.e. toe 5. Depending on how much the rat is handicapped in performing a recognisable withdrawal reflex (towel, denervated muscles required), pain response testing might provide more reliable results than withdrawal reflex testing.

Additionally, when assessing neural regeneration by functional tests, nerve samples for histological assessment should be harvested in sites close to the end organs examined, in order to determine whether reinnervation has actually taken place and minimise misinterpretation of functional results.

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Ambulatory testing on elevated beams and parallel bars reveals extent of plasticity after transsection and repair of the sciatic nerve in the rat

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Abstract. *Purpose:* In order to arrive at an overall assessment of integrated locomotor function after sciatic transsection and repair, it was decided to supplement measurements of static (SSI) and ambulatory (SFI) footprints by having rats traverse beams of different widths (8, 3, 2, 1.5, 1 cm) and two sets of parallel bars.

Methods: Eighteen rats were submitted to sciatic double sectioning and subsequent repair of a 14 mm gap by three different surgical methods: (A) autograft, (B) empty collagen tubes, (C) muscle-filled collagen tubes. The rats' footprints and their ambulatory abilities on beams/bars were assessed twice weekly for eight weeks. Ambulatory abilities were evaluated by noting speed and the number of times the affected hind limb was misplaced or slipped off the beam/bar.

Results: Group A, which exhibited the best results in SFI and SSI, also made most progress where ambulatory tests primarily focussed on motor or sensory regeneration. In the overall ambulatory evaluations, however, hardly any difference could be noted between groups, even though groups B and C experienced only very little or next to no neural regeneration, confirmed by morphometric assessments.

Conclusion: The progress in ambulatory tests made by groups B and C must primarily be attributed to plasticity at the cortical and subcortical level and underlines the need for functional tests to be supplemented by morphometric assessments in order to minimise misinterpretation of results. Additionally, this study confirms the proposition that SFI and SSI are assessments primarily concerned with motor function and should not be applied to rate overall function after sciatic section and repair.

Keywords: rat, sciatic, functional assessments, SFI, beam walking, plasticity

1. Introduction

The sciatic nerve in the rat is the most popular site for research on peripheral nerve regeneration, with many different methods for evaluation of regeneration being described. They can roughly be divided into two groups: functional and structural assessments. The former includes motor and sensory evaluation of peripheral nerve regeneration, the latter is purely concerned with the restoration of anatomical features. Interestingly, there is no or next to no correlation between the results of these two types of evaluation [23,47], the reason for this being that functional regeneration comprises the sum of all types of structural regeneration plus the imponderable factor plasticity.

Functional evaluations can further be subdivided in motor, sensory or autonomic tests. Overall assessments, such as gait analysis, usually consist of a combination of both motor and sensory aspects.

Footprint-measuring was and still is considered to provide a suitable overall assessment of functional restoration [11] reflecting the complex combination of sensory input, central integration and motor response [2,23,38,42]. This is probably the main reason why most research papers featuring functional evaluation of sciatic nerve regeneration include one of the three different methods used for footprint evaluation: the Sciatic Function Index (SFI), the Static Sciatic index (SSI) or the toe-spread factor. It has, however, been demonstrated that none of the parameters commonly used in footprint analysis is dependent on sensory input. Only the stride width, which is not assessed in SFI, SSI or the toe-spread factor, changes after sensory impairment in the rat [46]. This suggests that conventional footprint analysis primarily measures motor function [17,45,48] and needs to be supplemented by sensory assessments.

Sensory testing can be carried out in the form of nociceptive or proprioceptive testing. With the former, noxious stimuli are applied to the foot of the rat; with the latter, tests usually applied in examinations for “conscious” proprioception in small animal neurological examinations [10] have been adapted for rats. These comprise the tactile placing response [17], the hopping response [17], the extensor postural thrust and the grasping test [8,32]. The problem with the majority of tests in nociceptive and proprioceptive testing is that they rely on a motor response to a sensory stimulus. It can then become impossible to separate sensory and motor functions in these methods of assessment [32]. Furthermore, extensive sprouting

of the saphenous nerve makes it difficult to determine the most suitable point for measuring the return of sensory function [17].

Gait evaluation, when not limited to the measuring of footprints, provides an analysis of overall and integrated function. In locomotion assessments rats are scored as regards different aspects of their walking pattern and general carriage [29,38]. With ankle kinematics the focus has been shifted, concentrating on the angles of rats' ankles during different parts of the stance phase [43,44,48]. A different parameter of the gait, the stance factor, is determined by calculating the ratio between the amount of floor contact of the operated and the contralateral hind limbs [45]. And a further option for assessing the extent of functional nerve regeneration is provided by evaluating ground reaction forces [18].

Beam-walking, which up to now has primarily been applied in studies dealing with brain injury or drug-induced ataxia [16], is another potential method of gait evaluation. Different widths of beams are used depending on the aspect assessed. In order to gain information about gross motor function, the time taken for rats to negotiate an elevated flat 8 cm wide "runway" is measured [12,13]. More intricate motor function coupled with motor learning and hind limb coordination can be examined by getting the rats to traverse elevated parallel bars. This test involves recording and scoring the time taken to run along the bars and also the number of mistakes made by placing both hind paws on one bar, placing a hind paw beside a bar or swinging under the bars [12,13]. Similar tests involve measuring the number of times misplacement of paws occurs when the rat crosses a floor consisting of parallel bars of metal rods [30], a horizontal runway of metal bars [1] or simply a narrow beam [16,24].

In the following study 18 rats were subjected to double sciatic nerve transection with extraction of a 14 mm segment, and subsequent repair by guidance interponation. As the main focus was on measuring overall functional regeneration, the standard evaluation of sciatic regeneration by SFI and SSI was supplemented by measuring the time taken by the rats to transverse elevated beams of five different widths and also parallel bars. Additionally, the number of times the operated hind foot was misplaced or slipped off the beam was noted. At the end of the study, morphometric and electronmicroscopic assessments of the sciatic nerve were carried out and the volumes of the right and left gastrocnemius muscles were ascertained.

A comparison of the results obtained for ambulatory abilities, i.e. gait, with the results of the accompanying evaluations (SFI, SSI, sciatic morphometry and

volumes of the gastrocnemius muscle) revealed that rats with the best results in the accompanying evaluations exhibited most progress in both the specifically motor (speed on the widest beam) and the specifically sensory (hind limb placement on the narrowest beam and the parallel bars) categories of the beam/bar-walking assessments. In all the remaining ambulatory tests, however, the rats with the best results were ones, which made no or next to no progress in the accompanying tests. One can only surmise that plasticity, on either a cortical or subcortical level, has played the major role in functional regeneration in these rats. Furthermore the suggestion that SFI and SSI assessments are primarily concerned with motor functions and should not be regarded as overall assessments of nerve regeneration, can be confirmed by the results obtained in the present tests.

2. Materials and Methods

2.1. Operational procedures

Eighteen male Lewis rats (Charles River, Germany; 300 to 320 g) had a 14 mm segment of the sciatic nerve extracted and the defect subsequently repaired by one of the following three methods: group A (n=6) autograft repair, group B (n=6) repair with empty collagen tubes (length 2 cm) and group C (n=6) repair with collagen tubes filled with denaturated autologous muscle [28]. Muscle material was extracted from the serratus muscle. Control animals, which only experienced muscle extraction were evaluated for change of gait before muscle was extracted from animals in group C. No changes in footprints or balancing disabilities were noted (data not shown).

All lesions were set at the same place, with the distal end located 4 mm proximal to the submersion of the tibial part of the sciatic nerve into the gastrocnemius muscle. Lewis rats were chosen on account of their proven resistance to autotomy after sciatic lesions [5,6,19,34], their suitable anatomy of sciatic nerve [35] and their friendly nature [40], as many evaluations would require frequent handling.

2.2. Animal housing and physiotherapy

The rats were weighed weekly and inspected daily with regard to grooming, activity levels and signs of autotomy.

All rats were housed in groups of four on soft bedding in a temperature-controlled room with 12-hour light cycles, and had free access to standard rat food and water. Additionally they experienced 4 to 6 hours of “playtime” daily on weekdays in a 45 cm x 55 cm x 120 cm motor enriched cage in groups of eight to twelve individuals. The cage was customised to provide continuous physiotherapy by consisting of four different levels connected by oblique mesh ramps. A hammock-like construction was added, which provided the rats’ feet with additional stimuli due to its unsteady nature. Additionally, the sides of the cage could and were frequently used as climbing sources. Beams, foreign objects and healthy treats (pieces of carrot, cucumber or capsicum) were introduced into the cage in different places at frequent time intervals to provide the rats with constant stimuli to explore, climb and balance.

Animal studies were approved by the local animal care committee.

2.3. Training and evaluation

The rats were accustomed to being handled and trained multiple times on the balancing devices twice daily for five days as from nine days before the operation. By the end of the training week all animals exhibited zero faults when traversing the beams or bars. Two days before the operation (D-2) all reference values were obtained. The first post-op evaluation was on day 5 (D5), the next one on D7. Further examinations took place twice weekly until D56 with one of the evaluation days always on the full week after D0; a mean value of the two experimental values was calculated for each week. All evaluations followed the same routine, starting with ambulatory tests, then preceding to walking and static footprints. As the test for locomotor activity is sensitive to the circadian clock [24], testing was restricted to the morning to give all animals the same advantage/disadvantage.

All tests were conducted by the same researcher to avoid interrater variabilities. Measurements of walking and static footprints, however, were taken by two independent researchers. At the end of the study the individual measurements were compared blind and the average was applied for calculating the SSI and the SFI.

2.4. Assessments

2.4.1. Beam-balancing and parallel bars

For the balancing tests a construction consisting of a table structure (29 cm x 20 cm; height: 25 cm) and a box-like area (30 cm x 30 cm x 20cm), painted black on the inside and with a small entrance at the same height as the tabletop and a removable lid, were used (Fig. 1). The edge of the table and the entrance to the box were arranged at a distance of 95 cm and then connected by beams of different widths (8, 3, 2, 1.5 and 1 cm). Two sets of parallel bars were also used for this kind of testing. The distance between the centre of the bars was 2.5 cm [13], the bars being both either 1 cm or 0.8 cm in diameter. For the evaluation of their locomotor activity, the rats were placed onto the table and the time taken for non-hesitant traversal of the beam from the moment when the second hind foot was placed on the beam until the whole animal had disappeared in the box was measured. Mistakes where the paw of the operated hind limb was situated beside the beam in the weight-bearing phase (Fig. 2b) were rated in two different categories: either the paw was placed incorrectly (i.e. next to the beam) (1) or the paw was placed correctly, but could not or did not have the strength to grip and as a consequence slipped off the beam (2).

Fig. 1: Balancing construction

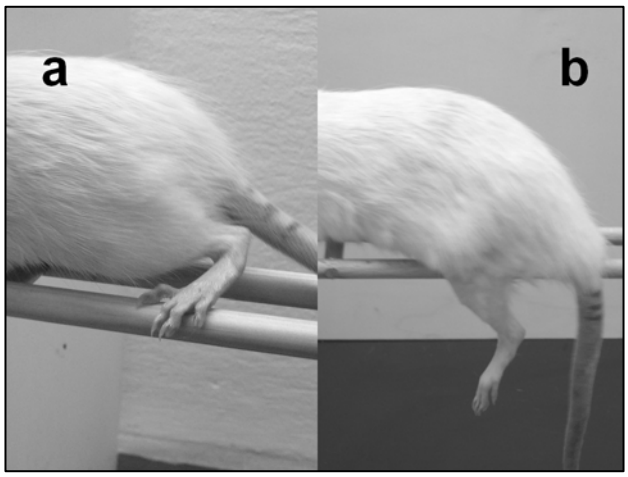

Fig. 2: Rat traversing the parallel bars.
a: correct placement of the hind limb;
b: incorrect placement.

The maximum score for mistakes in one run amounted to eight. For their morale the rats were first trained and evaluated on the widest beam and then

progressed to beams of decreasing size, finishing with the parallel bars. Sometimes it was found necessary on the very narrow beams to lightly touch the rats at the base of their tails so as to reassure them that they would not completely fall off. Placement of the hind limbs, however, was never hindered in any way.

2.4.2. Footprints (SFI and SSI)

Both static footprints and prints during walking were taken. For both sorts of prints the soles of the rats' hind paws were covered from the tip of their toes to the heel with non-toxic children's paint. For walking prints the rats were then placed onto the table end of the 8 cm balancing beam onto which an 8 cm wide and 100 cm long strip of paper had been clipped. This technique produces more usable footprints - especially on the operated side – than those obtained when letting the animals run in a corridor (unpublished observations). As there is no wall, the rats have nothing to lean on, and there is no chance of exorotated foot swings hitting the wall and thus producing no footprints. Also, whilst traversing the beam the rats subconsciously trust their unoperated (right) hind limb and keep to the right, enabling clear footprints to be obtained on the left (operated) side. As a result of having been trained and examined on this beam many times, the rats made their way towards the box in an unhesitant and confident manner. Runs were repeated until at least three well recognisable and distinct prints for each side had been acquired; at the most a rat had to undergo five runs. For static prints the rats were held upright, with one leg and the tail tucked under. The rat was then lowered onto a plain sheet of paper with the extended leg anticipating the ground and the print was taken much like stamping a piece of paper. Here also the procedure was repeated until at least 3 well recognisable and distinct prints for each side had been acquired.

For evaluation three footprints for each hind limb were chosen and the distances between toes 1 and 5 (toe spread) and toes 2 and 4 (intermediate toe spread) and the print length (only SFI) were measured as described by Varejão et al. [44] and Bervar [2]. The average of the measurements for each animal was then incorporated into the relevant formulas for SFI (SFI= − 38.3 x PLF + 109.5 x TSF + 13.3 x ITF − 8.8) and SSI (SSI= 108.44 x TSF + 31.85 x ITF − 5.49). The PLF, TSF and ITF were calculated by subtracting measurements taken from the physiological hind limb from those taken from the operated hind limb. The difference was then divided by measurements for the physiological side. SFI and SSI scores of

approximately −100 indicate total impairment, whereas scores around 0 can only be achieved in rats with complete function of the sciatic nerve [42].

2.4.3. Structural assessment of muscles and nerves

Eight weeks after the operation (D56) the rats were euthanised with a lethal dose of intraperitoneal pentobarbitone. The sciatic nerve was harvested from both hind legs from its most proximal accessible point near the major trochanter along with its tibial branch to the middle of the plantar side of the foot. The nerves were immediately cut into three segments, stretched on a piece of paper and immersed in 2.5% glutaraldehyde in Soerensen's (pH 7.4) phosphate buffer for 1 hour. After fixation, samples were rinsed with Soerensen's phosphate buffer and cut into 2 mm long transverse segments, which then underwent postfixation in 2% OsO_4 for 2 hours at room temperature, repeated buffer rinses and a graded alcohol series before being embedded in epoxy resin. For morphometric evaluations semithin sections (0.5 µm) were mounted on triethyloxysilane-coated slides and stained with p-phenylene diamine.

Morphometry was performed on cross-sections taken from mid-level of the collagen tube or autograft and mid-thigh level of the sciatic nerve on the contralateral side.

Digital images were taken of these sections with a Zeiss Axiovert 100® light microscope equipped with a PLANABO oil immersion objective (100x, n.A. 1.25), a CCD camera and a motorised stage. Pictures were assembled semiautomatically and picture processing was performed by the MT_O_P (Research System Inc., Boulder, CO, USA) software programme.

Electron microscopic assessments were performed on the segments containing the mid-level of the interponate. Slices with a thickness of 80 nm were contrasted with uranyl acetate and lead citrate, and then examined under a Zeiss-EM10 (Germany).

In addition to the nerves the gastrocnemius muscles were harvested on both sides and their volume was determined by water displacement.

2.5. Statistical analysis

Balancing times, scores for mistakes, SFI and SSI scores and gastrocnemius volumes were subjected to statistical analysis using Sigma Stat Software (SPSS). As there was fairly high variability between mean variation in each group, the

Welch-Test was applied to determine significant differences ($p<0.05$) between groups at week eight in the different methods of evaluation. For more detailed comparison of score differences between only two groups, the paired *t*-Test in combination with the Levene-test was used, and to determine whether significant progress had been made between weeks one and eight in any given group the paired *t*-Test was applied.

Morphometric results were assessed by the SAS system. The ANOVA was used to determine whether significant differences existed between absolute fibre numbers and the g-ratio of the different surgical groups. For a more detailed comparison of two groups the paired *t*-Test was applied.

3. Results

3.1. Ambulatory tests

3.1.1. Balancing times (Table 1)

On the widest beam (8 cm) the rats in group A finished fastest at week 8 (W8) after having exhibited a constant increase in speed over the eight evaluation weeks. Groups B and C displayed fluctuating balancing times, with group B not making any progress at all when comparing their balancing time from W1 with W8. The same finding was made in statistical analysis of balancing times. Group A was the only group to show significant progress between W1 and W8 (group A: $p=0.028$, group B: $p=0.517$, group C: $p=0.106$).

On all the other balancing devices (3, 2, 1.5, 1 cm, parallel 1 and parallel 0.8 cm) it was either group B or C which exhibited the fastest times by W8. Statistically, however, no significant differences could be noted between any of the groups at W8 on any of the balancing devices. Progress in balancing times could be observed in all groups when comparing times recorded in W1 after surgery and W8. In none of the groups however, was this progress significantly better than in any other group. During the course of the eight evaluation weeks the times taken to traverse the balancing devices often fluctuated; this was always the case with group B and with all beams except the 3 cm and 2 cm ones in group C. When a linear decrease in balancing times could be observed (mostly group A) this was often interrupted by an unexpected increase in the balancing time in week 5. From then on the decrease would begin all over again.

Table 1: Traversing times [s]

	Group	D -2	W1	W2	W3	W4	W5	W6	W7	W8	W8–W1
8 cm beam	A	3.16	**3.88**	3.44	3.18	3.05	3.28	3.13	3.00	**2.89**	– 0.99
	B	2.27	**3.27**	3.06	3.18	3.11	3.11	3.23	3.04	**3.37**	+ 0.10
	C	2.57	**3.34**	3.20	3,38	3.36	3.65	3.65	3.13	**3.03**	– 0.31
	P value	0.143								**0.038**	0.032
3 cm beam	A	3.75	**5.86**	4.62	3.93	3.78	4.17	4.37	4.04	**3.98**	– 1.88
	B	2.63	**5.12**	4.19	4.24	4.10	4.02	4.00	3.90	**4.20**	– 0.92
	C	3.09	**5.27**	5.19	5.22	4.52	4.64	4.07	4.12	**3.75**	– 1.52
	p value	0.053								**0.177**	0.618
2 cm beam	A	3.80	**6.31**	5.46	5.44	5.27	5.88	5.22	4.88	**5.33**	– 0.98
	B	2.82	**5.75**	4.99	4.70	5.73	5.49	5.32	4.59	**4.93**	– 0.82
	C	3.22	**5.79**	5.49	5.52	5.38	5.33	4.69	5.53	**4.31**	– 1.49
	p value	0.284								**0.100**	0.719
1.5 cm beam	A	4.73	**9.79**	8.65	6.43	9.14	7.98	6.82	6.12	**6.92**	– 2.87
	B	3.77	**7.08**	6.77	6.35	6.23	6.46	5.75	5.53	**5.44**	– 1.65
	C	4.07	**6.25**	6.18	6.58	5.80	6.02	5.89	5.50	**5.41**	– 0.85
	p value	0.457								**0.182**	0.311
1 cm beam	A	10.26	**25.51**	15.13	12.50	9.98	8.16	7.55	7.20	**7.59**	– 9.42
	B	6.34	**9.75**	7.12	7.64	7.74	7.49	7.77	7.15	**7.60**	– 2.15
	C	4.39	**7.76**	7.88	8.95	8.61	7.81	7.04	7.79	**7.06**	– 0.70
	p value	0.014								**0.387**	0.243
1 cm bars	A	7.02	**11.82**	9.04	8.46	8.10	8.64	8.59	8.39	**8.42**	– 3.40
	B	5.51	**9.21**	6.79	7.74	7.70	7.75	7.97	7.62	**8.46**	– 0.75
	C	5.90	**9.23**	9.18	9.37	8.54	9.73	8.28	8.07	**8.23**	– 0.10
	p value	0.091								**0.931**	0.365
0.8 cm bars	A	6.60	**10.61**	9.20	8.48	7.77	8.85	7.68	8.09	**8.77**	– 1.84
	B	5.62	**8.02**	8.16	7.64	8.49	7.80	7.51	8.19	**8.01**	– 0.02
	C	5.49	**8.82**	9.04	8.81	9.02	9.23	8.73	8.05	**7.77**	– 1.05
	p value	0.691								**0.280**	0.427

3.1.2. Mistakes

On the widest beam (8 cm) no rat at any given time of examination made any mistakes. On the narrower beams and the parallel bars the number of mistakes made increased with decreasing width of the beams. Most were made on the parallel bars, with no difference to be noted between the two different sizes of parallel bars used. Generally, when calculating the percentage of slips or misplacements in relation to the total number of mistakes, the animals exhibited a lot more slips, especially after W2.

Total mistakes (Figs. 3,4)

A comparison of the total number of mistakes made on all beams (3, 2, 1.5, 1 cm) in W1 and W8 shows that animals of all groups made progress. This progress was statistically significant ($p<0.05$, paired *t*-Test) on the 2, 1.5 and 1 cm beam for rats in groups A and B and on the 2 and 1 cm beams for rats in group C. With the parallel bars no significant difference could be noted between W1 and W8 for any of the rats.

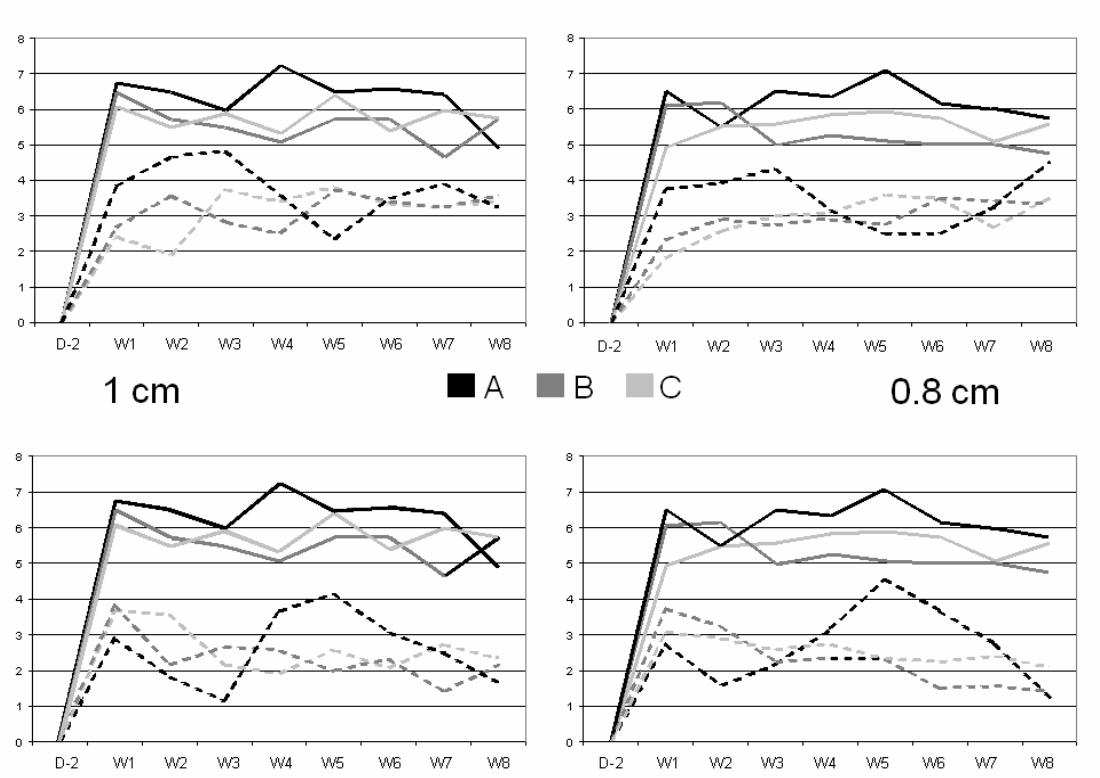

Fig. 3: Total, slip and misplacement mistakes on the parallel bars.
Total mistakes are denoted by continuous lines. In the top two pictures the broken lines depict slip mistakes, in the two bottom pictures they depict mistakes made by misplacement of the operated hind limb.

No group consistently exhibited less mistakes when compared to other groups with either the beams or the bars and no group finished with less statistically significant total mistakes than any other group at W8 on any of the beams and bars ($p>0.05$, Welch-Test).

Placing mistakes (Table 2, Fig. 3)

Hardly any placing mistakes were made in any case on the 3 and 2 cm beams. On all other balancing devices placing mistakes decreased in all animals over time,

though groups B and C made hardly any progress on the parallel bars. Group A exhibited more progress on the parallel bars, however their decrease in the number of mistakes they made was interrupted and set back by unexpectedly high amounts of placing mistakes during W4 and W5 (similar to balancing times). Despite this setback, at W8 group A could still be noted as the group with the least placing mistakes on the narrowest beam (1 cm) and both parallel bars. This finding is not significant however ($p>0.05$).

Statistically, it was only on the 1 cm beam that a significant reduction in placing mistakes could be noted for all groups (A: $p=0.008$; B: $p=0.030$; C: $p=0.016$), while group B also made progress on the 0.8 cm parallel bars ($p=0.005$).

Table 2: Misplacements

	Group	D -2	W1	W2	W3	W4	W5	W6	W7	W8	W8–W1
3 cm beam	A	0.00	**0.17**	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	– 0.17
	B	0.00	**0.00**	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	0.00
	C	0.00	**0.00**	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	0.00
	p value									**n.c.**	n.c.
2 cm beam	A	0.00	**1.67**	0.00	0.00	0.00	0.00	0.00	0.17	**0.00**	– 1.67
	B	0.00	**0.83**	0.08	0.17	0.08	0.25	0.00	0.00	**0.00**	– 0.83
	C	0.00	**1.17**	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	– 1.17
	p value									**n.c.**	0.80
1.5 cm beam	A	0.00	**3.17**	1.83	0.33	1.00	0.92	0.25	0.08	**0.92**	– 2.25
	B	0.00	**1.33**	1.83	0.33	0.17	0.00	0.08	0.00	**0.00**	– 1.33
	C	0.00	**0.67**	0.33	0.42	0.00	0.00	0.08	0.00	**0.00**	– 0.67
	p value									**n.c.**	0.58
1 cm beam	A	0.00	**5.58**	4.50	1.83	1.08	0.58	0.42	0.00	**0.25**	– 5.33
	B	0.00	**4.33**	3.00	1.92	1.50	0.58	1.17	0,75	**0.83**	– 3.50
	C	0.00	**2.17**	2.08	2.00	0.42	0.33	0.17	0.50	**0.33**	– 1.83
	p value									**0.350**	0.08
1 cm bars	A	0.00	**2.92**	1.83	1.17	3.67	4.17	3.08	2.50	**1.67**	– 1.25
	B	0.00	**3.83**	2.17	2.67	2.58	2.00	2.33	1.42	**2.17**	– 1.67
	C	0.00	**3.67**	3.58	2.17	1.92	2.58	2.08	2.75	**2.33**	– 1.33
	p value									**0.823**	0.95
0.8 cm bars	A	0.00	**2.75**	1.58	2.17	3.17	4.58	3.67	2.75	**1.25**	– 1.50
	B	0.00	**3.75**	3.25	2.25	2.33	2.33	1.50	1.58	**1.42**	– 2.33
	C	0.00	**3.08**	2.92	2.58	2.75	2.33	2.25	2.42	**2.08**	– 1.00
	p value									**0.825**	0.56

n.c. (not calculated)

Slips (Figs. 3, 4)

Only very few slip mistakes were recorded on the 3 cm beam, and they had been fully eliminated by the end of W5. On the next two beams (2, 1.5 cm) rats of all groups also made progress concerning their slip mistakes. On the narrowest beam (1 cm) groups B and C exhibited fluctuating amounts of slip mistakes while animals in group A actually showed an increase in their amount of slip mistakes. On the parallel bars an increase in slip mistakes was recorded in groups both B and C; here animals of group A exhibited fluctuating amounts of slip mistakes.

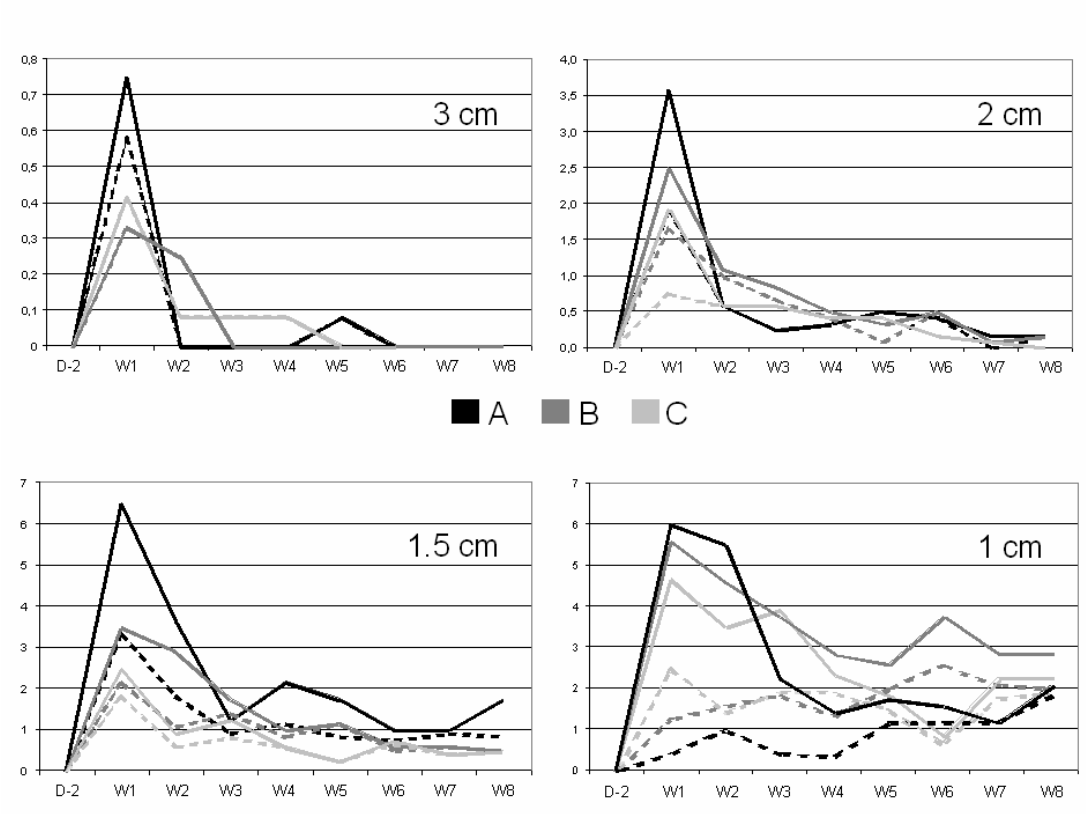

Fig. 4: Total and slip mistakes on the beams of different widths.
Total mistakes are denoted by continuous lines, slip mistakes by broken lines. Note the different scales for the mistakes.

Statistically, no group consistently exhibited less slip mistakes when compared to other groups at W8 ($p>0.05$. Welch-Test) and only group B made significant progress on the 2 cm ($p=0.00$) and 1.5 cm ($p=0.042$) beams over time in this category.

3.2. SFI and SSI (Table 3)

The results of both SFI and SSI evaluations were equal, with group A finishing best in both evaluations. Differences between groups, however, showed up more clearly in the SSI. In SSI, but not in SFI evaluations, a statistically significant difference could be noted between the three experimental groups in W8 (p=0.035) and also in the deduction of scores of W8 from W1 (p=0.033). Group A was the only group to make statistically significant progress between W1 and W8 (SFI: p=0.044; SSI: p=0.018; paired *t*-Test); the most dramatic change in the calculated values could be observed from W5 onwards. Neither group B nor group C exhibited any progress at all ($p>0.05$, paired *t*-Test), but as group C posted better values in SFI and SSI from W1, this group also finished significantly better than group B. This could clearly be shown in statistical analysis, however once again only in SSI scores (p=0.040).

Table 3: SFI and SSI scores

	Group	D -2	**W1**	W2	W3	W4	W5	W6	W7	**W8**	W8-W1
SFI	A	−9.31	**–101.5**	−97.71	−98.42	−94.12	−88.39	−85.77	−82.42	**–85.12**	+16.42
	B	−7.75	**–95.77**	−94.83	−94.70	−94.74	−96.71	−100.9	−101.1	**–97.43**	−1.66
	C	−13.54	**–81.39**	−99.05	−98.10	−98.83	−99.42	−99.29	−98.83	**–98.17**	+0.18
	p value	0.224								**0.109**	0.086
SSI	A	−5.70	**–93.67**	−93.81	−97.35	−96.98	−87.50	−74.22	−60.20	**–57.72**	+35.95
	B	−3.11	**–86.45**	−82.14	−81.87	−82.40	−85.07	−85.93	−85.52	**–85.65**	+0.81
	C	−5.19	**–73.86**	−76.00	−77.56	−81.43	−77.41	−77.46	−76.64	**–75.48**	−1.62
	p value	0.750								**0.035**	0.033

3.3. Morphometric and electron microscopic assessments (Table 4)

The mean number of total fibres at midlevel of the interponate was largest for group A, followed by group B. Four of the six rats in group C exhibited no regenerating myelinated fibres at all, which contributed to the very low number of mean total fibres in this group.

Statistically, a significant difference could be noted between the total fibre numbers for the three groups in the operated hind limb ($p<0.001$), with all groups exhibiting a significantly different amount of fibres when compared with one another ($p<0.05$). On the contralateral side no statistically significance between the total fibre numbers of the different groups was evident (p=0.0594).

Table 4: Morphometric assessments and gastrocnemius volumes [ml]

	Operated hind limb		Contralateral hind limb	
Group	Mean fibre number	Muscle volume	Mean fibre number	Muscle volume
A	11468	0.78	7485	2.27
B	3106	0.43	8535	2.25
C	151	0.55	7966	2.25
p value	0.0001	0.005	0.059	0.982

Electron microscopic assessments confirmed the impressions gained in morphometric evaluations. Those rats exhibiting countable myelinated fibres also showed regrowth of comparable numbers of unmyelinated fibres. In segments not containing any myelinated fibres at all, no evidence of the occurrence of unmyelinated fibres could be detected either.

3.4. Volumes of the gastrocnemius muscles (Table 4)

The figures for the combined volumes of the lateral and medial heads of the gastrocnemius muscle extracted from the contralateral, unoperated hind limb were the same for all groups (p=0.982). Volumes of muscles from the operated hind limb were largest for group A, followed by group C. The muscles from animals of group B exhibited the lowest volumes.

Statistically, a significant difference in mean volumes for the left gastrocnemius muscles could be shown between groups A and B (p=0.001) and A and C (p=0.010). No significant difference could be noted between volumes of groups B and C (p=0.057)

3.5. General health

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. One animal in group B developed a chronic ulceration on its heel in week 2, which did not regress throughout testing, but also did not provide any hindrance to activity. In clinical examinations this rat also appeared to be normal.

None of the rats displayed any signs of autotomy.

4. Discussion

In this study results of traversing times and mistakes on different sizes of beams and parallel bars were compared to the conventional SFI and SSI evaluations after double transsectioning of the sciatic nerve and repair of the subsequent gap by guidance interponation. The reason for doing so was that it has been suggested that both SFI and SSI evaluate only the motor aspect of regeneration; in contrast, gait in its every aspect, especially in more difficult and challenging conditions such as traversing beams of decreasing widths and parallel bars, requires extensive hindlimb coordination [12,13,24]. Gait was assessed by measuring speed and determining numbers of obvious stepping mistakes. Speed, obviously, is most dependent on motor function, especially of the thigh musculature in combination with the calf musculature, the latter being responsible for propulsion [18]. As opposed to this, foot mistakes could originate both from sensory or motor inabilities. This was differentiated by assessing whether the foot was primarily placed incorrectly (next to the bar or beam), i.e. there was no hind limb coordination, defined as a primary sensory inability with a motor component due to aberrant innervation, or whether the affected hind limb was placed correctly but could not grasp the beam or bar and therefore slipped off due to motor inabilities.
The results of this study indicate that, depending on the balancing device applied, particular attention could be paid to either one of the regenerational aspects (motor or sensory), although cortical integration is assumed to have played a major part in all assessments.

4.1. Traversing times

When getting rats to traverse a wide (8 cm) elevated runway, only crude motor function is assessed [12,13]. This axiom also applied to this study. Animals exhibiting the highest motor regeneration rates as regards SFI and SSI examinations and finishing the study with the largest gastrocnemius volumes (group A), also traversed the 80 cm long runway the fastest, and made linear progress during the eight weeks of evaluation. This makes sense when considering speed is positively linked with propulsion and the muscles in the affected lower hind limb responsible for this action are mostly the gastrocnemius muscles [18]. Interestingly however, even though severe atrophy that could be observed in these and other muscles of the conduit groups (B and C), the sprint times of rats in these

two groups were not much slower than those of animals in group A after eight weeks of regeneration. Furthermore, on all narrower balancing devices and the parallel bars, rats in groups B and C exhibited fastest balancing times when comparing absolute times (i.e. disregarding reference values) for traversing the distance. Although one would assume that mistakes (footfaults), regardless of whether they are of primarily motor or sensory origin, would have an impact on traversing times, this was not the case in any of the groups or on any of the balancing devices. An explanation for this might be that the rats were very aware of the “unreliability” of their operated hind limbs due to their high degree of activity and therefore placed less weight on this limb. This would mean that a misplacement or a slip of the hind limb would not result in a rat losing its balance.

4.2. Mistakes

Results showed that the gripping ability was equally poor in all groups. The number of slip mistakes was the same in all groups, comprising approximately 70% of total mistakes, which implies that different muscles or muscle groups (the intrinsic foot muscles) compared to those used for sprints and propulsion (gastrocnemius) and spreading of the feet (toe extensors [26,32]) must be activated for this kind of function, and that these were not innervated sufficiently or not at all in any of the examined groups by the end of the study. Analysis of the number of misplacement mistakes however reveals quite clearly (though this is not significant statistically speaking) that group A showed the best regenerational results by exhibiting the least number of misplacement mistakes on both sets of parallel bars and also the narrowest beam (1 cm). This result is not surprising, considering that group A easily exhibited the largest number of regenerating myelinated fibres in morphometric assessments of the interponate at its midlevel.

Interestingly though, animals in group A went through a patch of an increased misplacements during W4 and W5, setting them back behind the other groups. It could be speculated that these periods of extra mistakes (which in this case did occasionally coincide with higher traversing times) could be due to irregular and/or aberrant innervation both of muscles and sensory end organs, as these periods coincided with the times where first real progress in motor recovery in this group, as indicated by SFI and SSI evaluations, could be observed.

4.3. Overall results

Taking together the results in SFI, SSI, morphometric assessments and muscle volumes, animals in group A predictably finished best in those ambulatory tests which focussed primarily either on motor or sensory aspects. However, one also has to be aware of the fact that rats in groups B and C fared very well in the ambulatory tests, often even showing better results in the velocity tests and the placement of hind limbs on the wider beams. This was the case even though rats in these groups exhibited no return of motor function in SFI and SSI tests, a finding that could be confirmed by examination of the highly atrophied muscles of the lower limbs, especially of the calves. Morphometric assessments of the sciatic nerve also showed that these rats had no or only moderate numbers of regenerating myelinated axons.

4.4. Source of progress

With no or only very little regeneration able to be confirmed in morphometric assessments, it is safe to conclude that there was also no or next to no sensory input and motor output from and to regions below the knee in animals in groups B and C. Nociception returned in nearly all areas of the foot in these rats and has to be attributed primarily to extensive sprouting of the saphenous nerve (paper in preparation). Therefore one has to assume that all the progress made in the recovery of integrated locomotor function, discernible when comparing balancing times and amount of mistakes in W1 and W8, must be attributable to plasticity at the level of the cortex or at subcortical levels, such as the dorsal root ganglia and the spinal cord [3,7,22,25]. A similar scenario has been described only recently by Moon et al. [31]. Here rats recovered some motor function in their hind limbs after complete spinal cord transection, whilst no evidence for regeneration could be seen in histological assessments of the spinal cord. Motor enriched housing preserved and even further promoted motor recovery when compared to average basic housing.

4.5. Fundamentals of plasticity

Somatosensory system

Multiple studies have shown that representational maps in the mature primary somatosensory cortex are dynamic. They can be modulated to suit the use in

question [3] and changes are reversible [15]. Cortical changes can be observed both after changes of sensory input due to activity, behaviour and skill acquisition and after deafferentation following peripheral nerve injury [3,15,21,25]. Sciatic transsection and ligation, for example, can induce a very rapid change in the S-I hindpaw representational area, of which 85% is normally predominantly activated by the sciatic nerve and 15% by the saphenous nerve. Within one to three days after injury the area from the saphenous skin territory assumes an additional 23% to 26% of the total hind paw cortex and ends after seven to eight months of cumulative cortical reorganization in four stages with a representational area for the saphenous nerve, which does not significantly differ from the representational area of the total hindpaw [9]. Similar changes can be observed in the dorsal horn after the same kind of lesion. After chronic sciatic denervation due to sectioning and ligation, cells with saphenous receptive fields take over the former sciatic territory [27]. If both the saphenous and sciatic nerves are sectioned and ligated, neurons in the dorsal horn, which used to have receptive fields on the lower leg, within four to five days become sensitive when neighbouring intact areas, such as the medial thigh, perineum and lower back are stimulated [38]. These changes to the organisation of cortex and subcortical structures may however occur within minutes [15,25]. During epidural nerve blocks, for example, neurons originally responsible for the newly anaesthetised area responded to neighbouring, non-anaesthetised territories [7].

Motor system

In the motor cortex, in contrast to the somatosensory cortex, there is no somatotopically ordered representational map. Instead there are subregions, which function rather like a web interacting to trigger certain movements. It therefore seems that sites responsible for individual parts of the body are widely distributed and overlapping [37]. Loss of connection to the end organ and changes to the sensory input can alter the motor cortex [14,15,36], which similarly to the sensory cortex is remodelled throughout life [33]. In 1990 Sanes and his colleagues [36] showed that after nerve injury, the affected areas in the motor cortex exhibited an enlargement of adjacent areas, so that these became associated with new muscle groups. In this area the newly represented muscles required lower thresholds for activation. Changes to the target area of the motor cortex therefore mean that more attention can be paid by the cortex to non-affected muscles. This means that motor control for the target muscle could possibly be adjusted more finely [14].

Furthermore, increased output maps of the relevant muscles have been described to go hand in hand with faster reaction times for motor tasks [33].

Induction of plasticity

For cortical representation to be adjusted, it is extremely important that the subject in question possesses the appropriate behavioural stance. A high level of motivation, backed up by positive reinforcement is essential if functional sensibility is to be regained; in other words stimuli must be specific and closely associated with the desired functional result [25]. The same paradigm seems to be valid for motor recovery [41] and the early phase of nerve regeneration [42]. Neurotrophin levels and their receptors, for example, are activity-dependent and can influence sprouting in the rat spinal cord after injury [1].

Bearing the aforementioned arguments in mind, it was decided to provide the rats with the opportunity for daily physiotherapy in which the rats could participate according to their individual ability and motivation, instead of forcing them to swim for example. Voluntary movement was encouraged by exploiting the rats' natural curiosity by letting them explore foreign objects and recover healthy treats hidden in different corners of the large cage provided [31].

Mechanisms

Recovery to the original state can be achieved within 6 hours after 14 days of hypodynamia-hypokinesia of the hind limb [15]. This speedy return to the initial state indicates that original connection patterns persist and can be readopted simply by losing hold of a new map [22]. Two main mechanisms have been proposed to explain reorganisation on the various cortical and subcortical levels after a peripheral lesion. The first, resulting in short-term changes, consists of the unmasking of already present, but functionally inactive connections [15]. There are multiple factors which could produce this result, such as increased excitatory neurotransmission, upregulation of postsynaptic receptors, changes in membrane conductance, decreased inhibitory inputs or the removal of inhibition from excitatory inputs [7,9,20]. Ultimately, however, the underlying anatomical features define the extent to which short-term plasticity can take place [4]. The second main mechanism, resulting in long-term plasticity, requires either NMDA receptor activation [3,4], which results in long-term-potentiation and/or long-term-depression, or axonal sprouting [1] and synaptogenesis featuring alterations in sizes and types [7].

5. Conclusion

Functional compensation mediated by plasticity at a cortical and/or subcortical level plays an impressive role after sciatic sectioning and repair, especially in animals which experience only moderate or no neural regeneration at all, but are provided with extensive physiotherapy facilities on a voluntary basis. Assessments in the form of gait analyses on balancing devices with these animals might give the false impression that the affected anatomical structures have undergone extensive regeneration. This makes it imperative to apply multiple tests for evaluation concerned with different aspects of regeneration and to conduct morphometric assessments of affected nerves as close to the end organ as possible [6] in order to minimise misinterpretation of functional results. When applying additional tests primarily concerned with motor function, the SSI seems to be slightly more sensitive to minimal changes than SFI assessments.

The testing of ambulatory abilities on different balancing devices is not recommended for distinguishing the degree of regeneration achieved by different experimental groups after sciatic sectioning and repair. However, in order to assess compensational capacity after neural lesions this method of evaluation is reliable, cheap and feasible after very little training, and also is able to provide a focus on some of the different aspects of gait, namely sensory input or motor output, depending on the balancing device applied.

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

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Abstract

Functional evaluations in peripheral nerve regeneration studies in the rat are often complemented by electroneurographic assessment of the regenerating nerve.

In the first part of this study, which involved evaluating different interponates for bridging large peripheral nerve gaps, striking discrepancies between morphometric and electroneurographic examinations of the regenerating sciatic nerve were observed.

To shed light on this dichotomy, seven rats were subjected to permanent sciatic nerve transection in the second part of this study, and assessed electrophysiologically, histologically and by retrograde axonal tracing at different postoperative intervals (1 hour to 8 weeks).

The results of the histological examinations and retrograde tracing revealed that inspite 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 most probably 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 et al., 1989; Shenaq et al., 1989; Kanaya et al., 1996; Hadlock et al., 1999; Howard et al., 2000; Nichols et al., 2005); 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, 1985).

Recently, the reverse situation, i.e. almost full return of sensory function without any histological evidence of axonal regeneration, was observed after double transection and repair of the sciatic nerve, and this was primarily attributed to extensive sprouting of the saphenous nerve (Rupp et al., 2006a). In the same study, which had been carried out to assess the ability of biocompatible interponates to sustain neural regeneration across a 14 mm gap in the rat sciatic nerve, we came across striking discrepancies between electrophysiologic and morphometric examinations. After eight weeks of regeneration, statistically indistinguishable motor nerve conduction velocities (NCVs) were recorded in all rats, even though morphometric examination at mid-interponate level revealed that, whilst some rats experienced abundant regeneration, others showed none at all. Furthermore, the compound muscle action potentials (CMAPs), recorded from the gastrocnemius muscle (GM) after direct stimulation of the sciatic nerve proximal and distal to the lesion, exhibited a similar morphology in all rats. No correlation could be found between the number of myelinated fibres and the NCVs for the individual animals.

To shed light on the inconsistencies observed between morphometric and electrophysiologic assessments in the first part of this study, seven further rats were subjected to extraction of a 14 mm segment of 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. Evaluation after bridging the nerve defect (Part 1 of the study)

2.1.1. Study design

Twenty male adult Lewis rats (Charles River Laboratories, Germany; 320 to 340 g) were subjected to extraction of a 14 mm segment from the sciatic nerve and repair by autograft (group A; n=8), empty collagen tubes (group B; n=6), or collagen tubes filled with denatured autologous muscle (group C; n=6), according to a protocoll introduced by Meek and colleagues (1999).

The methods of postoperative evaluation included determination of the nerve conduction velocity (NCV) across the surgical site, and histological examination of the regenerating sciatic nerve eight weeks after the surgical procedures had taken place.

Lewis rats were chosen on account of their proven resistance to autotomy after sciatic lesions (Inbal et al., 1980, Panerai et al., 1987, Carr et al., 1992), and on account of their sciatic nerves being particularly suitable from an anatomical point of view (Rupp et al., 2006b).

2.1.2. Surgical procedure

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. A 14 mm segment was then excised from the sciatic nerve and the resulting gap was subsequently bridged by one of the three above mentioned interponates. 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.

Following the implantation of the interponate, 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.].

For postoperative analgesia, the rats received metamizol (200 mg/kg p.o.; corresponds to 3 drops of Novalgin®; Ratiopharm, Germany) upon waking up and

buprenorphine (50 µg/kg s.c.; Temgesic®; Essex Pharma, Germany) every 12 hours for three days.

2.1.3. Electrophysiologic examinations

Eight weeks after surgery the severed sciatic nerve was re-exposed in order to determine nerve conduction velocities (NCVs) across the surgical site. This was done by applying stimulation directly to the regenerating sciatic nerve proximal and distal to the lesion, and recording compound muscle action potentials (CMAPs) from the interosseus or gastrocnemius muscles. The contralateral hind limb served as control.

The rats were placed under the same general anaesthesia as for the previous surgical procedure, 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. The lateral insertion of the biceps femoris muscle, however, was left untouched. Care was taken to ensure that the rats' core temperature was maintained at between 36°C and 38°C.

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 sites lay proximal and distal to the interponate. On the unoperated, contralateral hind limb they were at mid-thigh level and at the medial malleolus.

The recording electrodes (monopolar needle electrodes) were placed subcutaneously on the plantar side of the foot, with the cathode over the interosseus muscles and the anode on the plantar side of the third toe. The ground electrode was inserted subcutaneously on the lateral side of metatarsus V. Additionally, the recording electrodes were placed further proximal in the operated hind limb. In this setting the cathode lay within the GM and the anode was placed subcutaneously over the GM. The ground electrode was positioned subcutaneously between the stimulating and recording electrodes, with the tip pointing towards the centre of the body.

All stimulations and recordings were completed with a Viking Quest electrodiagnostic unit and associated software (Viasys Healthcare; Germany). One Hz single pulses with a duration of 0.2 ms were delivered with increasing intensities until a potential could be recorded from the respective muscles. 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 and, subsequently, the NCVs were calculated from these values.

2.1.4. Morphometric studies

After completion of the electrophysiologic examinations the rats were euthanised by a lethal dose of intraperitoneal pentobarbitone. The sciatic nerve was harvested from both hind legs from its most proximal accessible point near the major trochanter along with its tibial branch to the middle of the plantar side of the foot. Whole trunk samples containing the area at the mid-interponate level and the area at the same level of the sciatic nerve on the contralateral side were stretched on a piece of paper and immersed in 2.5% glutaraldehyde in Soerensen's phosphate buffer (pH 7.4) for 1 hour. 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 hours at room temperature, repeated buffer rinses and a graded alcohol series before being embedded in epoxy resin. For morphometric evaluations semithin sections (0.5 µm) were mounted on triethoxysilane-coated slides and stained with p-phenylene diamine.

Morphometry was performed on cross-sections of the tibial nerve 0.5 cm distal to the calcaneus. Photographs were taken of these sections with a Zeiss Axiovert 100® light microscope equipped with a PLANAPO oil immersion objective (100x, n.A. 1.25), a CCD camera and a motorised stage. Pictures were assembled semi-automatically and picture processing was performed by the MT_O_P (Research System Inc., Boulder, CO, USA) software programme.

The morphometric parameters comprised total fibre counts (TFCs) and the fibre distribution.

2.1.5. Statistics

Electrophysiologic and morphometric data were assessed by the SAS system. The ANOVA procedure was chosen to determine whether there were any significant differences ($p<0.05$) between the NCVs and the TFCs of groups A, B and C in both the operated and contralateral side.

2.2. Determination of the source of electrical activity (Part 2 of the study)

2.2.1. Study design (Table I)

Seven male Lewis rats (Charles River Laboratories, Germany; 310 to 320g) 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.

Rats 2-1 and 2-2 were examined and euthanised on the same day as their operation. Rats 2-2-3, 2-4 and 2-5 were examined and euthanised one, three and five weeks, respectively, after the operation, and rats 2-6 and 2-7 were examined and euthanised eight weeks post-operatively.

Table I: Study design and tracer application in Part 2

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

2.2.2. Surgical procedures

In all rats the left sciatic nerve was exposed by the same approach as in Part 1, and the location and length of the segment excised from the sciatic nerve were also identical to Part 1. In direct contrast to the first part of this study, however, the proximal stump was provided with a firm ligature 1 mm proximal to its distal end in order to prevent regeneration. After that the biceps femoris muscle was sutured back into place. Type of anaesthesia and analgesia were the same as in Part 1.

2.2.3. Electrophysiologic examinations

Anaesthesia, equipment, approach, placement of electrodes, and perioperative management were the same as in Part 1. Five additional measures (A-E) were taken, however, in order to ascertain the source of electrical activity recorded from the GM of the operated hind limb in the first part of this study:

A: both monopolar and bipolar 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 (Gassel, 1964; Cuddon, 2002; Scholle et al., 2005).

B: the following four stimulation points (Fig.1) were added to the two original 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, 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 (Puidellívol-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.

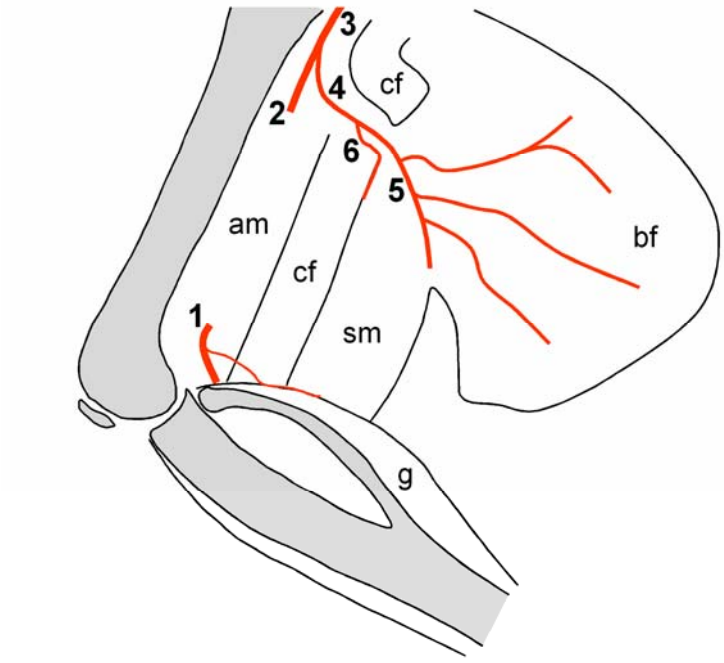

Fig. 1: Stimulation points in electrophysiologic examinations

Distal stump of the sciatic nerve (1), proximal stump (2), most proximal visible point of the sciatic nerve (3), musculocutaneous nerve of the hind limb (4, 5), first branch of the musculocutaneous nerve (6);

landmarks: biceps femoris muscle (bf), semimembranosus muscle (sm), adductor magnis muscle (am), caudofemoralis muscle (cf), gastrocnemius muscle (g).

C: 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 the results achieved in the first part of this study.
D: both insertion areas of the biceps femoris muscle were detached in three steps. In Step 1 only the aponeurosis between the biceps femoris muscle and the superficial gluteal muscle was detached in order to determine the location of the proximal and distal stumps of the sciatic nerve. 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.
E: the musculocutaneous branch of the sciatic nerve was transected working from distal towards proximal in order to locate the source of electrical activity more precisely.

2.2.4. Retrograde tracing studies

Eight days before euthanasia the GMs in all rats were injected with retrograde tracers Fast blue (Polysciences) and Fluorogold (Biotium). Rats 2-1, 2-3, 2-4, 2-5 and 2-6 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 2-2 and 2-7 the application sides were swapped (Table I).
Immediately after the electrophysiologic examinations, the rats were perfused transcardially with 2 ml of 2% lidocainhydrochloride (beta-pharm, Germany), followed by 100 ml of 0.1 M phosphate buffered saline (PBS; pH 7.4) and 250 ml 4% paraformaldehyde in 0.1 M PBS. The spinal cord segments L1 to S1 were removed according to Gelderd and Chopin (1977), postfixed in the same fixative for 6 hours and then transferred into 15% sucrose in PB for 15 hours. 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 a LP 420 nm barrier filter.

2.2.5. 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.

2.2.6. 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 2-3 to 2-7 and prepared for histological evaluations as described for Part 1. For evaluation, the semithin sections were stained with azur II-methylenblue-safranin.

2.2.7. Muscle volumes

After perfusion the GMs of both sides were extracted in rats 2-3 to 2-7 and the combined volumes of 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.

2.3. 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 hour light cycles, and had free access to standard rat food and water. Additionally, they experienced four to six hours of ‘playtime’ daily in a 45 cm x 55 cm x 120 cm cage in groups of 8 to 12 individuals. Animal studies were approved by the local animal care committee.

3. Results

3.1. Evaluation after bridging the nerve defect (Part 1)

3.1.1. Electrophysiologic assessments (Table II)

No CMAPs could be recorded in the interosseus muscles of the operated hind limbs in any of the rats. In the GM of the operated hind limb, however, CMAPs could be recorded in all the rats. The waveforms varied from virtually normal biphasic CMAPs (chiefly after stimulation of the distal stump) to polyphasic CMAPs with long durations (chiefly after stimulation of the proximal stump). The mean latency for CMAPs recorded from the GM after stimulation proximal to the gap was 1.22 ± 0.46 ms, for stimulation distal to the gap 0.39 ± 0.39 ms. The mean stimulation intensity required to elicit CMAPs after stimulation of the proximal stump was 3.02 mA and 5.8 mA after stimulation of the distal stump.

On the contralateral, unoperated leg all rats exhibited clearly distinct physiological CMAPs after supramaximal stimulation with an intensity of 1.9 mA. The mean latency for recordings from the plantar interosseus muscles after stimulation at midthigh level was 1.98 ± 0.21 ms, after stimulation at the medial tarsus the mean latency was 1.09 ± 0.18 ms.

Statistically, no significant differences in NCVs could be noted between the surgical groups either on the operated side ($p=0.41$) or the contralateral side ($p=0.38$).

3.1.2. Morphometric assessments (Table II)

The TFCs determined for the operated hind limb were found to differ widely in the rats examined. This resulted in a statistically significant difference between the groups for fibre counts ($p<0.0001$). On the contralateral side, however, no significant differences could be noted between the groups ($p=0.55$).

The distribution of myelinated fibre calibres varied considerably between the operated and the contralateral sides. On the healthy side the myelinated fibres reached diameters of up to 12 µm. Two peaks were notable in their distribution, namely a higher one at an approximate diameter of 7.0 to 7.5 µm and a smaller one at 3.5 to 4.0 µm.

On the operated side the largest fibres throughout all experimental groups reached a maximum diameter of 7 µm. The majority of fibres, however, had a diameter of 2.5 µm.

Table II: Electrophysiologic and morphometric results achieved in Part 1

Rat	Group		Operated hind limb		Contralateral hind limb	
			NCV [m/s]	TFC	NCV [m/s]	TFC
1-1	A		43.0	10253	48.0	8193
1-2	A		41.0	13480	60.0	7614
1-3	A		33.0	12733	51.0	6440
1-4	A		33.0	7962	59.0	7386
1-5	A		46.0	11255	56.0	7629
1-6	A		27.0	10552	59.0	7808
1-7	A		31.0	12828	71.0	8031
1-8	A		33.0	14079	66.0	8746
1-9	B		32.0	6288	60.0	9010
1-10	B		26.0	2928	47.0	8351
1-11	B		37.0	1582	54.0	8698
1-12	B		27.0	2565	45.0	8228
1-13	B		30.0	2169	57.0	7990
1-14	B		39.0	2621	55.0	6961
1-15	C		33.0	0	53.0	8766
1-16	C		34.0	0	55.0	8076
1-17	C		36.0	885	61.0	7305
1-18	C		27.0	0	52.0	6308
1-19	C		34.0	0	51.0	8510
1-20	C		38.0	21	51.0	8829
	A		35.88 ± 6.62	11643 ± 2026	58.75 ± 7.44	7731 ± 671
	B		31.83 ± 5.27	3026 ± 1664	53.00 ± 5.83	8206 ± 708
	C		33.67 ± 3.72	151 ± 360	53.83 ± 3.83	7966 ± 988
	TOTAL		34.00 ± 5.49	5610 ± 5396	55.55 ± 6.36	7944 ± 772
	p value		0.4100	<0.0001	0.1846	0.5453

3.2. Determination of the source of electrical activity (Part 2)

3.2.1. 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.

3.2.1.1. Operated hind limb: recordings from the interosseus muscles

Within one hour 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 one 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.

3.2.1.2. 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. In week 8 no CMAPs could be elicited in the GM anymore after stimulation with an intensity of 1.9 mA. This was also the case even when the aponeurosis of the biceps femoris muscle on the tibia had been left untouched (Step 1).

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.

b) Proximal Stimulation Points 2 to 5

Identical waveforms resembling CMAPs were recorded in the individual animals regardless of which of the proximal Points 2 to 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)

Stimulation of the first major branch of the musculocutaneous nerve resulted in the CMAPs recorded in the GM being less polyphasic than after stimulation of Points 2, 3, 4 and 5 (Fig.3).

When this branch was cut, the CMAPs elicited at proximal stimulation Points 2, 3, 4 and 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.

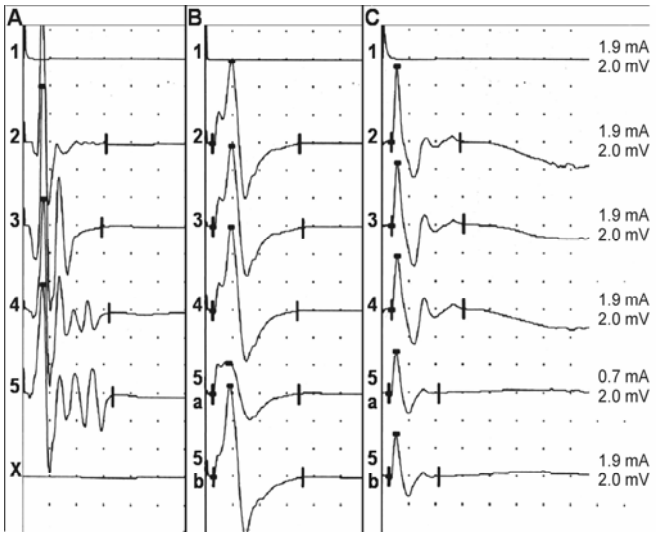

Fig. 2: Stimulation at the proximal stimulation points with gradual dissection of the biceps femoris muscle from its aponeurosis on the tibia (Rat 2-7)

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, 3, 4 and 5.
No CMAPs could be recorded after stimulation of the distal stump (1) with an intensity of 1.9 mA eight 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 cutaneus femoralis caudalis branch of the sciatic nerve.
Recordings A and B: monopolar electrodes; recording C: concentric electrode. Sensitivity: 2 mV.

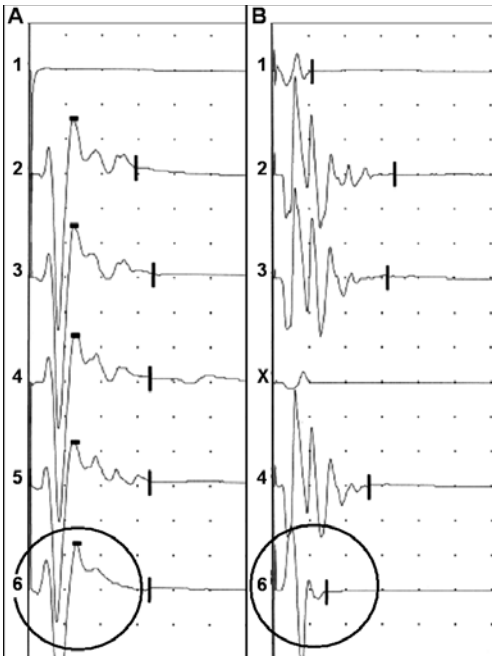

Fig. 3: Stimulation of the first major branch of the musculocutaneous nerve

A: Rat 2-4; Step 2
B: Rat 2-5: 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, 3, 4 and 5.
Note the small CMAP which could be elicited after stimulation of the distal stump with an intensity of 1.9 mA five weeks after surgery. Stimulation Point X was the cutaneus femoralis caudalis branch of the sciatic nerve.
Both recordings conducted with monopolar electrodes. Sensitivity: 2 mV.

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, 3 and 4 than before

the nerve was cut. 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 (even with monopolar electrodes) in the GM after stimulation at Points 2, 3 and 4. Stimulation at Point 6 resulted in clearly recognisable, almost biphasic CMAPs being recorded in the GM (Fig. 4).

Furthermore, transection of the caudofemoralis muscle in the midbelly 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.

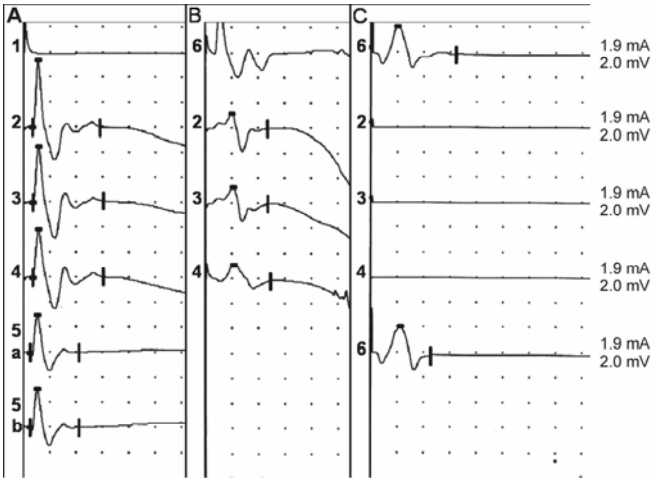

Fig.4: Transection tests after Step 3 (Rat 2-7)

A: Musculocutaneous nerve still intact
B: Transection of the musculocutaneous nerve near Stimuluation 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, 3 and 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 the stimulation to Point (6) still resulted in clearly recognisable CMAPs.
Recordings A and B: concentric electrode; recording C: monopolar electrodes. Sensitivity: 2 mV.

3.2.1.3. 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 recording electrode was placed within the GM, however, polyphasic CMAPs were also recorded on this side.

3.2.2. 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 properly had been correctly injected into either the lateral or the medial head or into both heads of the GM. In rat 2-7, however, some of the injected solution could be found in the biceps femoris muscle of the operated hind limb, the rest in the GM.

In rats 2-1 and 2-2 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 2-3, 2-4, 2-5, 2-6 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 GM.

In rat 2-7 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 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.

3.2.3. 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.

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 loses itself within the muscle (Fig. 5), maybe also providing innervation to the semitendinosus muscle at the same time.

All the later 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 2-5, 2-6 and 2-7 (five and eight weeks of chronic sciatic injury) very fine strands of neural tissue extending from the proximal stump towards the distal stump could be observed in the operated hind limb.

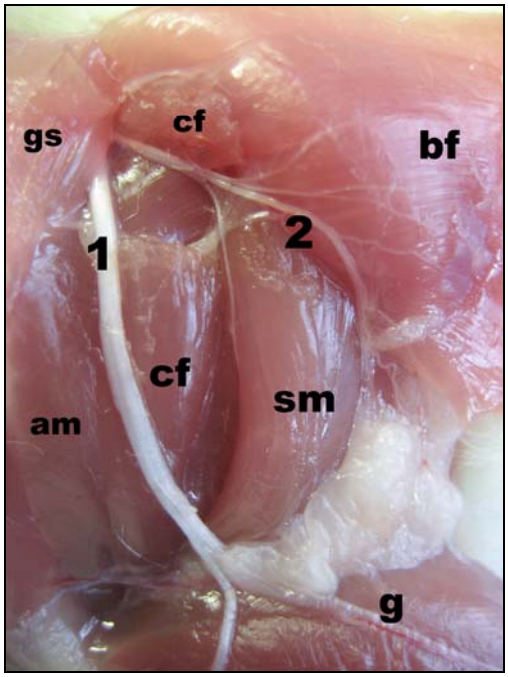

Fig. 5: Anatomical overview of the lateral aspect of the left thigh
The biceps femoralis muscle has been detached in both its insertions and folded toward caudal (Step 3). The caudofemoralis muscle has been transected and the border between caudofemoralis and semimembranosus muscle has been slightly 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), gastrocnemius muscle (g).

3.2.4. Examination of the distal stumps

Microscopic examination of the distal stump of rat 2-3 revealed that within one 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 2-4, three 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 2-5 to 2-7).

3.2.5. Gastrocnemius volumes

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 (Table III).

The gastrocnemius/body weight ratio after eight weeks of denervation reached only approximately 30% (rat 2-6) to 38% (rat 2-7) of the ratio ascertained for one week of denervation, and the ratio for operated to contralateral side decreased to 25% (rat 2-7) or even less (rat 2-6).

Table III: Gastrocnemius volumes in Part 2

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	
2-3		1.2	1.6	3.75	5.00	0.75
2-4		1.0	2.2	2.80	6.16	0.45
2-5		0.6	2.1	1.58	5.53	0.29
2-6		0.45	2.6	1.13	6.55	0.17
2-7		0.6	2.4	1.46	5.83	0.25

3.3. 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 contracture.

4. Discussion

4.1. Disparate results obtained in the electroneurographic and morphometric examinations in Part 1

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

The first is aberrant innervation of the GM by a different nerve (Gassel, 1964), such as one of the proximal branches of the sciatic (or even the tibial or peroneal) nerve, which supply the superficial, medial and deep gluteal muscles, the tensor fasciae latae muscle, the hamstring muscles (semitendinosus muscle, semimembranosus muscle, biceps femoris muscle), the quadratus femoris muscle and the caudofemoralis muscle (Greene, 1955; Hebel and Stromberg, 1976). In particular the musculocutaneous nerve, a minor branch of the sciatic nerve which has recently been described as possessing motor qualities in addition to contributing to the sensory innervation of the digits of rats' hind limbs (Puidellívol-Sánchez et al., 2000), seems a likely candidate.

The second possibility implies technical difficulties such as accidentally recording CMAPs from other muscles of the hind limb. This problem, also referred to as

"cross-talk" (Kuiken et al., 2003), has been reported after stimulation with high intensities and appears to be more prominent when high amplifications are used in recordings (Gassel, 1964). A combination of high intensities with high amplifications is usually required when assessing regenerating or demyelinated nerve fibres (Gassel, 1964; Röder, 1996). 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 by intramuscular and surface electrodes (Gassel, 1964; Gassel and Trojaborg, 1964), and of the knee extensor muscles (Farina et al., 2002; Farina et al., 2004) and lower limb muscles by surface electromyography (Perry et al., 1981). Given that it was possible to record CMAPs from GMs not experiencing any reinnervation (as proven by the morphometric assessments), the muscles in question must lie in relative proximity to the GM and must not be affected by denervation. The hamstring muscles, the gracilis muscle (Hebel and Stomberg, 1976) and also the caudofemoralis muscle (Greene, 1955) all fulfil these criteria. Stimulation of these muscles could occur either directly, as all of them except the gracilis muscle are innervated by a branch of the sciatic nerve and, therefore, are connected to the proximal stump or even indirectly due to spreading of the impulse (Gassel, 1964). A spreading of the impulse would have to be the case for the gracilis muscle, as this muscle is innervated by the obturator nerve (Greene, 1955), and for all other muscles following stimulation of the distal stump.

4.2. Source of electrical activity

Overall, the results of Part 2 of this study strongly suggest that the electrical activity recorded in the chronically denervated GM after stimulation of either the proximal or the distal sciatic nerve stump must be generated by surrounding muscles unaffected by denervation. These are stimulated either directly, or indirectly as a result of spreading of the impulse.

The observation that even in healthy subjects the activity of muscles in close proximity to the recording electrode contributes to the recorded potential has been documented in recordings from hand and foot muscles (Gassel, 1964) and lower limb muscles (Perry et al., 1981) in humans. As mentioned above, the contribution of other muscles is said to become particularly evident when the recordings are highly amplified (Gassel, 1964) and this was not the case in this study. Maybe strong amplification is not necessary for overwhelming neighbouring muscles in

small animals where limb muscles lie in closer proximity to one another than in larger species .
Be that as it may, three results, achieved in the electrophysiologic examinations, in particular lead one to assume that the surrounding muscles are the source of electricity observed in the the operated hind limb in both Part 1 and Part 2 of this study.

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 to 3), principally the biceps femoris muscle (Fig. 2).
The pronounced polyphasia observed in the initial examinations of the GM (Part 1 and Part 2 Step 1) after stimulation of either the proximal stump or other proximally located areas and branches of the sciatic nerve (Stimulation Points 2, 3, 4 and 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 do not in any way suggest that the GM of the operated hind limb in the second part of this study experienced reinnervation. This was also the case in many of the rats in Part 1.
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 both in Part 1 and Part 2 of this study match figures published for physiological values in rats (Ramerman et al., 1968; Sato et al., 1985; Chiu et al, 1988; Wolthers et al, 2005; Arnaoutoglou et al., 2006; Ja'afar et al., 2006) and the sciatic nerve of the contralateral side in the first part of this study did not itself exhibit any pathological features. Total myelinated fibre count and fibre distribution correspond well to values reported in literature (Shenaq et al., 1989; Varejão et al., 2004; Wolthers et al., 2005). 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 in Part 2) 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, 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 of shape in CMAPs ascertained in the second part of this study.

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, 3, 4 and 5 (Fig. 3); transection of this branch resulted in smaller CMAPs or none at all (again after stimulation of proximal Points 2, 3 and 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 this branch of the musculocutanous 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 mid-belly region, therefore, most probably had the effect of also injuring the branch of the musculocutanous 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 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. 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 Part 2, 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 in Part 2. 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 2-7.
Retrograde tracing studies revealed spinal cord segment L5 to be the primary motor pool of the GM. In the control animals (rats 2-1 and 2-2) and on contralateral sides, accumulation of tracer material was seen in the ventral horn neurons of spinal cord segment L5, located at the middle third of bony vertebra L1 (Gelderd and Chopin, 1977). In rat 2-7 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 & Iles, 1983). This suggests that in rat 2-7 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 the control rats 2-1 and 2-2, 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 2-3, 2-4, 2-5 and 2-6. In rat 2-7, 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 & 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 2-7 and this did not affect the action of tracers in any way.

4.3. 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 (Terzis et al., 1976; Dorfman, 1990; English et al., 2006). However, conduction in the distal stump after the application of strong stimulating impulses (mean 5.8 mA), which was ascertained in Part 1 of this study up to at least eight weeks after denervation, is not compatible with the results of the histological examinations of the interponate at its mid-level in many of the rats in Part 1. It was also ascertained that within 3 weeks of chronic denervation no more functional axons could be found in the distal stump (Part 2); moreover, motor endplates have been reported to deteriorate within two weeks of denervation in any case (Ijkema-Paassen et al., 2002). This means that in Part 1 (and most probably also in weeks 3 and 5 of Part 2) 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 (Gassel, 1964; Cuddon, 2002). 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 (both Parts 1 and 2). Transection tests in Part 2 revealed that the 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 (Fig. 5). 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.

4.4. Comparable cases in the literature

In view of the results ascertained in Part 2, it is important to note that it is probably only possible to regard morphometric and electrophysiologic assessments as redundant (Dellon & Mackinnon, 1989) if the electrophysiologic parameters are derived from direct recordings of the nerve under assessment. Recording of CMAPs from the gastrocnemius muscle after stimulation of the sciatic nerve has been applied in various other studies (Archibald et al., 1991; Hou and Zhu, 1998; Rodríguez et al., 2000; Valero-Cabré and Navarro, 2001; Meek et al., 2003; Negredo et al., 2004; Udina et al., 2004; Chen et al., 2005; Martins et al., 2005; English et al., 2006). In most cases the CMAPs were recorded with concentric needles (Archibald et al., 1991; Hou and Zhu, 1998; Martins et al., 2005), implanted electrodes (English et al., 2006) or microelectrodes (Rodríguez et al., 2000; Meek et al., 2003; Udina et al., 2004; Chen et al., 2005). Only microelectrodes probably record electrical activity in very direct proximity, a situation which could not be reproduced in this study even by utilising a concentric

needle electrode. As morphometric assessments were not undertaken at exactly the same time as all the electrophysiologic examinations (Rodríguez et al., 2000; Negredo et al., 2004; Udina et al., 2004), ascertained incomparable parameters (Chen et al., 2005), or were not conducted at all (Archibald et al., 1991; Valero-Cabré and Navarro, 2001; Meek et al., 2003; Martins et al., 2005; English et al., 2006), one cannot draw any conclusions as to whether these groups encountered the same inconsistencies as we did.

Notably, however, the NCVs calculated for all operated hind limbs in Part 1, correlated well with NCVs ascertained from regenerating sciatic nerves in other studies, even though in these the NCVs had been calculated from the latencies of nerve action potentials (Chen et al., 1995, Francel et al., 2003) or from CMAPs recorded from the plantar muscles (Chiu et al., 1988; Wolthers et al., 2005). The study by Francel and colleagues (2003) was the only one that applied comparable methods for morphometric assessments. The results revealed that those of their rats which had corresponding NCVs to ours, exhibited over 2.5 times more myelinated fibres at the mid-interponate level than the rats with the highest fibre density in Part 1. For the other three studies no direct comparison between the morphometric findings was possible, since either different sample areas were used (Chen et al., 1995; Wolters et al., 2005), or no morphometric evaluations (only histological assessments) were conducted at all (Chiu et al., 1988).

What did emerge clearly from the Part 1, however, was that the morphometric assessments were the only evaluation which permitted an accurate distinction between the different types of surgical procedures and indicated that the electrophysiologic assessments were incorrect.

4.5. Implications and areas requiring further research

Since rats in group A (Part 1) experienced extensive regeneration but were not distinguishable from the other groups in the electrophysiologic assessments, it becomes important to know how many nerve fibres are actually necessary to conduct and elicit a CMAP.

It has been estimated that in primates the presence of 4,000 to 5,000 myelinated axons > 5 µm is necessary to record a nerve action potential (comment by Kline to Oberle et al., 1997). Assuming that a similar number of fibres is required to elicit a recordable CMAP after direct stimulation of the corresponding nerve, not even the morphometric results of animals in group A of the first part of this study can fulfil

the requirements, as here the vast majority of nerve fibres were approximately only 2.5 µm in diameter. This diameter corresponds to NCVs of less than 20 m/s (Gasser and Grundfest, 1939; Hursh, 1939). Very few fibres reached a diameter of 7 µm (between eight and 37 fibres per rat, while one rat exhibited 332 fibres), which is the diameter approximately required to conduct at the speeds calculated for the operated hind limb in Part 1 (Gasser and Grundfest, 1939; Hursh, 1939). It has to be concluded that the results of the NCV studies for rats of group A, which on the whole experienced extensive regeneration, are (like groups B and C) primarily attributable to electrical activity generated in muscles which do not feature in the examination.

Further investigations of the exact requirements for conducting and eliciting CMAPs after stimulation of the corresponding nerve are, however, necessary.

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. 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.

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7. Discussion

7.1 Choice of animal model and specifically of rat strain

A wide variety of factors need to be considered when planning a peripheral nerve regeneration study. As outlined in Sections 2.2.2 and 2.2.3, rats are the preferred animal models in peripheral nerve regeneration studies. The choice of strain is important and will depend on the properties required in the rat since certain parameters, e.g. size, temperament, susceptibility to autotomy or even immunodeficiency, are strain-related (see Section 2.2.4.2).
Once the strain has been selected, a decision will have to be reached as regards age, weight/size and gender.

7.1.1 Anatomical investigations (Paper 1)

In the preliminary anatomical investigations the four rat strains most commonly featured in peripheral nerve regeneration studies (Wistar Han, Sprague Dawley, Lewis and Fischer 344), and a strain of nude t-cell deficient rats (amounting to a total of 210 adult rats) were examined regarding their anatomical suitability for peripheral nerve regeneration studies featuring the sciatic nerve (Fig.11). Anatomical suitability was defined as the amount of accessible undivided sciatic nerve, i.e. the length of the sciatic nerve before it branches into its two successors, the tibial and peroneal nerves. Additionally, the length of accessible sciatic nerve after it has divided into its two successors, but with both still contained in the same epineurium, was ascertained. These two measured sections of the sciatic nerve were not only expressed as percentages of the approximate femur length, but also as absolute lengths in millimetres.

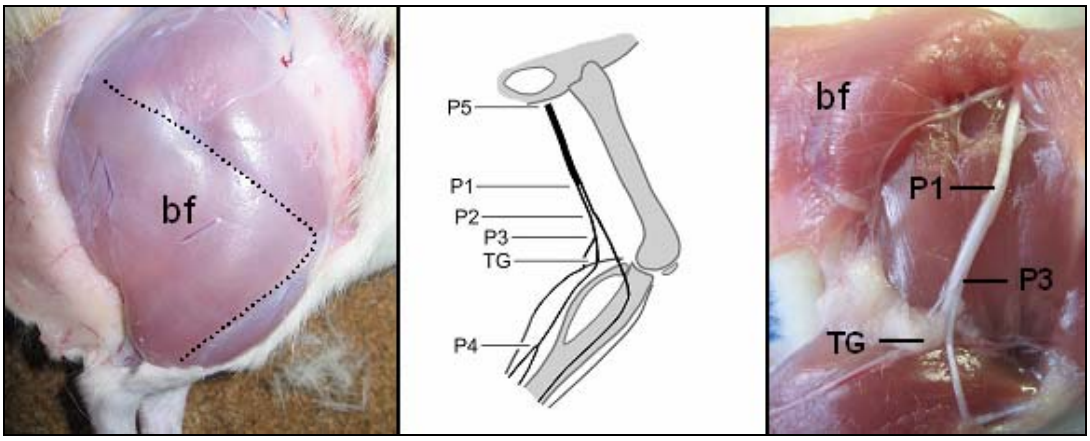

Fig.11 Anatomical overview: skinned right hind limb of a rat with the biceps femoris muscle (bf) still in place, anatomical landmarks assessed in Paper 1 (middle), demonstration of some of the landmarks after the biceps femoris (bf) muscle has been removed (right). Note that P2 cannot be clearly pinpointed from this angle.

7.1.1.1 Population examined

All rats examined were randomly donated as carcasses from various other studies. It can therefore be assumed that the assessments covered a representative cross-section of the adult laboratory rat population. By far the greater proportion of the rats were males (150 males, 60 females) as these feature more widely in research laboratories (personal communications with Charles River Laboratories, US) due to the fact that they are not affected by hormonal fluctuations, which can cause inconsistencies in a study otherwise based on standardised parameters. Additionally, they do not have any obvious negative traits, such as being strikingly more aggressive than females, are easy to keep in groups, and are slightly less expensive to acquire (see catalogue: www.criver.com). The reason why they are a little lower in cost is that rats are generally ordered by weight (in grammes) and as males grow faster than females they reach a suitable weight at a younger age. Another reason why female rats are more expensive is that they are the limiting factor in breeding programmes.

7.1.1.2 Results

The results revealed that the lengths of available sciatic nerve – where this was indeed accessible (see below) – were fairly constant in all strains of rats (Fig.12). It amounted to approximately one third of the length of the femur for stretches of undivided sciatic nerve, and up to nearly half of the femur length for stretches where the tibial and peroneal nerves were already present, but still enclosed by the

same epineurium. The average male adult Wistar Han and Sprague Dawley rats were the two largest strains with the longest femur lengths and the longest feet. Nevertheless it was ascertained that male adult Lewis rats had the greatest relative and absolute amount of sciatic nerve suitable for nerve regeneration studies (both undivided and divided), but only very marginally.

It should, however, be noted that in more than 68% of the hind limbs of the Fischer 344 rats the point at which the sciatic nerve divided into its two successors lay so far proximal that no sciatic nerve per se could be observed when the rat was dissected (Fig.12). This undesirable trait also featured, but to a lesser extent, in the other strains but least of all in the hind limbs of the Lewis rats (3.3%).

The same trends were observed in the female counterparts.

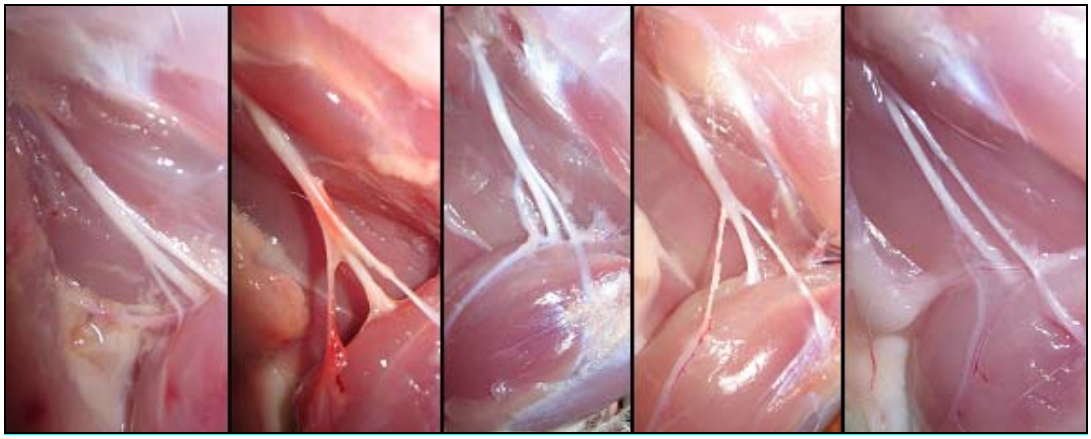

Fig.12 Division and branching of the sciatic nerve in nude, Wistar Han, Lewis, Sprague Dawley and Fischer 344 rats (from left to right).

7.1.1.3 Conclusions and practical implications

The above findings mean that from the anatomical point of view, Lewis rats are slightly more advantageous for peripheral nerve regeneration studies featuring the sciatic nerve, than the other strains examined (Wistar Han, Sprague Dawley, nude rats). The use of Fischer 344 rats is not advisable, for the reason given above.

A further advantage of the Lewis strain is that these rats do not exhibit the slightest inclination towards autotomy (PANERAI et al., 1987; CARR et al., 1992; STRASBERG et al., 1999), which means that they do not subject their denervated hind paws to automutilation (see 2.2.4.2). It should therefore be possible to perform footprint assessments when evaluating the extent of regeneration. The placid and trusting nature of this strain (STRASBERG et al., 1999), which represents a further

great advantage when carrying out evaluations, is the final reason why this strain of rats was chosen for the present regeneration study.

Male rats were selected for the reasons given under Section 7.1.1.1. On the basis of the results of Paper 1 it was calculated that the size or rather the weight of rats to be ordered should be around 300 g, as male Lewis rats of this size should normally have a mean femur length of 33.72 mm. This would mean that the length of undivided sciatic nerve should therefore amount to approximately 12.34 mm; the amount of undivided sciatic nerve plus divided sciatic nerve (with the successors still surrounded by the same epineurial sheath) should amount to approximately 16.37 mm. Considering the animals were to undergo extraction of a 14 mm segment of the sciatic nerve, a weight of 300 g was considered to be sufficient, particularly as the rats were to experience ten days of preoperative acclimatisation and training in which they would also grow and increase their weight by approximately 20 to 30 g.

7.2 Choice of methods for the assessment of sciatic nerve regeneration

Prior to commencement of the peripheral nerve regeneration study featuring the sciatic nerve of the rat (main study), an assessment plan for evaluating the extent of regeneration had to be devised.

7.2.1 Methods applied in comparable studies

Analysis of the 100 most recently published sciatic nerve regeneration studies conducted in rats and featuring methods to bridge a gap (pubmed; keywords: sciatic rat gap regeneration; 385 hits; most recent 100 between 1998 and 2007) revealed that 59% of the studies utilised morphometric assessments to determine the extent of regeneration. If purely qualitative visual evaluations (histology and electron microscopy) of the regenerating sciatic nerve are also included in counts, 88% of the studies examined featured a structural or even ultrastructural qualitative or quantitative assessment of the regenerating nerve (Table 3).

In the 100 most recently published sciatic nerve regeneration studies not solely featuring the bridging of gaps, but also straightforward sectioning and suture or crush injuries (pubmed; keywords; sciatic nerve rat regeneration; 2706 hits; most recent 100 between 2004 and 2007) the percentage of studies that featured assessment of the regenerating nerve under the microscope or electron microscope was roughly the same as above. Morphometric assessments were conducted in 63% of the studies. If purely qualitative assessments of the regenerating nerve under the light microscope or the electron microscope were included in counts, structural evaluations of the regenerating nerve were conducted in 91% of the studies examined (Table 3).

The percentages of other examinations applied were as listed in Table 3 (please note that individual studies might feature more than one type of examination).

7.2.2 Cut-off time

The cut-off time was set at eight weeks after the operation for two reasons. Firstly, studies have shown that if reinnervation is delayed for as little as two weeks, the denervated muscles can no longer regain their full muscle mass even after

Table 3: Assessments featuring in sciatic nerve regeneration studies in rats

	Sciatic gap studies	General sciatic regeneration studies
Somatosensory assessments	12%	8%
Analysis of prints	19%	38%
Electrophysiologic assessments (via CNAPs and CMAPs)	35%	47%
Nerve morphometry	59%	63%
Nerve histology	33%	29%
Nerve electron microscopy	9%	8%
Retrograde tracing	5%	12%
Muscle weight	11%	14%

Source: pubmed

sufficient neurotisation, whereas a delay of 12 months between lesioning and repair does not seem to affect the nerve at all (KOBAYASHI et al., 1997). This means that even if axonal regeneration takes place but only at a very slow rate, there is only a very slim chance that motor function and gait will be fully restored (KEUNE et al., 2006), which is the ultimate goal of nerve regeneration studies (WALKER et al., 1994; MARTINS et al., 2006). Secondly, it has been described that given enough time, differences between positive and negative controls become insignificant (KEUNE et al., 2006) due to the rats' extremely high capacity for regeneration (CARR et al., 1992; STRASBERG et al., 1999) and their short absolute limb length (KEUNE et al., 2006).

7.2.3 Assessment plan

In order to ensure comparability of the results of our study with those of related published studies, it was decided that at least one method of evaluation should be applied for each of the four following groups (explained in detail in Section 2.3):

- reinnervation of the distal stump
- reinnervation of the muscle
- restoration of anatomical features
- restoration of function

Table 4: Assessment plan

	Method	Specific assessment	Time-point
Reinnervation of the distal stump	Electroneurography	Nerve conduction velocity	Week eight
	Nerve morphometry	Total nerve fibre counts, nerve fibre density, nerve fibre distribution and diameters, g-ratio	Week eight
Reinnervation of the muscle	Observation	Use of hind limb during physiotherapy	Daily
	Electromyography	Compound muscle action potentials	Week eight
	Muscle volumes	Gastrocnemius muscle	Week eight
Restoration of anatomical features	Retrograde tracing	Motor pool from the gastrocnemius muscle and sensory reinnervation of the lateral and medial metatarsus	Week eight
	ENG, EMG, nerve morphometry	See above	Week eight
Restoration of function	Observation	See above	Daily
	Motor testing	Sciatic Function Index, Static Sciatic Index	Twice weekly
	Sensory testing	Pain reaction to pinching the feet in various points	Twice weekly
	Ambulatory testing	Time required and mistakes made when crossing elevated beams and parallel bars	Twice weekly

7.3 Additional considerations

Considering the fact that human patients would be treated in a stress-free environment and would receive every chance for physiotherapy and positive motivation after surgical intervention following injury to a peripheral nerve, it was decided to expose the rats to the same conditions. Otherwise it could be argued that the results obtained are distorted and falsely negative.

7.3.1 Physiotherapy and training

Physiotherapy was supplied on both a mandatory and a voluntary basis. The twice-weekly (mandatory) ambulatory assessments trained the rats' motor skills, while voluntary physiotherapy was provided by placing the rats daily for a few hours in a large motor enriched cage. Here they could participate in motor activities according to their individual ability and motivation.

Since all of the methods applied to assess the extent of reacquired function involved extensive handling of the rats, which potentially could result in chronic stress, the rats were familiarized with being handled twice daily for five days as from nine days before the operation (D-9 to D-5) (Fig.13). On these days the rats were also trained and conditioned to cross the elevated beams and parallel bars until they could perform this task in a confident and unhesitant manner and exhibited zero mistakes. Before commencement of the training the rats were allowed an additional four days (D-13 to D-10) to acclimatise and get their bearings in their new surroundings.

This procedure made it possible to ensure that all the reference values obtained two days before the operation (D-2) were derived from data collected in healthy and relaxed rats. A further reason for allowing the rats to acclimatise and for letting them gradually grow accustomed to the handling was to avoid the introduction of chronic stress, which would have resulted in increased plasma corticoid levels. Such changes to the adrenocortical axis have been shown to have a detrimental effect on axonal sprouting and elongation (VAN MEETEREN et al., 1997b; AMAKO & NEMOTO, 1998; VAN MEETEREN et al., 1998) and it was vital to avoid this effect at all costs.

Fig.13 Handling (top left) and **housing** of the rats in the motor enriched cage (right and bottom left).

7.3.2 Housing and voluntary physiotherapy

All rats were housed in groups of four on soft bedding in a temperature-controlled room with 12:12 hour light cycles, and had free access to standard rat food and water. The rats were kept in groups for two reasons. Firstly, rats are social animals which prefer living in groups (Fig.13). Therefore, being kept in groups will have a positive effect on their general well-being. Depriving rats of social contact with other rats will lead to physiological and behavioural abnormalities (MOON et al., 2006). Secondly, it has been shown that rats kept in groups are more active than those housed alone and will therefore exercise more on their own initiative (MEEK et al., 2004; MOON et al., 2006). Neurotrophin levels and their receptors, for example, are activity-dependent and can influence sprouting in the rat spinal cord after injury (BALLERMANN & FOUAD, 2006). The same has been proven for the proliferation and differentiation of oligodendrocyte progenitors and the maturation of oligodendrocytes. Consequently, this also renders myelination of the CNS highly activity-dependent (MC DONALD & BELEGU, 1996). Furthermore, it has been

shown that impoverished environments can even change gait characteristics in intact rats (MOON et al., 2006), and this had to be avoided at all costs.
For voluntary physiotherapy the rats were allowed 4 to 6 hours of 'playtime' daily on weekdays in a 45 cm x 55 cm x 120 cm motor enriched cage in groups of eight to twelve rats. The cage was customised to provide continuous physiotherapy by incorporating four different levels connected by oblique mesh ramps (Fig.13). A hammock-like construction was added, which provided the rats' feet with additional stimulus due to its unsteady nature. Furthermore, the sides of the cage could be and were frequently used as climbing opportunities. Beams, foreign objects and healthy treats (pieces of carrot, cucumber or capsicum) were placed in different parts of the cage at frequent intervals to provide the rats with constant stimuli to explore, climb and balance.

7.3.3 Surgical procedures and postoperative care

In 20 male adult Lewis rats (Charles River Laboratories, Sulzfeld, Germany; 320 to 340 g) 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. A 14 mm segment was then excised from the sciatic nerve and the resulting gap was subsequently bridged by autograft (Group A; n=8), empty collagen tubes (Group B; n=6) or collagen tubes filled with denatured autologous muscle (Group C; n= 6) (Meek et al., 1999a) (Fig.14). 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 gastrocnemius muscle.
Following the implantation of the interponate, 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.].
For postoperative analgesia, the rats received metamizol (200 mg/kg p.o.; corresponds to 3 drops of Novalgin®; Ratiopharm, Germany) upon waking up and

buprenorphine (50 µg/kg s.c.; Temgesic®; Essex Pharma, Germany) every 12 hours for three days.

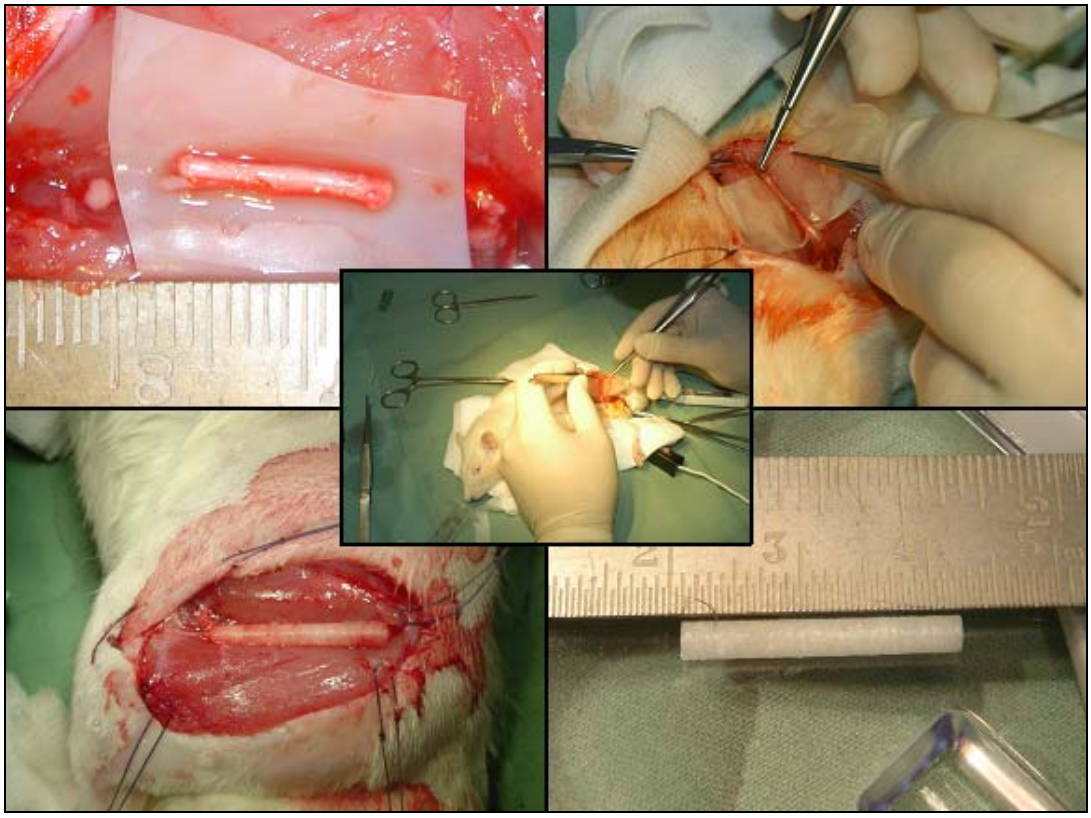

Fig.14 Surgical images (clockwise from top left): extraction of a 14 mm segment of the sciatic nerve, repair by autograft, empty collagen tube, collagen tube sutured into place.

7.4 Conclusions drawn after applying various methods of evaluation

Three major conclusions could be drawn after applying a variety of methods for the evaluation of sciatic nerve regeneration and comparing the results:

(1) Compensatory events in both the sensory (Paper 2) and the motor (Paper 3) systems play a major role where there has been very little regeneration or no regeneration at all. This means that caution should be exercised when interpreting functional evaluations.

(2) Some methods of evaluation might be affected by a methodological problem (logical inconsistency or flaw in implementation) (Papers 2, 3 and 4), which only becomes evident when other methods of evaluation, even those considered redundant, reach a completely different conclusion regarding the extent of regeneration.

(3) No sciatic nerve regeneration study in the rat should be conducted without histological assessments of the regenerating nerve (Papers 2, 3 and 4). Ideally these should be of a morphometric nature, thus yielding quantitative and easily comparable results, and should be carried out close to the end organ which has been subjected to functional evaluations.

7.5 Compensation and plasticity

Compensatory events and plasticity could be observed in both the sensory and motor systems after double transection of the sciatic nerve and repair though guidance interponation.

7.5.1 Sensory system

In Paper 2 the results of somatosensory assessments of the regenerating sciatic nerve are described and compared with results achieved in footprint analysis and in morphometric and electron microscopic assessments of the plantar extensions of the tibial nerve (Fig.15).

The somatosensory tests revealed that all animals experienced roughly the same amount of sensory reinnervation in their feet, regardless of their experimental group. This contrasted with the findings of assessments primarily concerned with motor function, such as footprint analysis, where rats in Groups B and C (collagen tube groups) did not improve at all. These groups also failed to show neural regrowth in morphometric and electron microscopic assessments of neural segments associated with the sensory reinnervation of the plantar side of the foot, that is the plantar extensions of the tibial nerve (GREENE, 1955; HEBEL & STROMBERG, 1976).

Retrograde tracing was able to prove that the saphenous nerve was the primary source of sensory reinnervation in those animals which experienced no sciatic regeneration (Fig.15). Contributions from the musculocutaneous nerve were also possible. This very proximal branch of the sciatic nerve has been reported to provide some sensory innervation to the hind paw in the rat under physiological circumstances (PUIGDELLÍVOL-SÁNCHEZ et al., 2000); however, it only seems to have a limited capacity for providing compensatory innervation of the foot by collateral sprouting after sciatic injury (PUIDELLÍVOL-SÁNCHEZ et al., 2005).

7.5.1.1 Comparable events described in the literature

Compensatory events on every level of the sensory nervous system have been extensively described in the literature.

Area of foot	Group	D 0	D 5	W 3	W 5	W 8
dorsal	A	0.00	9.71	7.75	6.31	3.69
	B	0.00	8.83	6.67	4.75	4.17
	C	0.00	7.67	6.08	4.92	4.50
	p value		0.003	0.066	0.039	0.842
lateral	A	0.00	11.57	10.94	9.38	4.63
	B	0.00	11.83	10.67	8.00	5.58
	C	0.00	10.83	8.50	6.42	5.25
	p value		0.040	0.005	0.003	0.363
plantar	A	0.00	14.57	11.50	8.19	2.56
	B	0.00	14.00	10.93	6.08	4.17
	C	0.00	13.50	8.67	6.42	4.00
	p value		0.152	0.020	0.084	0.309
total foot	A	0.00	35.86	30.19	23.86	10.89
	B	0.00	34.67	28.25	18.83	13.91
	C	0.00	32.00	23.25	17.75	13.75
	p value		0.006	0.005	0.010	0.464

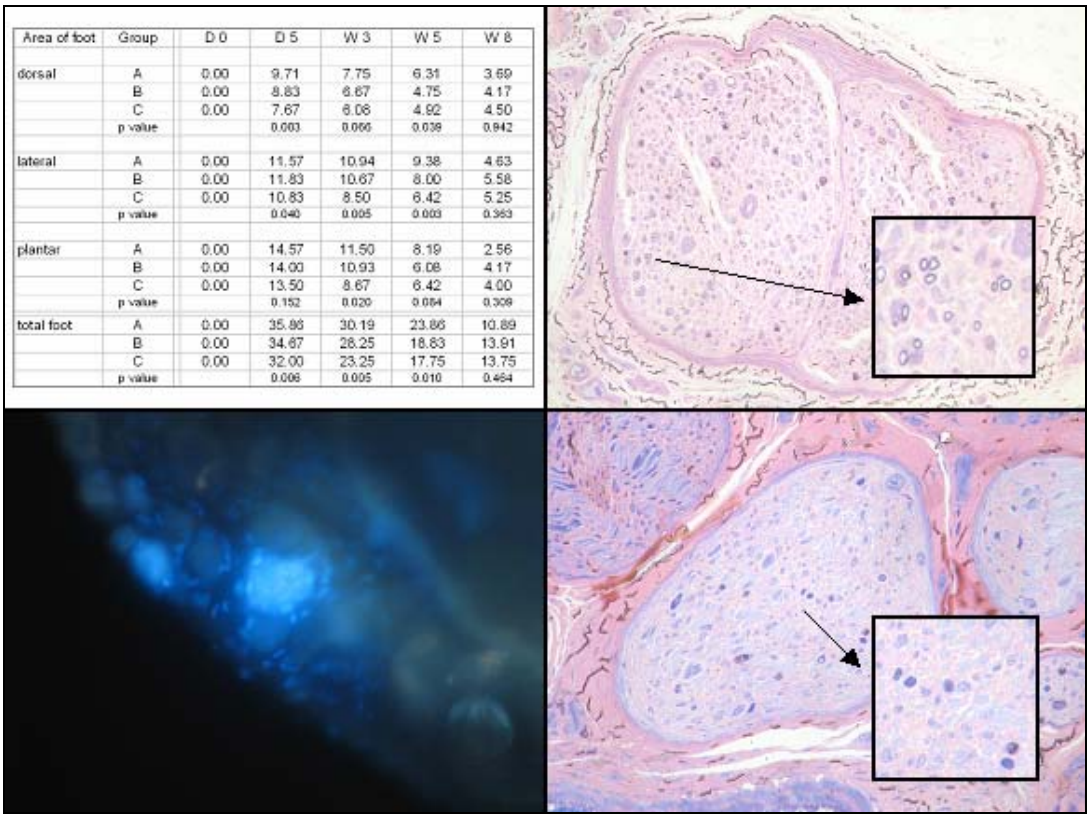

Fig.15 Evidence of collateral sprouting: scores achieved in the somatosensory evaluations (top left) – please note that there is no statistically significant difference between the three surgical groups at week 8; histological images of the plantar extensions of the tibial nerve of a rat in Group A (top right) and Group C (bottom right). In the top image small myelinated fibres can be seen, whereas in the bottom image there are none. Double labelled neuron in the dorsal root ganglion of L3 (bottom left) after injection of the tracers to the metatarsus of toes 1 and 5.

7.5.1.2 Compensatory events in the periphery – collateral sprouting

It was already well known in the 1940s (SPERRY, 1945) that collateral sprouting of neighbouring nerves takes place after injury to a sensory nerve; the extent to which sprouting of the saphenous nerve can take place in the periphery after damage to the sciatic nerve was first described by Devor and co-workers in 1979 (DEVOR et al., 1979). Various subsequent studies featuring chronic sciatic nerve ligation or section (MARKUS et al., 1984; KINNMAN & ALSKOGIUS, 1986; KINNMAN et al., 1992; KINNMAN & WIESENFELD-HALLIN, 1993), also serving as a model for chronic pain and hyperalgesia (KINGERY & VALLIN, 1989; ATTAL et al., 1994), confirmed these observations. Interestingly, however, the extent of collateral sprouting varies from one study to another, with the saphenous nerve never progressing into toes 4 and 5, and only into parts of toe 3, according to the study by Devor and co-workers (DEVOR et al., 1979). In contrast to this, the reports by

Kingery and Vallin (KINGERY & VALLIN, 1989) support the present findings, where toes 3 and 4 clearly exhibited reinnervation even in those rats with virtually no regenerating myelinated axons in plantar extensions of the tibial nerve.

The mechanisms underlying compensatory sprouting by neighbouring nerves in the event of damage to a sensory nerve have not been completely clarified as yet, but the following theories have been put forward. In the physiological state, territories are clearly defined by inhibitory signals, such as mutual growth-inhibiting factors which are transmitted between neighbouring nerves. If, however, these signals suddenly diminish in intensity, the inhibition is negated and the uninjured nerve encroaches into foreign territory by means of excessive sprouting (DEVOR et al., 1979). Alternatively, there have been suggestions that other chemical substances associated with the degeneration of a damaged nerve, or even a diminished or complete lack of feedback from the periphery to the central nervous system due to sectioning of a nerve, might be responsible for inducing sprouting of the neighbouring nerves (DEVOR et al., 1979).

Whatever the reason, the sprouting takes place rapidly and almost completely, as demonstrated in the present study. This ensures that the organism is protected from any potential harm which could arise if there is no full response to exogenous stimuli.

7.5.1.3 Central plasticity

In addition to triggering compensatory events in the periphery, injury to a peripheral nerve can also bring about changes in representational sensory maps on the cortical (CUSICK et al., 1990; SANES et al., 1990; KAAS, 1991; JONES, 2000; PELLED et al., 2006) and subcortical level (MARKUS et al., 1984; KAAS; 1991; CHEN et al., 2002; KOERBER et al., 2006). In the somatotopically organised sensory maps the adjacent body surface representations expand into the denervated area (MARKUS et al., 1984; CUSICK et al., 1990; KAAS, 1991; CALFORD, 2002). These alterations in the representational maps are directed towards improving sensory perception and discrimination, especially in areas adjacent to the area affected by denervation (CHEN et al., 2002).

Not only deafferentation, but also changes in sensory input due to activity, behaviour and skill acquisition can trigger dynamic remodelling of the representational maps in the mature primary somatosensory cortex, thalamus, brainstem and spinal cord (BUONOMANO & MERZENICH, 1998; DUPONT et al., 2001; CALFORD et al., 2002; CHEN et al., 2002; LUNDBORG et al., 2003;

KOERBER et al., 2006). The ability to reorganise the somatotopic representations in the cortex is, however, age-dependent; injury occurring at a younger age is often associated with more extensive reorganisation and a better functional outcome (CHEN et al., 2002). A return to the original state is possible (KAAS, 1991; CHEN et al., 2002) and, for example, can be achieved within 6 hours after 14 days of denervation in a hypodynamia-hypokinesia model in the rat hind limb (DUPONT et al., 2001).

Changes to the organisation of the cortex and subcortical structures may occur within minutes (DUPONT et al., 2001; LUNDBORG, 2003) or take much longer depending on the mechanisms involved. During epidural nerve blocks, for example, neurons originally responsible for the newly anaesthetised area quickly respond to stimulation of neighbouring, non-anaesthetised territories (CHEN et al., 2002).

The following observations have been made with specific reference to the sciatic nerve. On the spinal cord level, the saphenous nerve already seems to have spread into the representational area of the sciatic nerve and even further by day 21 after sciatic transection and ligation (MARKUS et al., 1984). If both the saphenous and sciatic nerves are sectioned and ligated, neurons in the dorsal horn, which originally had receptive fields on the lower leg, become sensitive to stimulation of neighbouring intact areas, such as the medial thigh, perineum and lower back, within four to five days (SELTZER and DEVOR, 1984). In the S-I area of the cortex, 85% of which is predominantly activated by the sciatic nerve and 15% by the saphenous nerve, it was shown that within one to three days after injury to the sciatic nerve, the area activated by the saphenous nerve annexes an additional 23% to 26% of the total hind paw cortex. Seven to eight months later, after cumulative cortical reorganisation in four stages, the area represented by the saphenous nerve does not differ significantly from the normal total hind paw representation (CUSICK et al., 1990).

In stark contrast to the very definite and exact findings given regarding the extent of compensatory events in the periphery, the scope of the present study did not extend to determining the amount of reorganisation on the central level of the sensory nervous system. However, in view of the fact that it has been possible to observe changes to the hind paw representational area in the somatosensory cortex with the aid of functional MRI even after crush injuries to the sciatic nerve (PELLED et al., 2006), one can surmise that reorganisation, especially in those animals not experiencing any sciatic regeneration, must have been extensive.

7.5.2 Motor system

In Paper 3 the ambulatory abilities of the rats in the three surgical groups (A: autograft; B: empty collagen tube; C: collagen tube filled with denaturated autologous muscle) are presented and compared with results obtained from footprint analysis (both static and walking), measurement of gastrocnemius muscle volumes, and morphometric and electron microscopic assessments of the sciatic nerve at mid-graft or mid-interponate level.

Ambulatory abilities were assessed by getting the rats to traverse elevated beams and parallel bars of different widths and noting the speed and the number of times the affected hind limb was placed next to the beam/bar or slipped off (Fig.16).

Rats in Group A, which exhibited the best results in SFI and SSI, had the largest gastrocnemius muscle volumes and displayed the most extensive regeneration of nerve fibres, also made most progress where ambulatory tests primarily focussed on motor regeneration (speed on the widest beam) or sensory regeneration

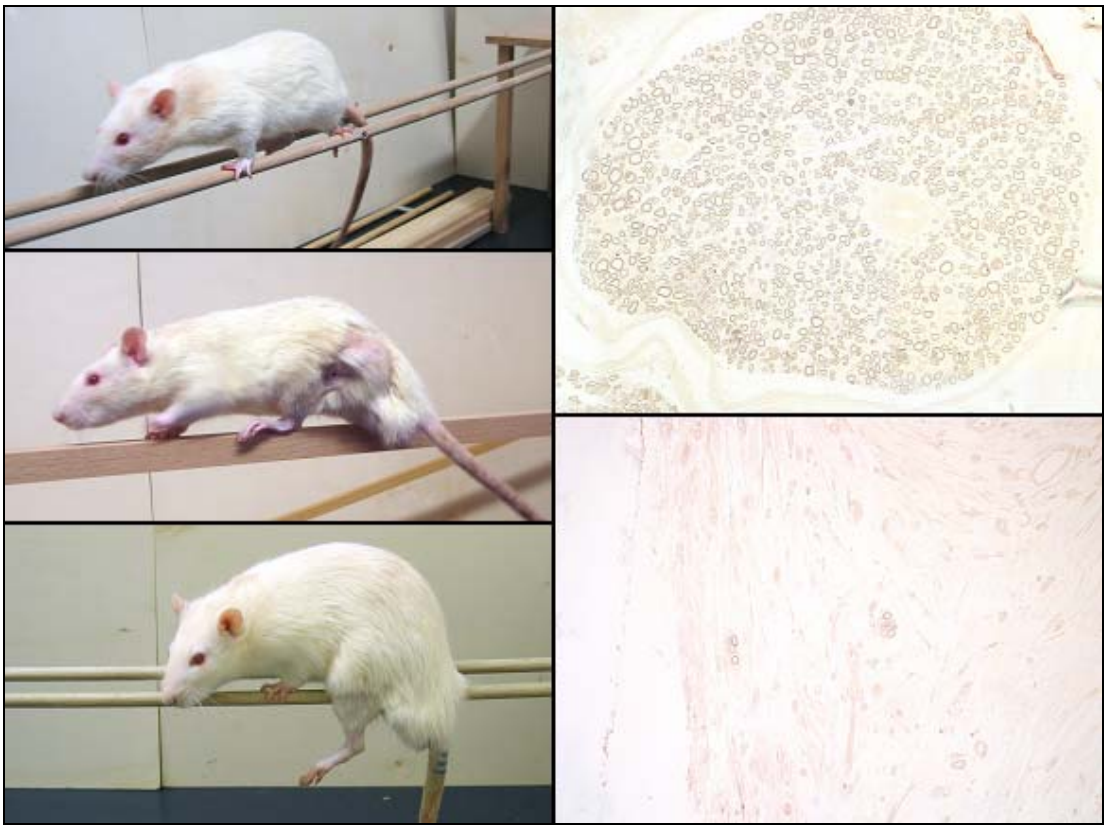

Fig.16 Left hand side: **ambulatory assessments:** correct placement of the healthy hind limbs (top), correct placement of the operated left hind limb (middle), incorrect placement of the operated left hind limb (bottom).
Right hand side: **morphometric evaluations of the regenerating sciatic nerve at mid-graft level:** the rat in Group A (top) exhibits large amounts of regenerating myelinated fibres, whereas the rat in Group C (bottom) has only very few.

(placement of the operated hind limb on the parallel bars). In the rest of the ambulatory evaluations, however, hardly any difference could be noted between groups, even though Groups B and C experienced only very little or no neural regeneration (Fig.16), a fact confirmed by morphometric, electron microscopic and footprint assessments. Consequently, the progress in ambulatory tests made by rats in Groups B and C had primarily to be attributed to plasticity at the cortical and subcortical level.

7.5.2.1 Comparable events described in the literature

The phenomenon of collateral sprouting, as described above for the peripheral sensory nervous system and clearly observed in the present study, does not seem to take place in the motor nervous system. Here the focus, both on the peripheral and central level, seems to be shifted instead on the remaining functioning end organs.

7.5.2.2 Compensatory events in the periphery – trick movements

From the end of the 19th century until the middle of the 20th century various researchers, including Kennedy (1897), Osborne (1909), Marina (1912, 1915), Perthes (1918, 1922), Foerster (1930), Stopford (1930), Bethe and Fischer (1931), Anokhin and colleagues (1935, 1940), were firmly convinced that complete readjustment of the nervous system on both the cortical and subcortical level was possible, after injury to the motor (and sensory) system resulting in aberrant innervation of the end organs (SPERRY, 1945). However, in the late 1930s and early 1940s these very optimistic opinions came under fierce attack by Ford and Woodhall (1938) and Sperry (1940-1943) after new studies featuring findings which stood in direct contradiction to earlier views were presented. Instead of complete and rapid reorganisation, nerve-muscle rearrangements were found to result in discoordinations with no sign of correction (SPERRY, 1945). Additionally, it was found that the earlier investigations did not analyse the action of individual muscles in detail, but merely the animals' general use of the hind limb in running, walking and other activities, and that control experiments had only very rarely been conducted. The term "trick movements" (also "compensatory", "supplementary" or "anomalous" muscle function) was introduced for situations which tend to lead to mistaken conclusions regarding the extent of motor recovery. The most popular mechanisms cited for the induction of trick movements have been described as follows:

(1) remaining sound muscles can often, with or without practice, be made to reproduce the actions previously performed by the affected muscles,
(2) after contracture of a joint, the ankle joint being the one most frequently affected, the direction of action of the affected muscles can often be attained simply by relaxation of the antagonist muscles,
(3) owing to the mechanical relations of tendons and ligaments of joints, the displacement of one joint can often cause a passive movement of a joint further distal (often seen in knees and ankles of mammals),
(4) movements of the heavier limb segments can simply be produced by gravity,
(5) movements especially of the distal joints can simply be produced by momentum,
(6) contractures can support the utilisation of a joint due to stabilisation, whereas flaccid paralysis would not be beneficial (SPERRY, 1945).

Transection of the sciatic nerve at the level described in the present study, resulted in all muscles distal to the knee being denervated. This means that of the mechanisms described above, mechanisms (1), (2) and (4) can be excluded. The mechanism for the induction of a trick movement most frequently observed was (5), i.e. the production of a movement, especially of the distal joints, simply by momentum.

Freshly operated animals tended to walk on the dorsum of the toes and paws of the operated hind limb for the first two or three days. After that, however, they quickly learnt that with an exaggerated flexion of the knee during the swing phase and relatively late positioning of the foot on the ground in the stance phase, it was possible to achieve appropriate positioning of the foot of the operated hind limb. Animals with extensive reinnervation (mostly Group A) would then proceed to a state, beginning approximately three weeks after the operation, in which slight to moderate contractures of the ankle and the toes could be seen (Fig.17), occasionally making it rather difficult to acquire clearly defined footprints from these rats. In contrast to this, rats experiencing no regeneration would continue to exhibit flaccid paralysis of the lower leg of the operated hind limb (Fig.17). Comparison of the gait patterns showed that the animals with flaccid paralysis appeared to walk more smoothly and also always were able to position their foot flat on the ground with the plantar side downwards (unpublished observations).

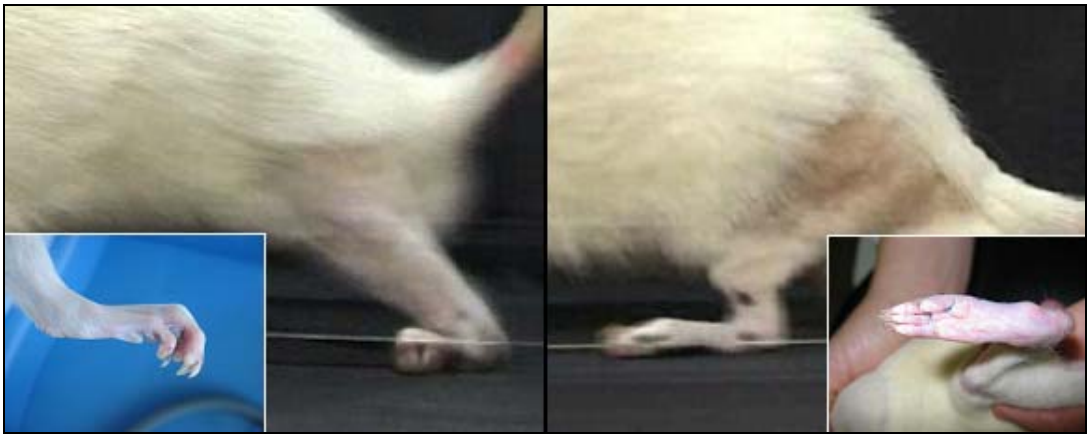

Fig.17 Images taken from videos of rats walking: the rat on the left experienced extensive neural regeneration and suffered from contractures, whereas the rat on the right had no regenerating fibres at all and exhibited flaccid paralysis of the operated hind limb.

These observations can be very misleading, since flexion contractures are closely connected with reinnervation of the distal target muscles. They seem to result from the natural imbalance which exists between flexor muscles and extensor muscles, with the flexor muscles being more powerful than their antagonists (CHAMBERLAIN et al., 2000). The imbalance might further further aggravated by there also being slower or less complete reinnervation of the antagonists (CHAMBERLAIN et al., 2000). Furthermore, rats suffering from contractures most probably also have to cope with the consequences of aberrant innervation, which can highten the impression of their gait being more choppy and less coordinated than that of rats “simply” displaying unchanging flaccid paralysis. This leads us back to Sperry’s remark (SPERRY, 1945), that instead of scoring the overall use of an operated hind limb, the individual muscles need to be analysed in detail in order not to be deceived by trick movements.

For this reason more detailed assessment of ambulatory abilities was carried out on balancing devices of varying widths, since the placement of the hind limb on narrower beams and parallel bars requires particularly intricate hind limb coordination and is closely associated with motor learning (DING et al., 2000, 2001).

The results of the ambulatory tests revealed that all surgical groups exhibited similar numbers of placement mistakes, which means that extensive reshaping of the motor representational maps on the cortical and subcortical level must have taken place.

7.5.2.3 Central plasticity

The motor cortex, unlike the sensory cortex, is not divided into a somatotopically ordered representational map. Instead it is divided into subregions which function rather like a web interacting to trigger certain movements (WEISS & KELLER, 1994; SANES & DONOGHUE, 2000). It therefore seems that sites responsible for individual parts of the body are widely distributed and overlapping (SANES & DONOGHUE, 2000). However, motor maps, just like sensory maps, still have a flexible relationship on the various cortical and subcortical levels with their target organs, the muscles (DONOGHUE et al., 1990; SANES et al., 1990; SANES & DONOGHUE, 2000), and can be remodelled throughout life (NUDO et al., 1996; NUDO et al., 1997). Similar to those changes affecting the organisation of the somatosensory maps, motor maps can also undergo changes due to peripheral nerve lesions (DONOGHUE et al., 1990; SANES et al., 1990; HUNTLEY, 1997; CHEN et al., 2002) or motor skill training (NUDO et al., 1997; SANES & DONOGHUE, 2000; REMPLE et al., 2001); these changes are also reversible, but not completely (NUDO et al., 1996). After peripheral nerve lesions an enlargement of the adjacent representations in the motor cortex can be observed; this results in affected areas in the motor cortex becoming associated with new muscle groups (DONOGHUE et al., 1990; SANES et al., 1990; HUNTLEY, 1997). Changes in the target area of the motor cortex therefore enable the cortex to devote more attention to non-affected muscles. This means that motor control for the new target muscle can possibly be adjusted more finely (DONOGHUE et al., 1990; CHEN 2002). In the present study the muscles in question most probably were the thigh muscles. It is very conceivable that fine-tuning of the quadriceps muscle, but especially of the biceps femoris, semimembranosus and semitendinosus muscles would lead the rat to being able to place the hind paw of the affected hind limb relatively selectively when walking, in addition to being able to execute a smooth gait pattern. This would especially be the case if the muscles below the knee were affected by permanent denervation, a situation that was seen in most rats of Groups B and C. Consistency in the peripheral changes would allow the CNS to adjust better to the new situation resulting in a better adaptation to actual conditions and therefore in a gradual decrease in placement mistakes, as was observed in all the rats examined. Animals in Group A went through a patch of increased misplacements during week 4 and week 5, setting them behind the other groups. It could be speculated that these periods of extra mistakes could be due to regular and/or aberrant reinnervation of both muscles and sensory end organs, as these periods coincided

with the times where first real motor recovery progress could be observed in this group, as indicated by SFI and SSI evaluations. Reinnervation of the end organs, whether physiological or aberrant, would render newly acquired maps useless and the process of reorganisation would begin all over again, resulting in increased placement mistakes until new fine-tuning had sufficiently taken place.

The alterations to the motor maps described above are established within hours and can persist for long periods following nerve injury (DONOGHUE et al., 1990; SANES et al., 1990; HUNTLEY et al., 1997). Two main mechanisms have been put forward to explain reorganisation on the various cortical and subcortical levels after a peripheral lesion. The first, resulting in short-term changes, consists of unmasking already present, but functionally inactive, connections (DONOGHUE et al., 1990; KAAS, 1991; HUNTLEY, 1997; DUPONT et al., 2001; CHEN et al., 2002; KOERBER et al., 2006). There are numerous factors which could produce this result, such as increased excitatory neurotransmitter release, dephosphorylation of receptors, upregulation of postsynaptic receptors, changes in membrane conductance, decreased inhibitory inputs, or the removal of inhibition from excitatory inputs (CUSICK et al., 1990; DONOGHUE et al., 1990; JACOBS & DONOGHUE, 1991; KAAS; 1991; LITTLE et al., 1999; SANES & DONOGHUE, 2000; DUPONT et al., 2001; CHEN et al., 2002). Ultimately, however, it is the underlying anatomical features which define the extent to which short-term plasticity can take place (CALFORD, 2002). The second main mechanism, resulting in long-term plasticity, requires either NMDA receptor activation (ABRAHAM & BEAR, 1996; NUDO et al., 1996; BUONOMANO & MERZENICH, 1998; CALFORD, 2002; WIELOCH & NIKOLICH, 2006), which results in long term potentiation and/or long term depression, or axonal sprouting (KAAS, 1991; CHEN et al., 2002; BALLERMAN & FOUAD, 2006; WIELOCH & NIKOLICH, 2006) and synaptogenesis (KOERBER et al., 2006) featuring alterations in sizes and types of synapses (CHEN et al., 2002). Axonal sprouting and synaptogenesis might be initiated and controlled by the expression of cell adhesion molecules, which are also very important for forming connections between neurons during embryonic development (FIELDS & ITOH, 1996).

It has been suggested that trophic factors prompt the reorganisation of motor maps on the spinal level, while changes to sensory inputs trigger alterations in the cortex (DONOGHUE et al., 1990).

As with the central plasticity of the sensory system, the scope of the present study did not extend to determining exact degree of changes to the cortical and

subcortical motor maps. On the basis of the results of the ambulatory assessments it is, however, safe to assume that the alterations were extensive.

The most probable reason why such an impressive display of functional plasticity could be observed in the present study, is that all rats received extensive physiotherapy, both on a mandatory (twice weekly in the stress–free ambulatory assessments) and voluntary (during their daily “playtime” in the motor enriched cage) basis. Positively reinforced movement has been proven to induce and promote plasticity (WIELOCH & NIKOLICH) and this phenomenon has in turn been used in recent years as a means of promoting functional recovery after extensive lesions to the nervous system (LANKHORST et al., 2001; NORRIE et al., 2005; BEHRMAN et al., 2006).

7.5.2.4 Induction of plasticity

For cortical representation to be adjusted, it is extremely important that the subject in question (human or animal) possesses the appropriate behavioural stance. A high level of motivation, backed up by positive reinforcement (VAN MEETEREN et al., 1998; BEHRMAN et al., 2006) is essential if functionality is to be regained; in other words any exercise which raises stress levels and leads to an increased glucocorticoid level, is detrimental to functional recovery, as this could accentuate the atrophy of denervated muscles and, as mentioned before, reduce axonal sprouting and elongation (VAN MEETEREN et al., 1998). Also stimuli must be specific and closely associated with the desired functional result (LUNDBORG, 2003).

The site and extent of the insult must be taken into consideration before introducing experience-driven re-learning by physiotherapy (VAN MEETEREN et al., 1998; LITTLE et al., 1999; NORRIE et al., 2005). With strokes, for example, it has been shown that it might possibly be detrimental to initiate physiotherapy within two days after the insult, whereas physiotherapy will not be effective after a gap of more than 30 days (WIELOCH & NIKOLICH, 2006). With spinal cord injuries, however, the improvement of motor recovery via training should be started as early as possible (NORRIE et al., 2005).

Apart from the importance of avoiding stress, as mentioned above and its implications for the restoration of function, training conditions also are important. Rigorous training performed under duress during the reinnervation phase of the muscle seems to impede the restoration of muscle contractile properties, whereas moderate training helps the muscles to regain their physiological properties at a

faster rate (VAN METEEREN et al., 1998) and has a positive influence on the early phase of nerve regeneration (VAREJÃO et al., 2001, 2003).
Functional recovery can also be promoted by “conditioning” the neurons for plasticity by applying indirect or direct electrical or transcranial magnetic stimulation (WIELOCH & NIKOLICH, 2006). The administration of amphetamines is also thought to enhance those mechanisms that stimulate brain activity and long term potentiation, thus stimulating axonal sprouting and the reorganisation of cortical maps (WIELOCH & NIKOLICH, 2006).

7.5.2.5 The concept of enriched housing

The aforementioned arguments have led to the introduction of the concept of “enriched housing”, first proposed as an experimental paradigm in the late 1940s (MEEK et al., 2004), for laboratory animals subjected to injury to the nervous system. Generally “enriched housing” means that the animals are housed in groups (instead of individually) in a large cage. The cage is equipped with miscellaneous objects such as logs, toys and running wheels (LANKHORST et al., 2001), which provide the animals with continuous and voluntary physiotherapy. Additionally, food and water are provided at different sides of the cage to stimulate locomotor activity (LANKHORST et al., 2001). These aspects were borne in mind when designing the motor enriched cage in which the rats were placed for a few hours daily for their voluntary physiotherapy. Over and above the regular objects in the cage, foreign objects such as cardboard boxes, paper bags and plastic reels were introduced at regular intervals in order to exploit the rats’ natural curiosity and desire to explore and destroy. Treats hidden in different corners of the cage further encouraged the rats to traverse the oblique ramps and beams, and they also very quickly learnt to use the sides of the cages as climbing opportunities (Fig.18).
Thus “enriched housing” spurs physical activity (LANKHORST et al., 2001) and provides the animals with sensory stimulation. The animals are encouraged to move, but are not placed under any duress, which could potentially trigger overuse of impaired limbs and has been shown to have adverse effects on functional recovery in various models of CNS injury (MOON et al., 2006). Additionally, “enriched housing” has a social component (WIELOCH & NIKOLICH, 2006), which can have a positive effect on the return of functionality as outlined in Section 7.3.2. The rats in the present study experienced these social benefits both in their “enriched housing” and in their standard everyday housing. As a result health and behaviour of the rats were normal throughout the study. All the animals exhibited a

Fig.18 Rats participating in voluntary physical activity

slight gain in weight, groomed themselves well, and had moderate to high levels of activity. None of the rats displayed any signs of autotomy, the reasons for this most probably being that they were Lewis rats (PANERAI et al., 1987; CARR et al., 1992; STRASBERG et al., 1999) and that they were housed in a beneficial environment (MEEK et al., 2004).

“Enriched housing” has been shown to change biochemical parameters, to enhance dentritic arborisation and gliogenesis, and to increase the number of neuronal stem cells and precursor cells in the subventricular zone and striatum (MEEK et al., 2004; WIELOCH & NIKOLICH, 2006; STEINER et al., 2006); rats in “enriched housing” develop better locomotion skills than those housed under standard conditions after contusion to the spinal cord (LANKHORST et al., 2001), and are less likely to suffer progressive loss of restored hind limb movement after spinal cord injury (MOON et al., 2006). This factor has also been shown to improve motor behaviour function in induced Parkinson’s disease in rats (STEINER et al., 2006).

From a functional point of view, all rats, even those experiencing no morphometrically detectable sciatic regeneration, made good progress during their eight weeks under observation. All rats were successfully able to perform motor tasks, such as traversing elevated beams or bars, climbing cages or simply running from Point A to Point B. The extent to which “enriched housing” and extensive physiotherapy impacted on this progress cannot be assessed as there was no control group with simple standard housing in the present study. The reason for this was to establish comparability with human conditions (see Section 7.3).

Until now, only one study has been carried out to compare the effects of “enriched housing” with those of standard housing after lesioning of the sciatic nerve in the rat. For evaluating the extent of regeneration, the walking pattern was scored and the stance factor (ratio of stance duration between operated and contralateral hind limb; see 2.3.4.1) was calculated (MEEK et al., 2004). No differences could be detected between the groups with either tests. However, since the authors themselves expressed doubts regarding the reliability of the stance factor (MEEK et al., 2004) and a subjective scoring of walking patterns is a method not really advisable as described above in Section 7.5.2.2 (trick movements), the findings are open to doubt. Furthermore, following injury to the sensory or motor cortex it has been shown that pre- and post-lesion environmental enrichment or deprivation influence recovery of beam-walking skills (GOLDSTEIN & DAVIS, 1990).

7.6. Methodological problems affecting certain types of evaluation

Somatosensory assessments, footprint analyses and electrophysiologic evaluations all encountered methodological problems.

7.6.1 Somatosensory evaluations

As indicated in Section 2.3.4.4 a), there are many different approaches concerning how and where the noxious stimuli should be administered.

The only generally accepted rule is that the medial aspect of the foot should be avoided, as the saphenous nerve caters for this region (DE LAHUNTA, 1977; DEVOR et al., 1979; DE KONING et al., 1986; VAREJÃO et al., 2004a).

It should also be borne in mind that by varying the intensity of stimuli applied, it is possible to trigger the subsequent reaction on two different levels: a subconscious withdrawal reflex or a conscious pain reaction (DE LAHUNTA, 1977; GAROSI, 2004). The withdrawal reflex is a true reflex which involves the central nervous system on the spinal level. The animal responds to the stimulation of nociceptors simply by withdrawing its leg. For a conscious reaction to pain, however, afferent signals have to reach the thalamus via the spinothalamic tracts and the medial lemniscus, and are further projected to the somaesthetic cortex via the internal capsule (DE LAHUNTA, 1977). Conscious pain perception occurs both on the thalamic and on the cortical level, and causes the animal to react with an accurate pain response such as licking its foot or vocalising its discomfort in addition to withdrawing its foot from the noxious stimulus (NAVARRO et al., 1994).

Presupposing that the afferents have been re-established after experimental nerve trauma, the question that now arises is whether a rat with residual sciatic dysfunction is capable of performing a recognisable withdrawal of the foot. This could be a problem if the effector muscles needed for this action are still denervated or incompletely or inappropriately reinnervated. Additionally, the common technique of wrapping the rat in a towel for immobilisation and restriction of vision during evaluation (Fig.19) (MASTERS et al., 1993; HU et al., 1997; VAREJÃO et al., 2004b) might not only hinder the rat from performing a withdrawal reflex, but might also make it more difficult for the examiner to recognise weak responses.

7.6.1.1 Implementation of somatosensory assessments

The somatosensory assessments in the present study were carried out by pinching the rats' feet with atraumatic forceps in a number of places. These amounted to 14 spots on the dorsal aspect, 13 spots on the lateral aspect, 5 spots on the medial aspect and 24 spots on the plantar aspect (Fig.19), a greater number than in comparable studies. The noxious stimuli were delivered by holding the rats gently, but firmly, with one hand (Fig.19), whilst pinching their foot with the other at a maximum force of 0.8 N per mm².

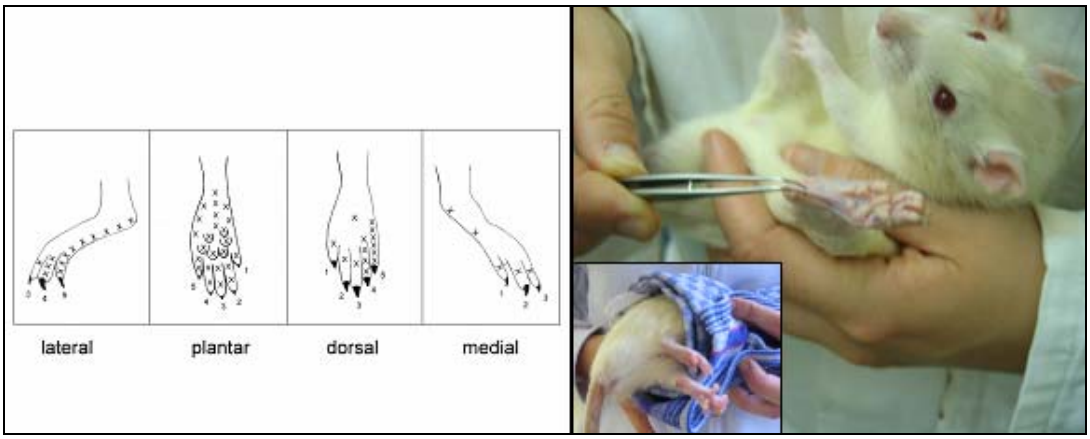

Fig.19 Implementation of somatosensory assessments: pinching points (left), fixation of the rat (right). The inset illustrates the way in which rats are commonly wrapped in towels for somatosensory evaluations.

Only conscious pain reactions were counted as a definite reaction to a noxious stimulus. These took the form of definite vocalisation of protest at the moment of being pinched, with or without retraction of the foot. Often the rats also attacked the source of stimulation, the forceps, or licked their foot in an attempt to alleviate discomfort. Only in one single case of more than 320 examinations did a rat attempt to bite the examiner.

7.6.1.2 Results of somatosensory and supplementary assessments

The somatosensory tests revealed that all animals experienced roughly the same amount of sensory reinnervation in the foot, regardless of their experimental group (Fig.15). Morphometric and electronmicrosopic evaluations revealed, however, that the vast majority of rats in Groups B and C experienced no neural regrowth in the plantar extensions of the tibial nerve (Figs.15, 20), which physiologically is associated with innervation of the plantar and lateral aspect of the foot (GREENE,

1955; HEBEL & STROMBERG, 1976). This contrasted strongly with the morphometric assessments of the same neural segments of rats in Group A (Figs. 15, 20), where all rats except one experienced neural regeneration.

With the help of retrograde tracing studies (Figs 15, 20) it could be proven that it was primarily the saphenous nerve and maybe also a very proximal branch of the sciatic nerve, the musculocutaneous nerve (PUIGDELLÍVOL-SÁNCHEZ et al., 2000, 2005), which were providing sensory reinnervation to the lateral metatarsus of toe 5.

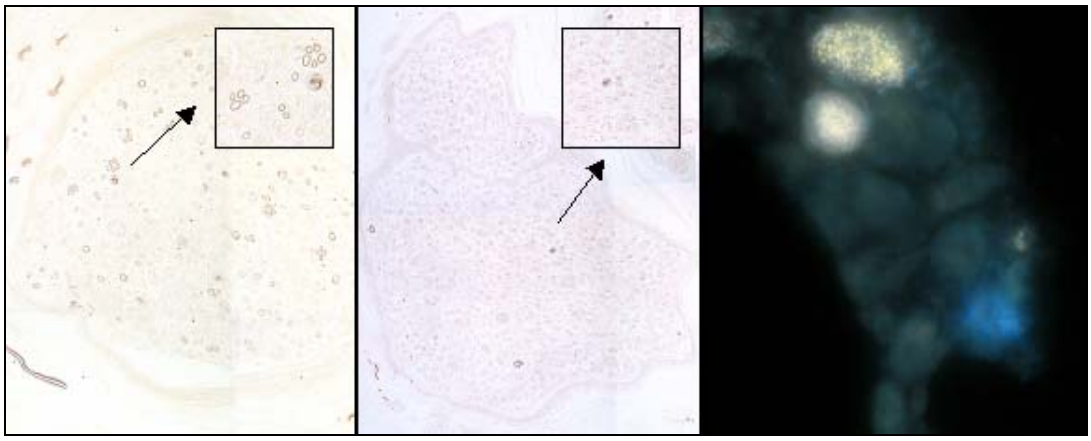

Fig.20 Results of the supplementary assessments: histological images of the plantar extensions of the tibial nerve in a rat in Group A (left) and a rat in Group C (middle). Note that there are no myelinated fibres in the neural segment of the rat in Group C. The picture on the right shows a dorsal root ganglion of L2 exhibiting fluorescence for both the tracers Fluorogold and Fast Blue. These had been injected at the medial metatarsus of toe 1 (Fluorogold) and the lateral metatarsus of toe 5 (Fast Blue).

7.6.1.3 Anatomical considerations

Physiologically, the saphenous nerve is responsible for the sensory innervation of the medial aspect of the foot, toes 1 and 2 and only the proximal phalanx of toe 3 (GREENE, 1955; PUIGDELLÍVOL-SÁNCHEZ et al., 2000). According to the literature, the peroneal nerve provides sensory innervation to the dorsum of the foot, the medial aspect of toe 1 and the adjacent aspects of toes 2, 3, 4 and 5; the tibial nerve provides sensory innervation to the plantar side of the foot and toe 1, to the lateral side of toe 5 and to the adjacent sides of toes 1 to 5 (GREENE, 1955; HEBEL & STROMBERG, 1976). In short, under physiological circumstances, the distal extensions of the tibial and peroneal nerves are responsible for innervation of the dorsal, plantar and lateral sides and all the toes of the hind paw in the rat, while the saphenous nerve only innervates the medial side of the hind paw and has

variable contributions to toes 1, 2 and 3. The musculocutaneous branch of the sciatic nerve, which has only recently been discovered and described, seems to contribute to all the toes; the exact extent and locations have unfortunately not as yet been clarified, however (PUIGDELLÍVOL-SÁNCHEZ et al., 2000). The musculocutaneous branch leaves the sciatic nerve shortly after the caudal gluteal nerve branches off. It then crosses the very proximal aspect of the thigh, running across the adductor magnus muscle, under the caudofemoralis muscle, and across the semimembranosus muscle towards the biceps femoris muscle and the popliteal fossa (PUIGDELLÍVOL-SÁNCHEZ et al., 2000).

7.6.1.4 Implications for both existing and future somatosensory assessments

(A) The observations described in Paper 2 mean that somatosensory assessments, even if they are conducted at the most lateral aspect of the foot, can produce unreliable results. Results obtained in both withdrawal reflex tests and pain response tests should, consequently, be treated with caution. The areas tested, and the likelihood that collateral sprouting of the saphenous nerve could have taken place must be taken into account.

The musculocutaneous nerve should also be kept under observation. This nerve runs in areas of the thigh which are not commonly subjected to injury in the average sciatic nerve regeneration study, and physiologically provides sensory innervation of the foot. Under pathological circumstances the musculocutaneous nerve is also capable of sprouting (PUIDELLÍVOL-SÁNCHEZ et al., 2005). Unfortunately, however, as noted above, the territory of this nerve not even under physiological circumstances has as yet been clarified.

This means that when conducting somatosensory evaluations, care should be taken to stimulate the rats only in areas where no sprouting of neighbouring nerves has so far been observed, i.e. toe 5.

(B) Sensory assessments based on pain responses are feasible to perform and produce reliable results.

Depending on how much the rat is handicapped in performing a recognisable withdrawal reflex (towel, required muscles denervated), pain response testing might produce more reliable results than withdrawal reflex testing.

(C) The results once again show the Lewis strain to be the most suitable rats for sciatic nerve regeneration studies. Due to their docile and friendly nature (STRASBERG et al., 1999) they allow themselves be examined single-handedly

even in a painful situation and do not respond to pain with adverse reactions against the examiner.

7.6.2 Analysis of footprints

The evaluation of footprints is one of the most popular methods for evaluating the functional aspect of sciatic regeneration, since footprint analysis has traditionally been regarded as an overall assessment of sciatic nerve regeneration consisting of sensory input, cortical integration and motor output (DELLON & MACKINNON, 1989; KANAYA et al., 1996; BERVAR, 2000; VAREJÃO et al., 2001b; SCHIAVETO DE SOUZA et al., 2004). Analysis of the 100 most recently published papers on sciatic nerve regeneration revealed that close to 40% of the studies used footprint analysis to determine the extent of functional regeneration (Table 3).
It is not easy to obtain well recognisable, characteristic and distinct footprints, as described in Section 2.3.4.3. Usually, prints from walking rats are acquired by coating the soles of their feet with (finger) paint or ink and inducing the rats to traverse a narrow corridor lined with strips of paper and leading to a darkened cage (Fig.21) (VAREJÃO et al., 2001b, 2004a). In the original version of this method developed by de Medinaceli and colleagues, the corridor was lined out with x-ray films and the rats' feet were dipped in x-ray developer (DE MEDINACELI et al., 1982). Various authors suggested using photographic paper and film developer instead. The high costs, the slippery surface and the potentially caustic effects of the developer made it necessary to search for alternatives, however (VAREJÃO et al., 2001b).
These days, either ink or paint and white paper are used, or in a more sophisticated version, the rats traverse a perspex corridor, and are filmed from underneath with a high-speed camera. If a mirror is placed at an angle of 45º below the runway, both the side view and the plantar view can be obtained from the animals in one film session. The footprints can then be assessed in stills created from the film (VAREJÃO et al., 2001a,b, 2004a).
However, as mentioned in Section 2.3.4.3, it has repeatedly been suggested that footprint analysis primarily measures motor function (WALKER et al., 1994; YU et al., 2001) and for an overall evaluation it needs to be supplemented by sensory assessments (HADLOCK et al., 1999).

7.6.2.1 Unpublished observations and implementations of footprint gathering

During preliminary tests with other rodents it could be observed that, whilst traversing the corridor, the animals would lean against the wall on their affected side (Fig.21). None or only very sketchy footprints of the affected hind limb could be acquired because the exorotated foot would hit the wall during the swing phase and then often be placed with the dorsum on the ground or not at all.

In view of this, the prints (SFI) in the present study were collected on a wide elevated beam, since all rats had learnt to traverse this beam for the ambulatory assessments. A fitting strip of paper was clipped onto the 8 cm wide beam and the rats were placed on this paper with the plantar aspects of their hind limbs covered from the tips of their toes to the heel with non-toxic children's paint (Fig.21). As a result of having been trained and examined on this beam many times, the rats made their way across the beam in an unhesitant and confident manner.

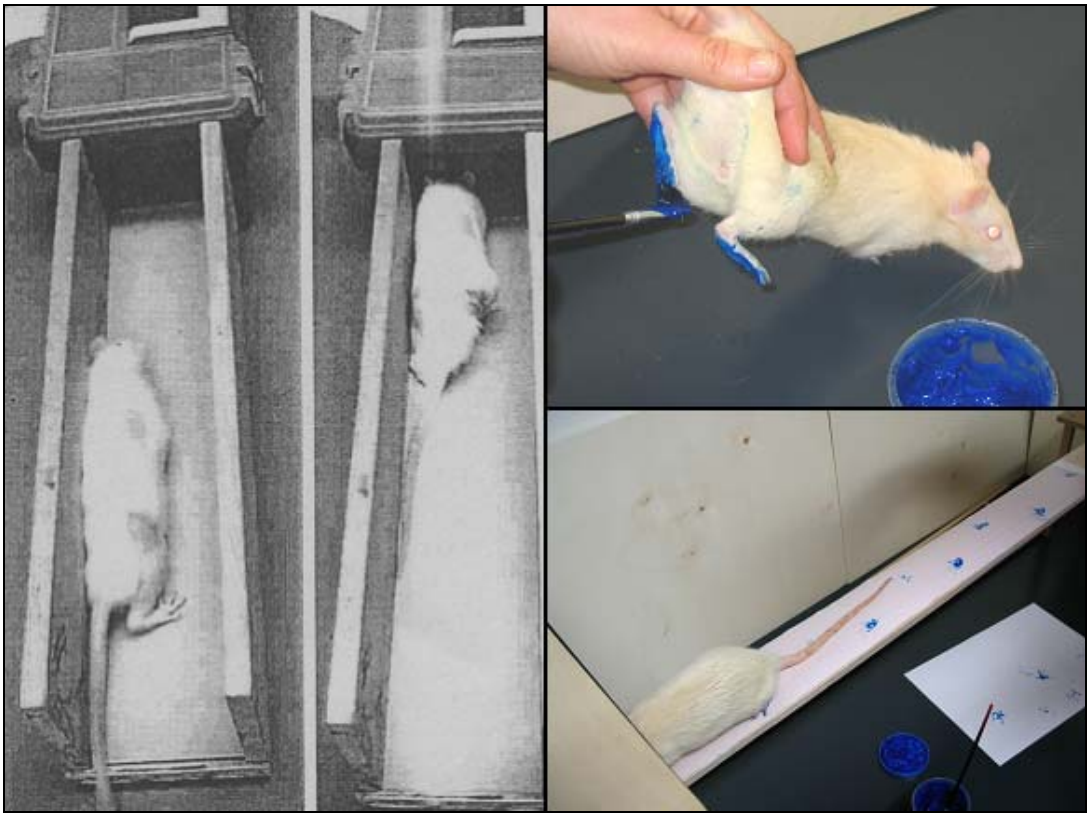

Fig.21 Footprint gathering: rat leaning against the wall of a corridor (Varejão et al., 2001b) (left), covering the rats' feet with children's paint (top right), rat traversing the 8 cm wide beam (bottom right).

An important factor was that there was no wall, which meant that the rats had nothing to lean on, and there was no chance of exorotated foot swings hitting the wall and there being no footprints. Furthermore, the rats subconsciously trusted their unoperated hind limb more and kept toward the unoperated side, enabling clear footprints to be obtained on the operated side.
For static prints (SSI) the rats were held upright, with one leg and the tail tucked under. The rat was then lowered onto a plain sheet of paper with the extended leg anticipating the ground and the print was taken much like stamping a piece of paper.

7.6.2.2 Results achieved in footprint analysis

The results achieved in the present study and presented in Paper 3, revealed that whilst moderate differences could be noted between the different groups regarding the results achieved in the footprint analyses (SFI and SSI), only very few, if any, differences could be noted between the different surgical groups in the overall ambulatory assessments (see also Section 7.5.2). Furthermore, the differences in regeneration between the groups showed up more clearly in the SSI evaluations than in the SFI results.

7.6.2.3 Implications for both existing and future footprint assessments

(A) The acquisition of footprints whilst getting rats to traverse an elevated beam of suitable width is a feasible procedure and produces more usable footprints than the traditional method of letting the rat walk up a corridor. No additional training is required for conditioning the animals.
(B) A comparison of the different surgical groups indicated that the SSI assessments are slightly more sensitive to minimal changes than the SFI assessments.
(C) This procedure confirmed moreover the proposition frequently met in the literature, that footprint assessments are primarily only concerned with motor function (WALKER et al., 1994; HADLOCK et al., 1999; YU et al., 2001) and cannot be regarded as an overall assessment of integrated motor function. For overall assessments other methods of evaluation concerned with gait, such as the ambulatory assessments carried out in the present study, or additional somatosensory tests have to be applied.

7.6.3 Electrophysiologic assessments of the regenerating sciatic nerve

Functional assessments in peripheral nerve regeneration studies are often complemented by electrophysiologic evaluations, even though these are occasionally considered to be redundant to morphometric assessments (DELLON & MACKINNON, 1989).

As indicated in Sections 2.3.1.2 and 2.3.2.3, assessments of the electrophysiologic qualities of the regenerating nerve can either be conducted on the nerve itself with the aid of compound nerve action potentials (CNAPs), or on a muscle innervated by the nerve with the aid of compound muscle action potentials (CMAPs). The latencies of CMAPs and CNAPs are measured and offset against the distances between stimulating and recording electrodes in order to calculate the individual nerve conduction velocity (NCV). In regenerating nerves the NCVs are typically lower as the axonal/fibre diameters are smaller (CRAGG & THOMAS, 1964; KANAYA et al., 1996; VLEGGEERT-LANKAMP et al., 2004). In muscles undergoing reinnervation, a temporal dispersion of the CMAP (increased area) with or without polyphasia can often be seen; this indicates that the regenerating axons are conducting at varying speeds due to their different sizes and different stages of myelination (CUDDON, 2002).

In electrophysiologic assessments of the regenerating sciatic nerve in the rat conducted with the aid of CMAPs, the recording electrodes are generally placed either subcutaneously to the interosseus muscles or within the gastrocnemius muscles; stimulation is provided proximal and distal to the lesion site.

7.6.3.1 Results achieved in electrophysiologic investigations

In the Part 1 of Paper 4, striking discrepancies between electrophysiologic assessments of the regenerating sciatic nerve and morphometric assessments of the same nerve at mid-graft or mid-interponate level are presented (Fig.22). In the morphometric assessments some rats exhibited extensive axonal regeneration, whereas others showed none at all. With the electrophysiologic assessments, which were conducted by providing stimulation proximal and distal to the lesion site and recording CMAPs from the gastrocnemius muscle, no differences could be noted between the different surgical groups. Furthermore, no correlation between the numbers of regenerating myelinated fibres and the NCVs of the individual animals could be found. Interestingly, however, the NCVs calculated in this study

corresponded well with NCVs calculated by different procedures in other studies on regenerating rat sciatic nerves.

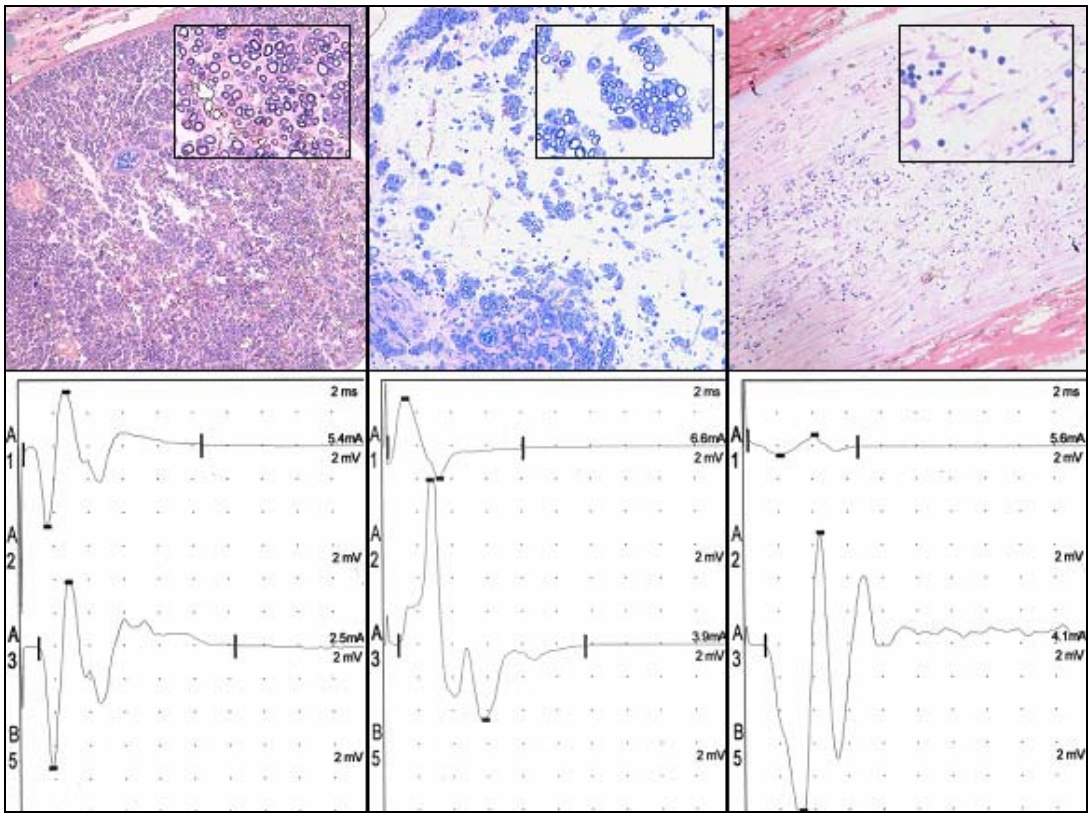

Fig.22 Results of electrophysiologic and histological investigations: the CMAPs recorded in the gastrocnemius muscle are very similar for all three rats (left: Group A; middle: Group B; right. Group C), whereas very different amounts of regenerating myelinated fibres can be observed in the histological images of the regenerating sciatic nerve at mid-graft level (same magnification in all images).

7.6.3.2 Follow-up study to investigate the source of electrical activity

Consequently, in the Part 2 of Paper 4, seven additional rats were subjected to a permanent sciatic nerve transection which featured the same surgical approach and parameters as the main study. At different time-points (1 hour to 8 weeks) after the insult to the sciatic nerve the rats were subsequently assessed electrophysiologically, histologically and by retrograde tracing.

The results of both the histological (Fig.23) and the retrograde tracing examinations revealed that no reinnervation of the gastrocnemius muscle, either physiological or aberrant, had taken place at any stage of the investigation. Furthermore, the electrophysiologic evaluations strongly suggested that the electrical activity recorded in the gastrocnemius muscle after stimulation of the

proximal or the distal stump was most probably generated by surrounding muscles unaffected by denervation. The muscles participating most in this phenomenon, which is also termed cross-talk (KUIKEN et al., 2003), were the biceps femoris muscle and the semimembranosus muscle, both of which are innervated by the musculocutaneous branch of the sciatic nerve (GREENE, 1955; HEBEL & STROMBERG, 1976; PUIGDELLÍVOL-SÁNCHEZ et al., 2000). They were stimulated either directly, or indirectly due to spreading of the impulse.

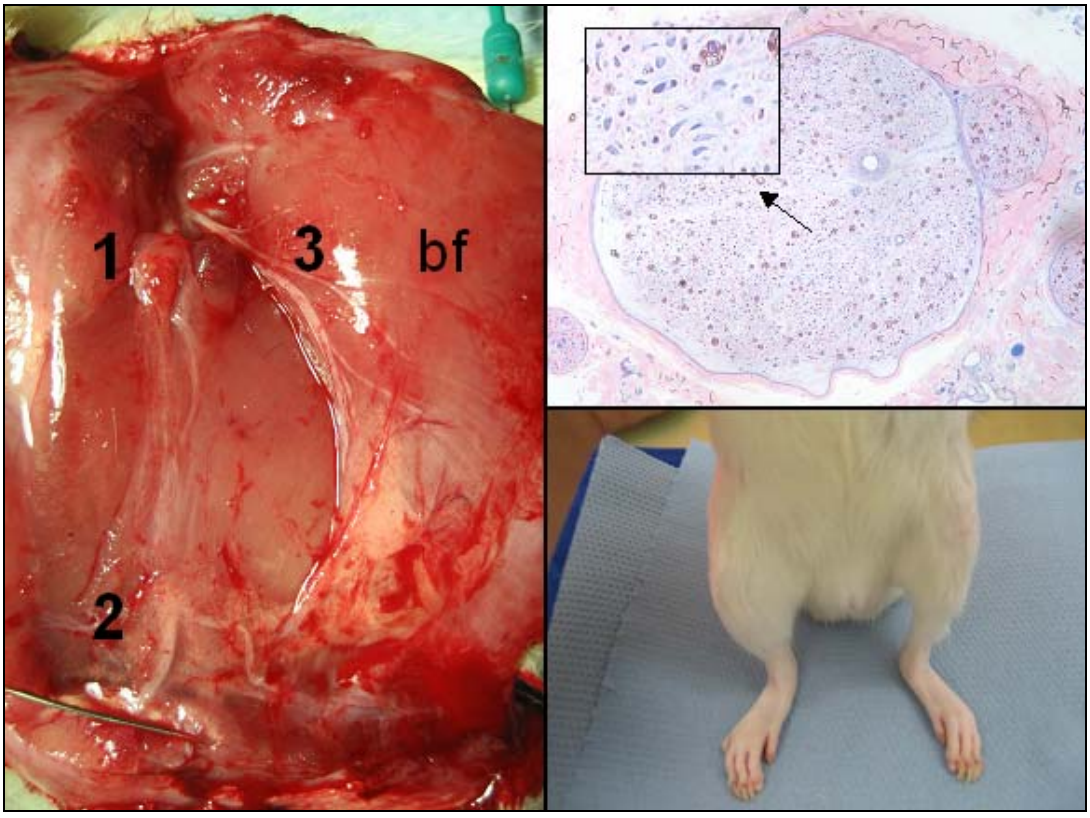

Fig.23 Results of the accompanying assessments in the follow-up study after eight weeks of chronic sciatic denervation: dissection of the lateral thigh after removing the biceps femoris muscle (bf) revealed thin neural strands trying to bridge the gap between the proximal (1) and distal (2) stump. The musculocutaneous nerve (3) appeared to be unaffected (left hand side).
Right hand side: in histological examinations of the distal stump (top), however, no myelinated axons could be observed. Extensive denervation atrophy of the muscles of the operated left hind limb (bottom) was also evident.

7.6.3.3 Implications for both existing and future electrophysiologic assessments

(A) Evaluation of sciatic regeneration by providing stimulation to the sciatic nerve and recording CMAPs from the gastrocnemius muscle should be treated with

caution. 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 muscles unaffected by denervation. It is very likely that even under physiological circumstances these muscles contribute to the CMAPs recorded in the gastrocnemius muscle.

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 via the musculocutaneous nerve, or indirectly as a result of the spreading of the impulse. This means that it is also important to set limits on the intensity of the stimulus applied.

The interosseus muscles are recommended as the “safest” muscles for recording CMAPs in sciatic nerve regeneration studies, as there are no other large muscles, which can interfere with the recordings, in close proximity to these,. It must, however, be borne in mind, that of all the muscles needing to be reinnervated, this group of muscles is situated at the longest distance from the regenerating sciatic nerve and therefore will be reinnervated last. On the other hand it is safe to assume that if this muscle group is reinnervated by regenerating axons, all the more proximal muscles will to some extent have been reinnervated, too.

(B) There is need for further research into the numbers of myelinated nerve fibres and fibre diameters required to conduct impulses and elicit CMAPs. At the moment it is not possible to determine whether the examinations performed on the rats exhibiting extensive regeneration were reliable, since although the number of fibres in these rats was most probably appropriate, their diameters corresponded with much lower NCVs.

7.7 Never evaluate without morphometry

The results reached in the present study very strongly indicate that no evaluations of sciatic nerve regeneration should be conducted without including morphometric assessments.

Compensatory events in the motor (Paper 3) or sensory (Paper 2) systems or even problems with the implementation of certain methods of assessment (Papers 2, 4) can lead to overestimation of the degree of regeneration. Morphometric assessments of the regenerating sciatic nerve or its successors, performed as close as possible to the end organ featured in the functional examinations (CHAMBERLAIN et al., 2000), minimise misinterpretation of functional assessments.

Without morphometric assessments, none of the conclusions reported in Papers 2, 3 and 4 would have been reached. These quantitative assessments of the regenerating nerve were not only the sole method of evaluation which permitted an accurate distinction between the different types of surgical procedures, but also indicated that the somatosensory and electrophysiologic evaluations were incorrect.

7.7.1 Groups of assessments employed in comparable studies

As mentioned before, analysis of the 100 most recently published papers featuring different methods to bridge gaps in the sciatic nerve in the rat (pubmed; keywords: sciatic rat gap regeneration; 385 hits; most recent 100 between 1998 and 2007) revealed that 59% of these studies applied morphometric examinations to determine the extent of regeneration in the sciatic nerve (Table 3).

Only 47% of the studies, however, applied a combination of functional evaluations (somatosensory examinations, footprint analysis), electrophysiologic assessments and sciatic nerve imaging (morphometry, histology and electron microscopy) (Table 5). In the 100 most recently published papers on sciatic nerve regeneration (pubmed; keywords; sciatic nerve rat regeneration; 2,706 hits; most recent 100 between 2004 and 2007) the percentage of studies that evaluated regeneration with functional, electrophysiologic and nerve imaging techniques was more than half (59%) of all studies examined (Table 5).

Table 5: Groups of assessments in comparable studies

	Sciatic gap studies	General sciatic regeneration studies
Purely functional (somatosensory, footprint) and electrophysiologic assessments	8 %	8 %
Purely nerve imaging (morphometry, histology and electron microscopy)	45 %	33 %
Functional tests, electrophysiological assessments and nerve imaging	47 %	59 %

Source: pubmed

7.7.2 Mean gap length in comparable studies

The mean gap length in the 100 studies examined amounted to 11.46 mm (minimum: 3 mm; maximum: 50 mm), with 66% of these studies featuring gaps of 10 mm or less. A gap of 10 mm in the sciatic nerve of rats has been shown to be the critical length to bridge in the rat model (MEEK et al., 2003). It has, however, been reported that larger gaps (10 to 15 mm) can be bridged without the support of a nerve graft due to the very good regenerational ability in rats (KEILHOFF et al., 2005) – an assertion which this study does not bear out; this assumption has, however, has resulted in it being regarded imperative to bridge gaps of a minimum of 15 mm when evaluating the qualities of new bridging materials (BELLAMKONDA, 2006).

Regardless of this, the two main reasons why such striking discrepancies between morphometric and functional or electrophysiologic evaluations have most probably not been described in literature before are (1) that the gap length featured in the present study was longer than the critical gap length, and (2) that, rather than providing support, the bridging material seemed to cause the regenerating axons to deteriorate. These two factors meant that very wide variations could be observed between the different groups in the morphometric assessments, whereas the extensive physiotherapy and enriched housing most probably led to increased compensatory mechanisms on the functional side.

7.8 Correlation between different parameters and methods of assessment

Various studies have been conducted on the degree of correlation between the individual outcome measurements in sciatic nerve regeneration studies with rats (DELLON & MACKINNON, 1989; WOLTHERS et al., 2005; MARTINS et al., 2006) with the aim of determining the “best” and most conclusive test (KANAYA et al., 1996).

7.8.1 Correlation of parameters

Comparisons of parameters have found, for example, that there is good correlation between nerve conduction velocities and fibre diameters (WOLTHERS et al., 2005) or myelin thickness (DELLON & MACKINNON, 1989; KANAYA et al., 1996), between the CNAP amplitudes and axonal counts (DELLON & MACKINNON, 1989; KANAYA et al., 1996), and between CNAP amplitudes and nerve conduction velocities (KANAYA et al., 1996). The SFI and the peroneal function index, a derivate of the SFI, correlated closely with the nerve fibre diameter (MARTINS et al., 2006) and the inverted g-ratio (KANAYA et al., 1996), but not with the nerve fibre number (DELLON & MACKINNON, 1989; SHENAQ et al., 1989). On the other hand axon counts agreed with muscle weights (KANAYA et al., 1996) and, not surprisingly, SFI and SSI correlated well (BERVAR et al., 2000). Interestingly, however, the CMAP amplitude did not tally at all with the number of myelinated fibres (WOLTHERS et al., 2005).

7.8.2 Correlation of methods

Comparisons of methods of evaluation have shown that the extensor postural thrust and the SFI correlate closely with one another after crush injuries (HADLOCK et al., 1999). Also electrophysiologic and morphometric assessments correlated appropriately in one study (DELLON & MACKINNON, 1989), but in a very recent study did not match at all (MARTINS et al., 2006). It should, however, be noted that in the latter study the researchers recorded CMAPs from the gastrocnemius muscle after stimulation of the regenerating sciatic nerve, a

technique that has been proven to be rather unreliable, as described in Paper 4. No correlation could be found between SFI, CMAP amplitudes or motor nerve conduction velocities (WOLTHERS et al., 2005). In MRI examinations of muscles, increased hyperintensity in T2-weighted images can be observed at the same time as electrophysiologic examinations detect spontaneous activity (WESSIG et al., 2004).

7.8.3 Observations in the present study

In the present study, the only two factors which showed any degree of correlation were the two footprint analyses, the SFI and SSI. These two evaluations and the morphometric assessments exhibited the same trend regarding the extent of regeneration.

7.8.4 Implications after the lack of correlation

The lack of correlation, especially between functional and electrophysiologic or morphometric data (KANAYA et al., 1996), indicates that all methods cater for different aspects of nerve function and regeneration (KANAYA et al., 1996; WOLTHERS et al., 2005); these are to some extent interlinked, but mostly not exclusively dependent on one another. Consequently no single evaluation method can be recommended as the most comprehensive and “best test” (KANAYA et al., 1996; VAREJÃO et al., 2004a).

7.9 Recommendations for future sciatic nerve regeneration studies in the rat

In conclusion, it is important to note the following points when choosing techniques for evaluating sciatic nerve regeneration in the rat:

(1) In the assessment of sciatic nerve regeneration, different methods relate more, others less, to different stages of regeneration (MARTINS et al., 2006) (see also Section 2.3).

When studying early nerve regeneration, e.g. axonal growth, the appropriate methods such as anterograde tracing or nerve morphology should be applied, as they are more sensitive to slight changes than techniques that have been developed to investigate events further down the chronological line, such as functional integration (DELLON & MACKINNON, 1989; KANAYA et al., 1996).

If, however, the extent of recovery of function is to be evaluated, overall functional assessments should be applied, preferably after exposing the rats to extensive physiotherapy and enriched housing since these have been shown to have a positive influence on motor behaviour in rats after lesions to the CNS (LANKHORST et al., 2001; MOON et al., 2006; STEINER et al., 2006).

(2) Financial circumstances and personal preferences and skills also play a role in choosing the batch of test methods to be used. There is no point in attempting to carry out somatosensory or proprioceptive tests if one does not feel confident and familiar with handling the animals and is not acquainted with the procedures, while morphologic and electrophysiologic evaluations of muscles and nerves require expertise and practice.

(3) A variety of methods should be chosen (VAREJÃO et al., 2004a) in order to ensure comparability and reproducibility of results, and to cover every aspect of regeneration.

(4) All sciatic nerve regeneration studies should include morphometric assessments of the regenerating sciatic nerve performed as close as possible to the end organ examined (CHAMBERLAIN et al., 2000).

Table 6: Recommendations for future peripheral nerve regeneration studies

	Method	Specific assessment
Reinnervation of the distal stump	Electroneurography	Compound nerve action potentials recorded directly from the sciatic nerve
	Nerve morphometry	Total nerve fibre counts, nerve fibre density, nerve fibre distribution and diameters, g-ratio
Reinnervation of the muscle	Electromyography	Compound muscle action potentials recorded from the interosseus muscles
	Muscle volumes	Gastrocnemius muscle
Restoration of anatomical features	Retrograde tracing	Motor pool from the gastrocnemius and interosseus muscles; sensory reinnervation of the medial metatarsus and the lateral aspect of toe 5
Restoration of function	Motor testing	Sciatic Function Index, Static Sciatic Index
	Sensory testing	Pain reaction to pinching the lateral aspect of toe 5
	Overall (ambulatory) testing	Time required and mistakes made when crossing elevated beams and parallel bars

8. Summary

The sciatic nerve of the rat is the site most often used in studies to evaluate new surgical techniques and peripheral nerve regeneration in general.

Prior to such a study, a preliminary anatomical investigation was carried out in which 150 male adult rats of five different strains (Sprague Dawley, Fischer 344, Wistar Han, Lewis and Nude) were assessed to determine the length of sciatic nerve available for nerve regeneration studies. The results established that all the rat strains examined, except the Fischer 344 strain, were equally suitable for peripheral nerve regeneration studies focussing on the sciatic nerve.

In the subsequent main study, a 14 mm segment was extracted from the sciatic nerve of 20 male adult Lewis rats and the defect was repaired either by autograft, newly developed collagen tubes (bovine collagen type I), or collagen tubes filled with denaturated autologous muscle.

The extent of functional regeneration was evaluated by means of ongoing ambulatory assessments, footprint analysis, and somatosensory tests. Additionally, after eight weeks of regeneration, electrophysiologic assessments were performed and the gastrocnemius muscle volumes were determined; the regenerating nerves and contralateral controls were subjected to morphometric and electron microscopic evaluations and retrograde tracing studies of the sensory and motor systems were also conducted.

The results revealed that in all the functional tests, except the footprint analysis, all rats, regardless of their surgical group, had experienced virtually the same amount of regeneration, whereas in the morphometric and electronmicroscopic assessments only the autograft rats exhibited large numbers of regenerating fibres.

The uniformity of the results of the functional assessments was attributed on the one hand to compensatory events (plasticity) which were evident in both the peripheral and central nervous systems, and on the other to methodological problems in the implementation of somatosensory and electrophysiologic investigations.

Consequently new guidelines have been proposed for future somatosensory and electrophysiologic investigations. Furthermore, in order to minimise misinterpretation of functional results, it is strongly advised, that sciatic nerve regeneration studies should always incorporate morphometric assessments of the regenerating nerve as close as possible to the end organ subjected to functional testing. When choosing suitable methods of evaluation for nerve regeneration studies, it is important to bear in mind that most methods cater for different aspects of regeneration, which means that they do not correlate apart from very obviously linked parameters. To ensure comparability and reproducibility of results, it is therefore important to select a variety of methods in line with the relevant scientific objectives.

Funktionelle, elektrophysiologische und morphometrische Beurteilung peripherer Nervenregeneration nach Überbrückung eines 14 mm langen Defekts im N. ischiadicus der Ratte

Experimentelle Studien zur Beurteilung neuer Operationstechniken und dem Regenerationsverhalten peripherer Nerven werden am häufigsten am N. ischiadicus der Ratte durchgeführt.

Vor der Durchführung einer derartigen Studie wurden in einer anatomischen Vorstudie 150 männliche adulte Ratten fünf verschiedener Stämme (Sprague Dawley, Fischer 344, Wistar Han, Lewis und nackte T-cell defiziente Ratten) hinsichtlich ihres Anteils des für Nervenregenerationsstudien nutzbaren N. ischiadicus untersucht. Die Ergebnisse ergaben, dass alle untersuchten Stämme, bis auf die Fischer 344 Ratten, gleich geeignet für entsprechende Studien sind.

Daraufhin wurde in der Hauptstudie 20 männlichen Lewisratten ein 14 mm langes Segment aus dem N. ischiadicus entnommen. Der daraus resultierende Defekt wurde entweder mit einem autologen Nervenstück, mit Kollagenröhren aus bovinem Kollagen Typ I oder mit denselben Kollagenröhren, gefüllt mit denaturierter autologer Muskulatur, überbrückt.

Eine Beurteilung der funktionellen Regeneration erfolgte mittels Fußabdruckanalysen und koordinativen und somatosensorischen Tests. Zusätzlich wurden nach acht Wochen Regenerationszeit elektrophysiologische Untersuchungen durchgeführt, die Gastrocnemiusvolumina bestimmt und die regenerierenden Nerven und ihre kontralateralen Kontrollen morphometrisch und elektronenmikroskopisch beurteilt. Darüberhinaus wurde die Wiederherstellung der sensorischen und motorischen Systeme mit der Hilfe von retrograden Tracern überprüft.

Während alle drei Versuchsgruppen in allen Tests (ausser den Fußabdruckanalysen) einen ähnlich hohen Grad an funktioneller Wiederherstellung aufwiesen, konnten in den morphometrischen und ultrastrukturellen Untersuchungen nur bei den Ratten, die ein autologes Nervenstück erhalten hatten, große Mengen an regenerierenden Nervenfasern gefunden werden.

Die Homogenität der Testergebnisse in den funktionellen Untersuchungen konnte sowohl auf offenkundige kompensatorische Ereignisse des peripheren und zentralen Nervensystems (Plastizität), als auch auf methodologische Probleme in

der Durchführung der somatosensorischen und elektrophysiologischen Tests zurückgeführt werden.

Für die Durchführung von somatosensorischen und elektrophysiologischen Untersuchungen wurden folglich neue Richtlinien vorgeschlagen. Zudem wird davor gewarnt, Nervenregenerationsstudien am N. ischiadicus ohne eine morphometrische Beurteilungen des Nervs in nächster Nähe des durch funktionelle Tests untersuchten Endorgans durchzuführen.

Hinsichtlich der Wahl der Beurteilungsmethoden für künftige Nerven-regenerationsstudien am N. ischiadicus der Ratte kann nur betont werden, dass die meisten Evaluationsmethoden unterschiedliche Aspekte der Nerven-regeneration hinterfragen und daher, bis auf wenige Parameter, nicht miteinander korrelieren. Um die Vergleichbarkeit und Reproduzierbarkeit einer Studie zu wahren, muss daher eine auf die zu untersuchenden Fragen sorgfältig abgestimmte Auswahl an Beurteilungsmethoden angewandt werden.

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Data for the preliminary anatomical investigations

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
011 l	f	fischer	175	31			18	9	14	
011 r	f	fischer	175	30			15	14	9	
017 l	f	fischer	284	36			prox	10	20	20
017 r	f	fischer	284	36			18	9	18	
018 l	f	fischer	250	36			prox	7	16	26
018 r	f	fischer	250	36			prox	9	12	26
019 l	f	fischer	237	33			prox	6	18	27
019 r	f	fischer	237	33			prox	8	20	27
039 l	f	fischer	230	34			15	7	19	
039 r	f	fischer	230	34			11	8	14	
040 l	f	fischer	212	33			prox	10	20	23
040 r	f	fischer	212	33			13	7	12	
041 l	f	fischer	216	34			19	8	21	
041 r	f	fischer	216	34			prox	7	16	22
042 l	f	fischer	195	33			prox	12	19	23
042 r	f	fischer	195	33			13	8	18	
043 l	f	fischer	248	34			prox	9	18	26
043 r	f	fischer	248	34			14	8	12	
044 l	f	fischer	232	35			prox	10	16	25
044 r	f	fischer	232	35			prox	8	17	25
046 l	f	fischer	262	34			21	7	24	
046 r	f	fischer	262	34			prox	8	21	22
047 l	f	fischer	252	34			5	8	21	
047 r	f	fischer	252	34			19	6	20	
050 l	f	fischer	240	35			12	10	25	
050 r	f	fischer	240	35			8	6	18	
051 l	f	fischer	260	35			prox	9	18	25
051 r	f	fischer	260	35			prox	6	16	23
052 l	f	fischer	275	35			10	8	27	
052 r	f	fischer	275	35			prox	8	25	23
055 l	f	wistar	250	32			7	6	19	
055 r	f	wistar	250	32			11	6	18	
056 l	f	wistar	215	31			9	8	24	
056 r	f	wistar	215	31			19	7	29	
057 l	f	wistar	218	31			10	5	20	
057 r	f	wistar	218	31			15	5	15	
058 l	f	wistar	205	30			10	4	13	
058 r	f	wistar	205	30			9	5	23	
059 l	f	wistar	212	30			10	6	15	
059 r	f	wistar	212	30			9	3	19	
060 l	f	wistar	215	31			11	5	16	
060 r	f	wistar	215	31			15	4	11	
061 l	f	wistar	215	31			11	5	18	
061 r	f	wistar	215	31			14	5	26	
062 l	f	wistar	205	30			13	7	26	
062 r	f	wistar	205	30			9	7	12	
063 l	f	wistar	219	31			14	5	11	
063 r	f	wistar	219	31			11	5	17	
064 l	f	wistar	255	34			11	6	22	
064 r	f	wistar	255	34			11	7	13	
065 l	f	wistar	192	30			13	4	18	
065 r	f	wistar	192	30			18	9	7	
066 l	f	wistar	251	34			13	6	25	
066 r	f	wistar	251	34			12	4	30	
067 l	f	wistar	193	30			15	5	26	
067 r	f	wistar	193	30			13	4	30	
068 l	f	wistar	203	31			10	4	21	
068 r	f	wistar	203	31			15	7	8	
069 l	f	wistar	204	31			11	4	16	

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
069 r	f	wistar	204	31			11	5	12	
070 l	f	wistar	212	32			18	6	17	
070 r	f	wistar	212	32			13	8	20	
071 l	f	wistar	226	32			15	9	23	
071 r	f	wistar	226	32			11	5	28	
072 l	f	wistar	215	32			16	8	11	
072 r	f	wistar	215	32			12	4	9	
075 l	f	lewis	230	35			5	5	28	
075 r	f	lewis	230	35			8	5	19	
076 l	f	lewis	260	35			10	4	24	
076 r	f	lewis	260	35			6	4	13	
077 l	f	lewis	205	34			11	11	21	
077 r	f	lewis	205	34			11	7	26	
078 l	f	lewis	220	34			6	6	20	
078 r	f	lewis	220	34			9	8	19	
081 l	m	lewis	310	37	38	15	7	7	16	
081 r	m	lewis	310	37	38	7	5	4	12	
082 l	m	s-d	260	36	40	14	9	6	19	
082 r	m	s-d	260	36	40	14	8	12	15	
083 l	m	s-d	225	37	40	18	12	12	24	
083 r	m	s-d	225	37	40	15	7	7	18	
084 l	m	s-d	228	37	40	14	10	6	17	
084 r	m	s-d	228	37	40	13	7	4	21	
085 l	m	s-d	405	39	42	9	8	9	19	
085 r	m	s-d	405	39	42	11	0	10	19	
086 l	m	s-d	400	39	43	15	8	10	23	
086 r	m	s-d	400	39	43	18	9	14	6	
087 l	m	s-d	372	36	43	16	10	9	16	
087 r	m	s-d	372	36	43	18	12	11	12	
088 l	m	s-d	395	36	43	17	6	5	11	
088 r	m	s-d	395	36	43	prox	11	9	21	27
089 l	f	wistar	300	36	36	11	9	6	14	
089 r	f	wistar	300	36	36	10	6	7	22	
090 l	f	wistar	310	37	37	12	7	7	18	
090 r	f	wistar	310	37	37	10	8	6	30	
091 l	m	s-d	345	37	41	12	9	6	17	
091 r	m	s-d	345	37	41	14	9	10	16	
092 l	m	s-d	375	39	42	10	8	6	18	
092 r	m	s-d	375	39	42	12	9	9	12	
093 l	m	s-d	344	38	42	11	7	5	20	
093 r	m	s-d	344	38	42	10	7	9	20	
094 l	m	s-d	367	37	41	9	5	5	13	
094 r	m	s-d	367	37	41	16	7	10	12	
095 l	m	s-d	368	37	42	prox	8	5	15	24
095 r	m	s-d	368	37	42	prox	9	7	16	22
096 l	m	s-d	380	39	42	20	12	9	18	
096 r	m	s-d	380	39	42	17	9	8	19	
097 l	m	nackt	210	31	38	10	7	9	14	
097 r	m	nackt	210	31	38	12	7	5	12	
099 l	m	nackt	260	37	38	12	5	6	5	
099 r	m	nackt	260	37	38	10	5	4	5	
100 l	m	s-d	349	40	41	13	8	6	21	
100 r	m	s-d	349	40	41	16	8	5	24	
101 l	m	s-d	470	45	42	21	10	12	24	
101 r	m	s-d	470	45	42	19	9	10	18	
102 l	m	s-d	450	45	43	13	11	9	17	
102 r	m	s-d	450	45	43	12	10	7	18	
104 l	m	s-d	350	35	41	12	5	6	13	
104 r	m	s-d	350	35	41	12	8	9	16	
105 l	m	s-d	350	36	41	16	8	7	17	
105 r	m	s-d	350	36	41	17	8	7	11	
106 l	m	s-d	350	35	42	14	7	9	28	
106 r	m	s-d	350	35	42	11	10	10	22	
107 l	m	s-d	340	37	41	9	7	8	18	
107 r	m	s-d	340	37	41	10	8	6	22	

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
108 l	m	s-d	320	38	39	7	5	7	21	
108 r	m	s-d	320	38	39	9	7	9	24	
109 l	m	s-d	396	38	42	11	8	11	18	
109 r	m	s-d	396	38	42	13	10	10	14	
110 l	m	s-d	348	37	43	13	7	7	15	
110 r	m	s-d	348	37	43	11	6	7	19	
111 l	m	s-d	359	38	42	10	3	6	18	
111 r	m	s-d	359	38	42	12	6	10	15	
112 l	m	nackt	230	35	38	8	6	8	17	
112 r	m	nackt	230	35	38	10	8	8	18	
113 l	m	fischer	310	34	38	prox	prox	8	12	24
113 r	m	fischer	310	34	38	23	19	10	17	
114 l	m	s-d	370	34	42	11	5	7	18	
114 r	m	s-d	370	34	42	12	6	6	12	
115 l	m	nackt	240	31	38	17	7	7	12	
115 r	m	nackt	240	31	38	8	3	5	12	
116 l	m	lewis	390	43	39	14	9	10	13	
116 r	m	lewis	390	43	39	10	8	8	25	
117 l	m	s-d	378	38	40	15	9	8	31	
117 r	m	s-d	378	38	40	13	8	6	31	
118 l	m	s-d	396	39	40	16	9	10	10	
118 r	m	s-d	396	39	40	13	8	8	18	
119 l	m	s-d	380	37	40	16	14	5	22	
119 r	m	s-d	380	37	40	12	7	6	31	
120 l	m	s-d	385	38	41	12	9	6	13	
120 r	m	s-d	385	38	41	12	10	5	17	
121 l	m	nackt	220	30	36	7	5	6	5	
121 r	m	nackt	220	30	36	8	3	4	5	
122 l	m	nackt	224	31	37	10	6	5	6	
122 r	m	nackt	224	31	37	9	4	6	3	
123 l	m	nackt	220	32	38	7	5	6	11	
123 r	m	nackt	220	32	38	9	5	5	7	
128 l	m	lewis	260	34	38	11	8	8	21	
128 r	m	lewis	260	34	38	prox	prox	7	18	24
129 l	m	fischer	230	34	39	prox	prox	5	16	24
129 r	m	fischer	230	34	39	prox	17	6	17	
130 l	m	lewis	350	39	38	6	3	5	20	
130 r	m	lewis	350	39	38	11	9	6	20	
131 l	m	fischer	250	35	38	prox	prox	10	20	21
131 r	m	fischer	250	35	38	prox	prox	10	16	23
133 l	m	nackt	290	38	37	13	6	7	9	
133 r	m	nackt	290	38	37	13	5	7	7	
136 l	m	lewis	460	41	37	11	8	8	19	
136 r	m	lewis	460	41	37	18	12	13	21	
137 l	m	lewis	460	43	40	10	6	7	20	
137 r	m	lewis	460	43	40	13	8	8	18	
138 l	m	fischer	260	34	38	12	8	8	17	
138 r	m	fischer	260	34	38	prox	prox	10	17	22
139 l	m	fischer	260	35	38	prox	prox	5	13	24
139 r	m	fischer	260	35	38	10	6	7	17	
140 l	m	fischer	260	34	38	prox	prox	5	17	20
140 r	m	fischer	260	34	38	10	8	7	15	
141 l	m	lewis	440	40	40	10	7	5	31	
141 r	m	lewis	440	40	40	14	10	11	23	
142 l	m	fischer	220	31	37	6	4	7	21	
142 r	m	fischer	220	31	37	5	3	3	17	
143 l	m	nackt	250	34	37	11	4	5	12	
143 r	m	nackt	250	34	37	10	5	5	15	
144 l	m	fischer	270	34	36	prox	prox	6	17	20
144 r	m	fischer	270	34	36	prox	prox	6	22	20
145 l	m	fischer	280	35	39	6	4	8	22	
145 r	m	fischer	280	35	39	prox	prox	8	21	22
146 l	m	fischer	290	35	40	prox	prox	9	18	22
146 r	m	fischer	290	35	40	prox	prox	10	20	22
147 l	m	fischer	260	32	40	prox	prox	7	20	21

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
147 r	m	fischer	260	32	40	prox	prox	8	17	21
148 l	m	fischer	240	31	39	9	5	5	18	
148 r	m	fischer	240	31	39	prox	prox	5	18	20
149 l	m	fischer	360	38	40	18	10	11	17	
149 r	m	fischer	360	38	40	17	10	12	20	
150 l	m	lewis	340	37	41	12	8	8	19	
150 r	m	lewis	340	37	41	13	11	10	21	
151 l	m	lewis	420	38	41	18	10	12	24	
151 r	m	lewis	420	38	41	16	8	10	24	
153 l	m	fischer	140	30	38	prox	prox	8	14	18
153 r	m	fischer	140	30	38	prox	prox	6	14	18
154 l	m	fischer	130	30	37	prox	prox	2	16	18
154 r	m	fischer	130	30	37	prox	prox	5	15	18
157 l	m	fischer	160	28	36	8	6	4	15	
157 r	m	fischer	160	28	36	prox	prox	4	15	18
158 l	m	fischer	180	28	37	prox	prox	6	12	18
158 r	m	fischer	180	28	37	prox	14	7	13	
159 l	m	fischer	130	29	37	7	4	5	16	
159 r	m	fischer	130	29	37	prox	prox	6	15	20
160 l	f	lewis	270	30	37	13	5	7	21	
160 r	f	lewis	270	30	37	prox	8	7	20	
161 l	f	lewis	320	33	39	17	6	9	22	
161 r	f	lewis	320	33	39	9	7	7	22	
162 l	m	lewis	440	39	40	12	5	8	24	
162 r	m	lewis	440	39	40	15	8	7	24	
163 l	m	lewis	460	41	41	15	6	7	30	
163 r	m	lewis	460	41	41	12	8	10	29	
164 l	f	lewis	250	30	36	7	3	5	21	
164 r	f	lewis	250	30	36	8	8	7	28	
165 l	f	lewis	280	31	37	11	5	6	14	
165 r	f	lewis	280	31	37	15	7	8	21	
166 l	m	lewis	450	38	40	16	8	9	18	
166 r	m	lewis	450	38	40	15	8	7	31	
167 l	m	lewis	490	41	42	13	8	10	22	
167 r	m	lewis	490	41	42	15	10	13	23	
168 l	m	lewis	460	39	40	15	10	5	14	
168 r	m	lewis	460	39	40	18	10	10	31	
169 l	m	lewis	450	38	39	16	10	9	18	
169 r	m	lewis	450	38	39	18	7	8	28	
170 l	m	lewis	420	40	38	16	6	5	25	
170 r	m	lewis	420	40	38	prox	7	8	25	
171 l	m	lewis	500	42	41	16	8	9	24	
171 r	m	lewis	500	42	41	16	6	8	25	
172 l	m	lewis	520	42	41	13	8	7	21	
172 r	m	lewis	520	42	41	20	10	11	31	
173 l	m	fischer	190	31	37	11	8	5	15	
173 r	m	fischer	190	31	37	prox	17	5	18	
174 l	m	fischer	210	32	40	10	10	7	13	
174 r	m	fischer	210	32	40	prox	prox	6	16	22
177 l	m	fischer	200	30	39	prox	prox	6	19	18
177 r	m	fischer	200	30	39	prox	prox	5	16	18
178 l	m	fischer	200	32	37	prox	15	5	16	
178 r	m	fischer	200	32	37	8	5	6	19	
179 l	m	fischer	210	30	40	5	3	5	19	
179 r	m	fischer	210	30	40	prox	prox	5	14	19
182 l	m	fischer	380	39	37	13	13	6	20	
182 r	m	fischer	380	39	37	9	5	5	16	
183 l	m	fischer	210	32	37	prox	prox	7	18	18
183 r	m	fischer	210	32	37	16	8	4	12	
184 l	m	fischer	215	32	38	prox	prox	5	16	18
184 r	m	fischer	215	32	38	prox	prox	6	15	17
185 l	m	fischer	215	32	39	prox	prox	7	19	24
185 r	m	fischer	215	32	39	prox	prox	7	21	24
186 l	m	fischer	215	31	38	prox	prox	6	20	23
186 r	m	fischer	215	31	38	prox	prox	6	19	23

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
187 l	m	fischer	230	32	39	prox	prox	6	20	25
187 r	m	fischer	230	32	39	prox	prox	5	18	25
190 l	m	fischer	150	26	37	prox	prox	4	15	18
190 r	m	fischer	150	26	37	prox	prox	5	18	18
191 l	m	nackt	300	36	41	6	3	7	12	
191 r	m	nackt	300	36	41	prox	15	7	7	21
192 l	m	nackt	250	32	38	5	2	6	15	
192 r	m	nackt	250	32	38	5	3	5	8	
193 l	m	nackt	250	32	39	15	10	9	9	
193 r	m	nackt	250	32	39	prox	prox	7	8	20
194 l	m	nackt	250	31	36	10	4	5	12	
194 r	m	nackt	250	31	36	12	6	8	4	
195 l	m	lewis	250	33	38	10	4	4	16	
195 r	m	lewis	250	33	38	10	5	6	18	
196 l	m	nackt	260	34	40	0	0	5	7	
196 r	m	nackt	260	34	40	6	2	8	14	
197 l	m	nackt	250	35	38	5	4	6	12	
197 r	m	nackt	250	35	38	7	3	8	13	
198 l	m	nackt	260	34	39	prox	15	6	22	
198 r	m	nackt	260	34	39	4	4	5	12	
199 l	m	nackt	245	33	38	5	2	4	13	
199 r	m	nackt	245	33	38	10	8	6	12	
202 l	m	s-d	325	37	44	9	6	5	16	
202 r	m	s-d	325	37	44	12	8	5	25	
207 l	m	lewis	294	36	42	10	7	7	15	
207 r	m	lewis	294	36	42	9	4	5	18	
210 l	m	lewis	270	32	37	5	3	6	24	
210 r	m	lewis	270	32	37	8	5	6	19	
211 l	m	nackt	230	32	38	8	5	7	13	
211 r	m	nackt	230	32	38	10	4	5	10	
212 l	m	nackt	350	36	40	18	16	9	13	
212 r	m	nackt	350	36	40	prox	prox	13	15	22
213 l	m	nackt	238	34	37	5	4	4	21	
213 r	m	nackt	238	34	37	prox	prox	6	13	18
214 l	m	nackt	375	39	40	prox	10	9	14	
214 r	m	nackt	375	39	40	prox	prox	6	12	23
215 l	m	lewis	280	35	40	6	6	6	24	
215 r	m	lewis	280	35	40	12	11	4	22	
216 l	m	nackt	190	32	38	5	1	7	14	
216 r	m	nackt	190	32	38	7	1	8	8	
217 l	m	lewis	200	33	40	9	8	8	12	
217 r	m	lewis	200	33	40	12	5	5	17	
218 l	m	nackt	250	34	40	prox	prox	8	14	21
218 r	m	nackt	250	34	40	7	4	5	16	
219 l	m	nackt	250	34	37	3	2	4	16	
219 r	m	nackt	250	34	37	10	5	6	20	
221 l	m	wistar	420	40	40	7	5	7	21	
221 r	m	wistar	420	40	40	5	3	9	30	
222 l	m	lewis	210	31	39	7	6	4	18	
222 r	m	lewis	210	31	39	9	7	5	21	
223 l	m	nackt	225	34	36	prox	prox	6	15	20
223 r	m	nackt	225	34	36	6	1	5	21	
224 l	m	lewis	240	34	38	5	5	5	21	
224 r	m	lewis	240	34	38	6	4	4	20	
225 l	m	lewis	255	34	38	6	4	4	20	
225 r	m	lewis	255	34	38	7	5	5	20	
226 l	m	nackt	230	33	37	5	2	9	12	
226 r	m	nackt	230	33	37	4	4	4	15	
227 l	m	nackt	175	31	37	2	2	4	22	
227 r	m	nackt	175	31	37	4	2	4	17	
228 l	m	lewis	220	35	38	4	4	6	28	
228 r	m	lewis	220	35	38	11	6	5	23	
229 l	m	nackt	315	36	41	5	2	3	8	
229 l	m	nackt	315	36	41	5	2	3	8	
229 r	m	nackt	315	36	41	5	2	5	13	

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
229 r	m	nackt	315	36	41	5	2	5	13	
231 l	m	lewis	210	32	39	9	8	6	21	
231 r	m	lewis	210	32	39	9	6	5	19	
233 l	m	wistar	480	43	38	20	19	11	25	
233 r	m	wistar	480	43	38	17	11	9	24	
237 l	m	nackt	285	35	37	11	6	6	6	
237 r	m	nackt	285	35	37	10	5	5	9	
238 l	f	wistar	286	34	39	10	7	9	24	
238 r	f	wistar	286	34	39	16	8	9	22	
239 l	f	wistar	230	31	38	7	5	6	23	
239 r	f	wistar	230	31	38	16	7	9	23	
240 l	f	wistar	248	32	39	14	10	12	22	
240 r	f	wistar	248	32	39	prox	prox	9	20	24
241 l	f	wistar	218	31	37	12	8	10	23	
241 r	f	wistar	218	31	37	10	4	6	23	
242 l	f	wistar	238	31	39	12	10	9	21	
242 r	f	wistar	238	31	39	18	8	7	22	
243 l	m	wistar	374	39	42	10	6	7	24	
243 r	m	wistar	374	39	42	15	7	8	22	
244 l	m	wistar	372	36	39	11	10	10	24	
244 r	m	wistar	372	36	39	17	6	6	23	
245 l	m	wistar	377	38	41	13	8	7	23	
245 r	m	wistar	377	38	41	prox	prox	15	24	24
246 l	m	wistar	383	37	42	14	10	9	25	
246 r	m	wistar	383	37	42	15	6	8	25	
247 l	m	wistar	332	37	42	11	7	7	23	
247 r	m	wistar	332	37	42	15	5	8	24	
248 l	f	wistar	247	32	37	15	7	8	18	
248 r	f	wistar	247	32	37	19	5	8	21	
249 l	f	wistar	259	33	39	13	6	7	22	
249 r	f	wistar	259	33	39	prox	5	6	18	22
250 l	f	wistar	248	34	40	16	6	9	23	
250 r	f	wistar	248	34	40	5	3	6	22	
251 l	f	wistar	214	33	39	11	6	7	23	
251 r	f	wistar	214	33	39	8	4	7	23	
252 l	f	wistar	232	33	40	10	10	11	25	
252 r	f	wistar	232	33	40	15	8	7	24	
253 l	m	wistar	395	38	43	14	14	13	25	
253 r	m	wistar	395	38	43	8	5	6	24	
254 l	m	wistar	248	40	43	11	8	8	25	
254 r	m	wistar	248	40	43	16	6	7	25	
255 l	m	wistar	377	36	43	13	4	5	25	
255 r	m	wistar	377	36	43	prox	prox	8	26	24
256 l	f	wistar	228	32	40	15	8	9	23	
256 r	f	wistar	228	32	40	12	4	5	23	
257 l	f	wistar	260	34	40	7	6	5	27	
257 r	f	wistar	260	34	40	16	5	7	23	
258 l	f	wistar	262	33	40	14	5	6	24	
258 r	f	wistar	262	33	40	15	4	5	22	
259 l	m	wistar	414	38	45	11	3	5	25	
259 r	m	wistar	414	38	45	17	3	6	25	
260 l	m	wistar	321	35	42	15	6	8	12	
260 r	m	wistar	321	35	42	10	6	7	11	
261 l	f	wistar	212	30	38	11	4	5	16	
261 r	f	wistar	212	30	38	5	4	5	18	
262 l	f	wistar	253	33	36	10	6	6	17	
262 r	f	wistar	253	33	36	15	4	5	17	
263 l	f	wistar	241	32	36	11	4	6	25	
263 r	f	wistar	241	32	36	11	4	5	25	
264 l	m	wistar	395	36	42	7	6	5	25	
264 r	m	wistar	395	36	42	15	6	6	26	
265 l	f	wistar	262	32	39	prox	prox	4	23	22
265 r	f	wistar	262	32	39	prox	prox	5	20	22
266 l	m	wistar	408	33	40	6	5	5	24	
266 r	m	wistar	408	33	40	7	5	5	23	

Rat r/l	m/f	strain	weight	D0	foot	D1	D2	D3	D4	D5
			[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
267 l	m	wistar	412	37	44	12	7	6	25	
267 r	m	wistar	412	37	44	prox	prox	9	25	24
268 l	m	wistar	424	39	44	12	5	7	23	
268 r	m	wistar	424	39	44	11	5	6	22	
269 l	m	wistar	450	38	43	10	5	6	24	
269 r	m	wistar	450	38	43	8	5	8	26	
270 l	m	wistar	390	35	41	10	6	7	22	
270 r	m	wistar	390	35	41	9	3	5	22	
271 l	m	wistar	439	35	41	prox	prox	7	21	26
271 r	m	wistar	439	35	41	19	8	8	23	
272 l	m	wistar	460	38	42	13	7	10	23	
272 r	m	wistar	460	38	42	10	6	7	23	
273 l	m	wistar	485	38	44	prox	10	10	27	24
273 r	m	wistar	485	38	44	12	7	10	19	
274 l	m	wistar	434	36	41	16	9	10	24	
274 r	m	wistar	434	36	41	19	7	10	22	
275 l	m	wistar	413	37	43	13	8	10	23	
275 r	m	wistar	413	37	43	7	4	5	25	
276 l	m	wistar	467	38	43	12	6	7	25	
276 r	m	wistar	467	38	43	7	5	6	23	
277 l	m	wistar	365	36	41	10	5	7	23	
277 r	m	wistar	365	36	41	6	3	3	25	
278 l	m	wistar	477	38	43	15	6	6	20	
278 r	m	wistar	477	38	43	8	7	6	25	
279 l	m	wistar	431	39	44	12	7	8	23	
279 r	m	wistar	431	39	44	12	8	8	25	
280 l	m	wistar	505	40	45	12	5	6	25	
280 r	m	wistar	505	40	45	19	5	6	25	
281 l	m	wistar	438	40	42	17	10	9	23	
281 r	m	wistar	438	40	42	23	7	7	26	
282 l	m	wistar	431	38	44	12	7	7	25	
282 r	m	wistar	431	38	44	10	5	5	26	23
283 l	m	lewis	250	34	39	7	6	6	26	22
283 r	m	lewis	250	34	39	9	4	4	26	23
284 l	m	lewis	283	34	40	11	10	8	23	23
284 r	m	lewis	283	34	40	15	8	8	21	23

On the following pages:

Data for the main study

n.c. means not calculated

RAT	V1 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,23	5,12	4,21	3,63	3,84	4,92	4,82	4,84	4,78	3,62	2,97	2,44	3,47	3,13	3,03	2,5	3,28
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
		s		4,86	7,22	7,82	6,75	6,71	5,94	5,9	5,13	4,51	5,43	3,17	6,03	4,78	3,94	4,66	3,63	4,06
	2 cm	mistakes	total	0	5	2	0	0	0	0	0	0	0	0	1	8	3	0	1	1
			placing	0	0	0	0	0	0	0	0	0	0	0	0	8	3	0	0	0
			slip	0	5	2	0	0	0	0	0	0	0	0	1	0	0	0	1	1
		s		5,00	8,06	7,35	6,75	6,88	5,84	7,42	6,66	4,78	7,50	5,55	6,52	6,12	5,47	3,57	3,91	4,25
	1,5 cm	mistakes	total	0	8	8	0	0	8	4	2	1	0	0	0	2	1	0	0	0
			placing	0	6	8	0	0	5	1	2	1	0	0	0	0	0	0	0	0
			slip	0	2	0	0	0	3	3	0	0	0	0	0	2	1	0	0	0
		s		7,66	17,73	14,69	10,28	11,62	9,67	13,85	9,85	8,77	7,87	6,50	8,31	5,50	5,72	4,78	4,22	4,97
	1 cm	mistakes	total	1			4	8	8	8	2	4	0	0	1	8	1	0	0	1
			placing	1			4	8	8	8	2	2	0	0	0	8	0	0	0	0
			slip	0			0	0	0	0	0	2	0	0	1	0	1	0	0	1
		s		29,07	0	0	17,80	20,19	21,66	19,80	13,87	13,44	12,43	7,43	6,84	4,47	8,25	7,50	8,79	5,31
	II 1 cm	mistakes	total	0	7	8	5	5	5	7	7	6	3	5	4	6	6	2	1	1
			placing	0	3	3	2	3	2	3	0	2	0	0	0	5	3	0	0	0
			slip	0	4	5	3	2	3	4	7	4	3	5	4	1	3	2	1	1
		s		9,96	16,46	16,09	11,26	10,66	8,81	11,52	9,53	7,06	7,31	8,03	6,32	7,10	9,84	6,37	5,71	5,19
	II 0,8 cm	mistakes	total	0	3	3	8	3	5	6	5	6	7	3	5	7	2	2	1	1
			placing	0	3	2	1	1	2	0	0	2	0	0	0	2	0	0	0	0
			slip	0	0	1	7	2	3	6	5	4	7	3	5	5	2	2	1	1
		s		7,55	17,38	12,31	13,46	9,66	9,71	9,45	11,68	6,71	9,05	7,00	7,34	7,84	5,72	6,96	7,41	5,72
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	11	12	12	11	10	9	11	10	10	7	7	6	7	6	4	5
		0 - 15	l plantar	0	15	14	15	13	13	13	10	10	8	9	10	10	7	3	1	0
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	10	10	10	7	7	8	6	5	5	6	5	3	4	1	0
Prints		mm	r TS	18,3	18,50	19,3	19,6	18,6	17	19	20,3	19	18,6	20	19,6	22	21,6	21,6	20,3	19,6
		mm	l TS	19		6	6,3	6,6	6,3	7	7,3	7,6	7,6	8	11,3	6,3	10	15,6	17,3	16
		mm	r ITS	10,6	12	11	12,6	14,3	12	11	12	10,6	12	13	12,6	13,6	14,6	14,3	14,3	13
		mm	l ITS	10,6		5	4,6	4,6	4	4,3	4,3	4,3	4	4	4,6	5	5	4,6	6,3	6,6
	SSI (mean)			-2,02	n.c.	-97,80	-96,75	-99,99	-96,80	-91,62	-95,50	-90,48	-94,88	-94,36	-77,41	-102,22	-65,20	-55,20	-40,89	-43,24
		mm	r TS	19,6	19,6	20,3	20,6	20,3	20,3	19,6	20,3	20	20,3	20	20,6	22,3	20,6	21,3	21	21,3
		mm	l TS	19,6	3	5,6	7	6,6	8	8,6	9	9,6	8	7,6	8			10	9	
		mm	r ITS	9	11	10,3	10,6	11,6	10,3	10,3	9,6	10,3	10	10,3	10,6	11	11	10,3	10,3	9,3
		mm	l ITS	9	3,3	4,6	4	4	5	4,6	4,6	5	5	4,6	4			6	6	
		mm	r PL	20,2	24,3	27,6	27,6	26,3	28	28	29,6	26,3	28,3	27	26,3	22	22	22,6	23	27,3
		mm	l PL	21,3	32	36,3	38,5	38,3	37,3	40,3	38	38	37,3	33,6	37			34	30	
	SFI (mean)			-11,10	-110,81	-100,58	-103,12	-105,01	-89,76	-97,48	-90,40	-95,44	-96,67	-97,99	-96,21	n.c	n.c.	-93,70	-101,40	n.c.
ENG		m/s	r	48																
		m/s	l	43																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	8193		N. plantaris		r	1725		
		ml	l	1								l	10253				l	1086		

RAT	V2 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,64	3,55	4,06	5,35	3,9	3,25	2,91	2,98	3,25	3,65	2,53	3,29	2,28	2,25	2,81	2,78	2,88
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,55	4,89	4,97	5,25	6,43	4,54	3,88	3,17	3,81	4,72	3,72	4,16	3,78	3,87	4,31	3,75	3,31
	2 cm	mistakes	total	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
		s		2,70	6,56	4,60	5,92	7,44	6,56	5,94	6,34	4,76	6,31	5,65	4,43	4,32	4,69	5,66	4,13	4,92
	1,5 cm	mistakes	total	0	4	0	3	2	2	0	6	0	0	0	0	0	2	0	1	1
			placing	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
			slip	0	3	0	2	2	2	0	5	0	0	0	0	0	2	0	1	1
		s		5,07	7,99	8,82	11,95	14,46	8,10	5,65	10,34	7,15	6,38	5,64	7,16	6,04	8,15	5,06	5,78	7,18
	1 cm	mistakes	total	0			5	1	1	3	2	2	2	0	3	0	2	0	1	1
			placing	0			5	1	1	3	2	2	1	0	2	0	0	0	0	0
			slip	0			0	0	0	0	0	0	1	0	1	0	2	0	1	1
		s		5,75	0	0	23,77	17,25	13,56	21,25	18,82	14,34	9,69	5,47	9,22	7,87	6,44	7,13	8,21	6,35
	II 1 cm	mistakes	total	0	6	7	7	7	6	8	6	8	4	6	7	6	6	6	6	6
			placing	0	2	2	2	1	1	0	2	1	0	4	3	1	1	0	0	1
			slip	0	4	5	5	6	5	8	4	7	4	2	4	5	5	6	6	5
		s		7,08	12,28	13,09	10,87	14,61	11,06	10,57	7,66	8,62	8,94	8,94	7,16	7,69	8,13	7,44	7,22	7,37
	II 0,8 cm	mistakes	total	0	7	5	5	4	4	6	6	8	5	7	6	4	4	7	7	7
			placing	0	2	2	0	1	1	0	4	3	0	2	2	0	0	2	0	2
			slip	0	5	3	5	3	3	6	2	5	5	5	4	4	4	5	7	5
		s		6,51	10,01	10,78	12,68	9,84	9,03	8,38	10,00	8,37	8,41	7,44	6,41	6,91	7,44	7,32	8,96	7,00
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	10	12	12	12	12	11	11	10	10	8	9	9	7	6	7	5
		0 - 15	l plantar	0	15	14	14	13	13	12	7	7	6	7	7	4	7	4	3	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	10	10	10	8	8	6	6	6	6	6	4	6	6	6	5
Prints		mm	r TS	19,6	18,60	20,3	19,6	20	19,6	20,6	22	20,3	20,6	21	19	20,3	19,6	20,3	20	21
		mm	l TS	19,6	6	8	6	6,6	6,6	7	7	7	7,3	7	7,3	7,3	8	9,6	8,6	9
		mm	r ITS	12	13	13	10,6	12	11,6	10,6	13,6	14	14,6	13,6	11	13	12,6	13,6	12,3	12
		mm	l ITS	12	3,3	3,6	3,6	3,6	3,6	4	4	4,3	4,3	4,3	4,3	4	4	4,6	4	4,3
	SSI (mean)			-8,47	-95,80	-93,32	-97,68	-97,47	-97,07	-95,59	-98,08	-95,65	-95,77	-102,65	-93,73	-99,68	-89,75	-83,43	-83,75	-86,47
		mm	r TS	19,6	20	20,6	20	20	20,6	20	21	20	20	20,3	19,6	20,3	20	20,6	19,6	20,3
		mm	l TS	19,6	6	5,6	6,3	6,6	6,6	8,3	9	8,3	10	11	11,3	9,6	8,3	7	8	7,3
		mm	r ITS	10	11	10,3	10,6	11	10	10	10,3	10	10	9,3	10	10	9,6	10	10,3	11
		mm	l ITS	10,3	3	3,3	3,6	4,6	4	4	4,6	5,3	5	6,3	6,6	5	5,3	4	4,3	5,3
		mm	r PL	21	26	27,6	28	28,6	26,3	26,3	28,3	27	28	30	27,6	26,6	29	26,6	27	26,3
		mm	l PL	22,6	34	28,3	35,5	35	34	37	35,3	35,3	27	39	38	38,6	36	37	38	38,6
	SFI (mean)			-11,94	-101,11	-104,24	-99,04	-90,07	-101,98	-96,31	-91,11	-89,36	-79,30	-76,41	-80,86	-83,32	-83,12	-97,78	-96,43	-103,97
ENG		m/s	r	60																
		m/s	l	41																
Postmortem	Gastrocnemius	ml	r	2,4		Morphometry: myelinated fibres				N. ischiadicus		r	7614		N. plantaris		r	1816		
		ml	l	0,6								l	13480				l	0		

RAT	V3 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	6 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,89	3,39	5,21	3,69	4,10	3,95	3,84	3,84	3,56	3,44	4,09	3,03	3,47	3,25	3,22	3,09	2,94
	3 cm	mistakes	total	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		5,27	6,64	6,92	5,47	4,09	3,63	4,18	4,09	4,15	3,69	4,31	4,81	6,13	5,03	3,78	4,31	4,00
	2 cm	mistakes	total	0	8	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	3	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,85	8,99	7,03	4,94	4,59	4,56	5,81	5,59	4,86	6,4	5,59	4,56	5,97	4,37	5,31	4,18	5,22
	1,5 cm	mistakes	total	0	8	8	8	5	5	2	1	0	0	0	0	0	0	0	2	0
			placing	0	1	8	8	3	0	1	0	0	0	0	0	0	0	0	0	0
			slip	0	7	0	0	2	5	1	1	0	0	0	0	0	0	0	2	0
		s		4,76	8,14	8,21	5,8	9,16	5,75	6,24	6,80	9,88	6,71	7,6	7,23	5,29	5,94	6,16	8,44	5,07
	1 cm	mistakes	total	0		8	8	7	1	8	5	0	1	0	0	0	0	0	2	1
			placing	0		8	8	2	1	8	3	0	1	0	0	0	0	0	0	0
			slip	0		0	0	5	0	0	2	0	0	0	0	0	0	0	2	1
		s		12,92	0	24,18	18,81	10,06	10,62	13,90	13,62	6,69	8,90	10,25	6,60	7,32	7,66	7,32	8,06	7,03
	II 1 cm	mistakes	total	0	7	8	7	5	6	4	6	7	6	7	4	7	7	6	4	4
			placing	0	2	2	2	0	1	0	2	1	0	0	0	0	1	4	0	1
			slip	0	5	6	5	5	5	4	4	6	6	7	4	7	6	2	4	3
		s		5,18	13,88	11,66	7,69	7,59	7,12	6,99	9,56	7,87	5,37	7,37	6,56	7,06	7,84	7,72	7,31	7,88
	II 0,8 cm	mistakes	total	0	5	5	5	7	8	5	3	5	6	6	5	4	7	7	8	5
			placing	0	1	1	0	0	0	0	0	0	3	0	1	0	1	4	0	1
			slip	0	4	4	5	7	8	5	3	5	3	6	4	4	6	3	8	4
		s		4,64	12,73	8,41	6,97	8,00	8,45	6,12	6,53	7,63	7,09	8,38	6,78	6,28	6,72	7,59	8,85	7,35
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	12	10	10	11	8	9	10	11	9	8	7	6	5
		0 - 15	l plantar	0	13	14	15	12	9	10	9	7	9	7	10	10	4	8	5	5
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	10	10	9	7	8	7	7	7	8	7	7	7	7	3	5
Prints		mm	r TS	19,6	19,60	19,3	19	20	20	20,6	20	21,3	21,3	21,3	21	21,6	20	21,6	20,6	20
		mm	l TS	19,6	7	6	6,5	6,6	7	7	7,6	7,6	9,6	10,6	13,6	16,6	17	19,3	19,6	19
		mm	r ITS	11,6	12	9,6	10	10,6	13,3	11	11,6	11,6	12,6	13,6	12	11,3	12,6	15	12,3	13
		mm	l ITS	12,3	4	4	4	4,3	4	4	4	4	4,3	4,3	4,6	4,6	6,3	6,6	6,6	7
	SSI (mean)			-2,00	-96,58	-96,04	-92,18	-95,25	-96,98	-96,61	-94,93	-91,36	-88,98	-79,12	-64,81	-45,73	-35,11	-33,38	-22,08	-24,30
		mm	r TS	21		20	20,3	20,6	21,6	22,6	21	22,3	20	21,3	21,3	21,3	22,6	22,6	23	21,6
		mm	l TS	20		6	6,6	8,6	7,6	8,6	9	9	10,3	13	14,6	15,3	13	15,6	15,3	15,5
		mm	r ITS	7,6		8	9	9,6	10,3	10,6	10,3	10	9,6	9,3	9,6	8,6	9	10	9,6	11,3
		mm	l ITS	10		3	4	4,6	4,6	5,3	4,6	5	6,3	5,3	5	4,6	7,6	6	4,3	5,5
		mm	r PL	21,6		20	25,6	26,5	24	28,6	29	24,3	27	27	24	25,3	26	26,3	25	28,3
		mm	l PL	22,3		35	36,6	35,6	37,3	38,6	35,6	28	36,6	40	38	39	38	37,3	38	40
	SFI (mean)			-13,05	n.c.	-121,26	-104,18	-94,14	-96,46	-96,79	-84,75	-101,67	-81,87	-83,60	-71,53	-70,68	-69,01	-66,22	-70,45	-65,44
ENG		m/s	r	51																
		m/s	l	33																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	6440		N. plantaris		r	1407		
		ml	l	0,8								l	12733				l	1		

RAT	V4 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,90	4,06	5,56	3,69	4,06	4,25	3,69	3,16	3,47	3,21	3,59	2,84	3,94	3,34	2,88	3,25	3,00
	3 cm	mistakes	total	0	8	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
			placing	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	6	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		s		4,91	9,75	7,31	5,52	4,68	5,46	4,25	4,97	3,66	4,44	5,15	4,56	4,38	4,12	4,06	4,25	3,65
	2 cm	mistakes	total	0	8	0	0	0	0	0	0	0	3	2	2	1	0	0	0	0
			placing	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	7	0	0	0	0	0	0	0	3	2	2	1	0	0	0	0
		s		6,64	9,60	7,36	5,61	6,03	6,79	4,73	5,92	5,10	7,85	6,72	6,58	5,53	4,91	4,87	4,75	4,62
	1,5 cm	mistakes	total	0	8	8	2	2	0	0	0	8	3	1	2	0	0	2	4	0
			placing	0	8	3	0	2	0	0	0	8	0	0	0	0	0	0	3	0
			slip	0	0	5	2	0	0	0	0	0	3	1	2	0	0	2	1	0
		s		7,01	18,97	11,73	8,30	7,82	7,50	6,94	5,37	21,10	10,17	9,66	8,81	5,59	4,25	7,03	7,47	5,18
	1 cm	mistakes	total	0		8	8	8	2	1	4	0	3	0	1	1	1	1	1	1
			placing	0		8	8	8	2	1	4	0	3	0	1	1	0	0	0	0
			slip	0		0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
		s		15,67	0	38,68	19,53	21,41	17,65	10,81	13,30	9,00	11,41	6,34	7,97	7,07	7,12	6,88	6,47	5,22
	II 1 cm	mistakes	total	0	6	8	8	8	6	7	8	8	7	7	6	8	8	8	5	4
			placing	0	2	3	3	0	1	1	0	5	5	4	4	4	3	7	1	2
			slip	0	4	5	5	8	5	6	8	3	2	3	2	4	5	1	4	2
		s		7,33	18,73	14,13	8,58	9,21	8,03	9,59	8,02	8,75	7,13	8,53	5,81	8,31	7,06	7,87	7,87	7,07
	II 0,8 cm	mistakes	total	0	8	4	7	8	7	8	6	8	8	6	6	8	5	8	7	4
			placing	0	8	1	5	2	2	4	0	6	7	6	4	4	2	4	1	1
			slip	0	0	3	2	6	5	4	6	2	1	0	2	4	3	4	6	3
		s		12,18	20,97	13,21	13,95	10,94	10,46	10,25	9,19	7,59	10,16	7,53	6,41	6,54	6,19	8,16	8,03	7,18
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	12	12	12	10	11	11	11	11	8	9	7	7	5
		0 - 15	l plantar	0	15	14	14	13	10	10	7	9	6	10	7	7	10	7	5	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	10	9	8	8	10	7	8	8	7	6	6	7	6	6	4
Prints		mm	r TS	19,3	19,60	21	20	19,6	20,6	21,3	19,6	20,3	20,6	21	21,6	21,6	20	21,3	22	20,6
		mm	l TS	19	6	6,3	6	6,6	7,3	6,6	7,3	7,6	6,6	8,6	9,3	12,6	16,3	15	14,6	14,6
		mm	r ITS	12,6	13	11,5	13	11,6	11,6	14,3	14,6	14,6	13,3	13,6	14	13,6	14,6	15,6	14,6	11,3
		mm	l ITS	10	3,6	3,6	3,6	4	4,3	4	4	4	4	4	4,3	6,3	7,6	7	8	8
	SSI (mean)			-1,29	-91,68	-101,62	-99,74	-94,02	-95,40	-103,84	-98,12	-101,93	-98,66	-88,63	-91,17	-75,74	-39,31	-51,37	-57,33	-47,50
		mm	r TS	20,6			21	20,6	21,6	22	21,6	21,6	21	20	21,6	21,6	21	22	22	21
		mm	l TS	19,6			6	4	6	7,6	8	8	8,6	9,3	9,6	11	10,6	11,3	10	12
		mm	r ITS	10			11	11,6	12	13	12	11,3	11	11	11	11,6	10,6	10	11,6	11
		mm	l ITS	10			4	3,5	4,3	4,6	5	4,6	5	4,6	5	5	6	7,6	7,3	7,3
		mm	r PL	18,6			30	27,6	28	28	29	29	27	26,6	28,3	27,6	27	27,3	27	27,6
		mm	l PL	20			34	34,4	37,3	38	38	38,3	27,5	35,6	36,3	38	36,3	37,6	37,3	34,3
	SFI (mean)			-11,97	n.c.	n.c.	-98,33	-114,24	-103,99	-102,11	-99,53	-97,16	-89,00	-90,03	-83,79	-84,08	-84,70	-83,67	-91,16	-73,97
ENG		m/s	r	59																
		m/s	l	33																
Postmortem	Gastrocnemius	ml	r	2,5		Morphometry: myelinated fibres				N. ischiadicus		r	7386		N. plantaris		r	1422		
		ml	l	0,8								l	7963				l	415		

RAT (A)	V5 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,09	3,43	3,06	2,27	3,16	2,56	2,85	2,5	2,22	2,34	3,56	1,97	3,38	2,28	2,22	1,78	2,66
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,13	5,37	4,29	4,39	3,32	3,20	2,81	3,91	3,41	3,78	3,55	3,69	4,47	3,44	3,34	2,90	3,53
	2 cm	mistakes	total	0	0	7	0	2	1	0	1	1	0	0	0	1	2	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
			slip	0	0	7	0	2	1	0	1	1	0	0	0	1	0	0	0	0
		s		3,26	5,27	4,97	4,97	5,28	5,78	4,18	5,91	3,72	4,06	4,97	4,19	6,19	4,84	3,78	4,78	9,34
	1,5 cm	mistakes	total	0	7	7	7	2	0	3	1	5	0	1	3	0	1	1	0	0
			placing	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
			slip	0	7	7	7	2	0	3	1	4	0	1	2	0	1	1	0	0
		s		3,56	7,97	5,44	6,34	7,58	3,94	6,19	6,50	8,69	5,44	6,28	8,63	5,28	4,19	4,34	6,50	4,72
	1 cm	mistakes	total	0	8	8	7	1	0	0	0	0	2	0	3	2	2	2	6	1
			placing	0	8	8	2	1	0	0	0	0	0	0	0	0	0	0	2	0
			slip	0	0	0	5	0	0	0	0	0	2	0	3	2	2	2	4	1
		s		8,05	10,93	6,10	8,23	10,94	14,15	8,56	7,16	5,90	7,65	5,82	6,63	6,09	4,75	5,37	7,97	5,87
	II 1 cm	mistakes	total	0	8	8	8	7	8	8	8	7	8	8	8	7	6	5	4	3
			placing	0	6	3	0	2	0	8	8	6	6	8	8	0	4	0	1	1
			slip	0	2	5	8	5	8	0	0	1	2	0	0	7	2	5	3	2
		s		7,70	12,56	10,26	8,64	9,51	9,38	11,12	7,31	9,44	9,00	10,65	9,07	10,72	11,56	6,71	9,56	7,59
	II 0,8 cm	mistakes	total	0	8	8	8	4	7	6	7	7	7	8	8	5	6	1	4	2
			placing	0	4	3	1	1	0	4	7	5	4	8	7	1	4	1	0	0
			slip	0	4	5	7	3	7	2	0	2	3	0	1	4	2	0	4	2
		s		5,23	9,95	5,63	8,95	9,85	8,04	10,17	7,82	6,47	8,81	10,78	10,07	10,53	10,28	6,19	8,81	8,56
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	12	12	11	11	9	9	10	8	6	6	5	6	4
		0 - 15	l plantar	0	14	14	14	11	11	11	10	10	9	7	5	4	5	6	4	2
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	9	7	7	9	5	6	5	6	6	4	4	6	6	5	3
Prints		mm	r TS	20	17,00	18,6	18	18,6	17	20,6	19,6	19	17,3	18	19	19,6	20,3	20,3	19,3	19,3
		mm	l TS	19,6	6	6	6,6	6,6	6	6,3	6	6	6,6	9	8	9,3	7	7	7	7
		mm	r ITS	9,6	10,3	11,6	10,6	10	11,3	12,3	11,6	10,6	9	11,6	9,6	11,6	11,3	11,6	12	12,3
		mm	l ITS	10	4	4,6	4	3,6	4	4	4	4	4	4,3	4,3	4,6	5,3	4	4,6	3,6
	SSI (mean)			-10,54	-89,55	-102,59	-95,95	-94,70	-99,15	-103,31	-103,82	-99,20	-91,27	-86,54	-89,56	-79,19	-88,64	-97,06	-90,14	-92,82
		mm	r TS	21	22	21,6	21,6	22	22	20,6	20,3	21,6	21,6	22,3	22	21,6	21,6	21,6	21	21,3
		mm	l TS	21	7,6	7,3	7,3	9	7,6	8	9	8	8,3	8,3	6	8			8	
		mm	r ITS	9,6	10	9,6	8,6	10	9	9,6	9	9,6	8,6	10	9,6	9	10,3	10	10	10
		mm	l ITS	10	4,3	4,6	5	5,3	5	4,6	4,3	5	5	4,6	4,3	4,6			5	
		mm	r PL	30	28	26,3	26	25	28,6	28,6	30,3	24,6	26,6	27,6	27,6	28,6	30,3	28	28,3	28
		mm	l PL	31,6	36,6	35,6	36	36,3	36,6	37,6	39,3	36	36,6	35,3	35,6	39,6			34	
	SFI (mean)			-9,69	-97,88	-102,03	-95,61	-97,29	-95,38	-94,53	-94,55	-98,39	-97,63	-99,26	-105,77	-108,02	n.c.	n.c.	-96,53	n.c.
ENG		m/s	r	56																
		m/s	l	46																
Postmortem	Gastrocnemius	ml	r	2		Morphometry: myelinated fibres				N. ischiadicus		r	7629		N. plantaris		r	1903		
		ml	l	0,9								l	11255				l	518		

RAT	V6 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,97	4,54	3,58	2,69	3,04	3,03	2,91	2,63	2,63	2,62	3,68	2,81	3,10	3,04	3,07	2,35	2,81
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,16	5,17	7,56	5,09	4,95	4,18	4,16	4,35	2,75	4,07	4,94	4,81	3,65	4,78	3,44	3,81	4,09
	2 cm	mistakes	total	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0
		s		2,97	5,53	6,57	5,34	6,37	6,53	4,66	5,91	3,41	4,41	6,63	5,43	4,57	4,56	4,68	4,37	7,41
	1,5 cm	mistakes	total	0	6	6	3	0	3	0	1	0	8	3	2	1	1	2	1	8
			placing	0	0	1	0	0	3	0	0	0	8	1	2	0	0	1	0	8
			slip	0	6	5	3	0	0	0	1	0	0	2	0	1	1	1	1	0
		s		3,22	11,16	9,53	9,41	8,15	9,4	8,03	8,03	9,97	15,15	8,74	6,44	8,72	8,72	7,94	6,06	14,44
	1 cm	mistakes	total	0	8	8	3	2	4	0	1	2	2	6	2	1	1	0	1	2
			placing	0	3	8	1	2	2	0	1	1	0	2	0	0	0	0	1	0
			slip	0	5	0	2	0	2	0	0	1	2	4	2	1	1	0	0	2
		s		7,95	15,58	18,81	11,91	19,06	10,32	15,62	11,44	7,19	7,81	10,28	9,82	8,00	10,25	7,69	9,50	10,00
	II 1 cm	mistakes	total	0	5	8	8	8	5	4	6	7	7	8	7	6	6	4	5	7
			placing	0	4	7	8	3	0	0	2	3	5	8	2	2	2	1	3	1
			slip	0	1	1	0	5	5	4	4	4	2	0	5	4	4	3	2	6
		s		7,06	8,34	9,11	9,34	10,49	8,07	7,05	7,28	7,21	6,59	13,32	9,75	9,88	8,65	6,60	8,21	10,34
	II 0,8 cm	mistakes	total	0	8	9	2	6	8	6	5	6	8	8	6	8	6	8	7	7
			placing	0	6	5	2	1	5	3	1	2	5	5	5	6	1	2	1	2
			slip	0	2	4	0	5	3	3	4	4	3	3	1	2	5	6	6	5
		s		6,22	8,54	8,66	6,62	8,65	9,25	6,16	6,38	7,25	7,66	12,19	7,60	9,91	10,75	8,47	8,53	11,00
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	12	11	10	11	9	9	8	8	5	3	4	3	2
		0 - 15	l plantar	0	15	14	14	13	12	11	11	10	12	7	10	8	10	6	1	0
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	9	10	9	9	7	4	4	4	4	5	3	4	3	2	2	0
Prints		mm	r TS	20,3	17,30	19,6	21	20,3	19,6	19,6	21	20	18	21,3	20	22	21	20	22	20,3
		mm	l TS	20	6,6	6,3	6,6	6	7	7	6,3	6,6	9	13,3	15	14,3	15,3	13,6	14,6	15,6
		mm	r ITS	13,6	10,6	11,6	12,6	12,6	10	11,3	14,3	14,3	11,3	11,3	13,3	15,6	13,6	11	13,3	11
		mm	l ITS	12,3	4,3	4,3	4	4	3,6	4,3	4	4,3	4,3	5,3	6	6,3	6,3	5	6,3	7
	SSI (mean)			-10,05	-88,82	-99,64	-101,32	-101,30	-93,13	-88,73	-104,98	-93,47	-87,42	-64,95	-49,07	-62,34	-51,42	-57,08	-57,86	-46,05
		mm	r TS	20	20	20,6	20	21	20	20	20,6	22	21,3	20,3	22	22	21	20,6	21	19,6
		mm	l TS	20	7	8,6	9,3	8	9	9,6	8,6	10	11	11	12,3	12	10,3	8,3	10,3	9
		mm	r ITS	10,6	12	11,6	11	11	10,6	11,6	12	11,6	11,6	11,6	11,6	10,6	9,6	10,6	11,3	10,3
		mm	l ITS	11,3	4,3	4	5	4,6	5	5	4,6	5	4,6	5,3	6	7	5,3	5	4,6	5
		mm	r PL	18,6	24,6	22	25,6	25,6	24	26,6	25,6	25	26	28,3	25,6	26	26,3	28,3	26,6	26,3
		mm	l PL	19,3	35	31,6	35,3	37	36,6	38,3	36,6	38,6	38,3	39,6	36,6	36,6	37	33,3	35,6	36
	SFI (mean)			-6,64	-104,57	-96,57	-97,29	-95,29	-96,81	-96,46	-96,62	-97,00	-89,78	-83,86	-78,59	-84,69	-91,62	-88,91	-86,50	-89,70
ENG		m/s	r	59																
		m/s	l	27																
Postmortem	Gastrocnemius	ml	r	2,3		Morphometry: myelinated fibres				N. ischiadicus		r	7808		N. plantaris		r	1994		
		ml	l	0,6								l	10552				l	487		

RAT	V7 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	6 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,14	7,60	7,75	4,78	6,96	4,84	5,02	5,99	4,26	3,28	4,13	2,52	2,65	2,97	4,73	4,38	3,92
	3 cm	mistakes	total	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		s		2,65	8,80	10,57	10,09	10,41	9,54	8,84	7,15	5,97	5,61	5,34	4,67	7,03	5,21	4,86	4,54	5,64
	2 cm	mistakes	total	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,41	9,25	11,91	8,02	9,39	8,51	8,09	6,28	9,66	4,86	10,29	9,20	5,24	5,71	5,97	5,25	6,44
	1,5 cm	mistakes	total	0		5	3	2	1	0	1	0	0	0	0	0	0	0	1	0
			placing	0		5	0	0	1	0	0	0	0	0	0	0	0	0	0	0
			slip	0		0	3	2	0	0	1	0	0	0	0	0	0	0	1	
		s		4,02	0	13,67	11,28	9,91	17,71	9,62	12,18	11,63	14,15	13,63	9,17	18,88	8,13	8,95	5,82	12,25
	1 cm	mistakes	total	0		8	8	6	5	4			1	2	3		8	5	7	3
			placing	0		5	4	2	1	1			0	1	2		1	3	4	1
			slip	0		3	4	4	4	3			1	1	1		7	2	3	2
		s		9,87	0	22,07	20,35	22,50	15,51	19,81	0	0	30,50	36,37	18,88	0	28,36	0	9,66	11,13
	II 1 cm	mistakes	total	0	8	8	8	8	8	5	5	7	7	6	6	4	5	8	4	5
			placing	0	4	5	3	2	3	3	2	5	3	3	2	1	4	5	3	3
			slip	0	4	3	5	6	5	2	3	2	4	3	4	3	1	3	1	2
		s		6,83	17,30	12,95	11,30	9,92	10,58	12,1	10,43	9,14	9,29	10,75	11,14	9,92	11,59	9,24	12,46	7,56
	II 0,8 cm	mistakes	total	0	8	8	8	7	7	7	7	5	6	8	5	6	6	6	4	4
			placing	0	6	3	4	3	4	3	2	2	3	2	2	4	3	1	1	2
			slip	0	2	5	4	4	3	4	5	3	3	6	3	2	3	5	3	2
		s		4,02	14,74	13,99	11,03	10,26	9,95	10,01	10,23	9,77	8,74	9,15	10,89	9,31	8,50	10,91	8,88	8,34
Nociception		0 - 2	r medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 2	r rest	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 12	l lateral	0	12	12	12	12	12	12	11	10	10	10	9	5	7	5	3	2
		0 - 15	l plantar	0	15	15	14	14	13	13	12	10	9	10	11	6	5	5	5	2
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	10	10	10	9	9	8	8	7	7	5	4	5	4	6	5
Prints		mm	r TS	19,3	20		22		19,5	18	18,5	17	22	18,6	19,6	17,3	19	18,6	20	17,6
		mm	l TS	19	10		6	7	6	6,5	6,5	7	7	7	9	10	14,6	11,3	11,6	13
		mm	r ITS	11,6	13		15		12	8,6	11	14	13	9,6	11,3	12,3	12,3	11,6	13,6	11,3
		mm	l ITS	12	5		3	3	3	4	4	4	4	4,3	4,3	4,6	5	5,3	5	4,6
	SSI (mean)			-6,03	-79,26	n.c.	-109,79	n.c.	-104,40	-91,76	-96,05	-91,98	-101,43	-90,65	-83,82	-71,14	-49,46	-65,30	-71,13	-52,67
		mm	r TS	20	28	22,3	29,6	29,6	30,3	30	29,6	32	28,6	30,6	29	30,6	27,3	25,6	27	23,6
		mm	l TS	21	35	35,3	37	37	37,6	38,6	34,4	34	36,6	35,6	39	36	38,3	36,3	37,6	38,3
		mm	r ITS	20,3	22	23	20,6	22,6	19,6	20,2	19,6	18,6	20,3	20,6	22	20,60	20,6	22,3	22	22
		mm	l ITS	20,6	9	8	6	7,6	7	6,6	6,6	6,6	7	8	12	12	12,6	13	14,6	13
		mm	r PL	10	12	10	10,3	10,6	9,6	9	9,6	10,6	10	9,6	10	10,6	10	10	10	10,6
		mm	l PL	9,6	5	4,6	4	4	4	4	4,6	4,3	4	4,6	4,6	5	5	5,6	5,3	6
	SFI (mean)			-9,63	-90,84	-109,72	-104,12	-99,33	-96,18	-100,89	-94,57	-89,74	-99,23	-88,96	-78,96	-68,30	-73,41	-76,33	-66,92	-83,22
ENG		m/s	r	66																
		m/s	l	33																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	8746		N. plantaris		r	1721		
		ml	l	1,1								l	14079				l	432		

RAT	V8 (A)	Autograft		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,46	3,19	2,93	2,75	2,60	2,44	2,47	2,90	3,50	2,88	3,78	3,57	3,91	4,26	3,41	3,69	3,47
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,47	4,13	3,28	3,25	2,97	3,22	3,60	3,28	3,80	3,78	3,85	4,40	3,65	4,16	4,09	5,19	4,97
	2 cm	mistakes	total	0	8	4	0	0	0	1	1	1	0	1	0	1	0	0	1	0
			placing	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	4	0	0	0	1	1	1	0	1	0	1	0	0	1	0
		s		3,40	3,78	5,44	4,42	4,56	3,59	6,19	6,29	5,47	3,97	7,95	4,78	6,06	4,22	6,63	6,15	4,03
	1,5 cm	mistakes	total	0	8	8	8	2	0	0	4	0	2	3	4	0	2	1	1	3
			placing	0	8	8	8	0	0	0	2	0	0	2	0	0	0	0	0	0
			slip	0	0	0	0	2	0	0	2	0	2	1	4	0	2	1	1	3
		s		4,75	11,70	7,81	8,75	6,10	3,75	5,69	8,07	7,79	6,13	7,91	8,00	4,63	5,47	6,16	6,03	6,15
	1 cm	mistakes	total	0	0	8	8	8	4	3	0	1	2	3	5	1	3	2	4	4
			placing	0	0	8	8	8	2	2	0	0	0	0	1	0	0	0	0	0
			slip	0	0	0	0	0	2	1	0	1	2	3	4	1	3	2	4	4
		s		11,19	7,41	19,53	10,00	10,56	6,47	7,13	5,60	6,65	7,29	7,06	8,19	5,85	7,79	8,00	7,25	9,13
	II 1 cm	mistakes	total	0	7	3	2	3	4	6	8	8	3	7	7	6	7	8	5	6
			placing	0	0	2	0	1	0	2	6	8	3	7	7	6	4	3	5	4
			slip	0	7	1	2	2	4	4	2	0	0	0	0	0	3	5	0	2
		s		7,78	10,65	7,18	6,78	5,16	6,25	6,28	6,25	9,21	9,81	8,00	12,60	8,50	10,65	10,43	8,81	11,82
	II 0,8 cm	mistakes	total	0	7	4	4	6	8	5	8	7	8	8	7	7	6	7	4	7
			placing	0	0	0	3	3	3	4	5	5	8	7	7	7	6	6	1	6
			slip	0	7	4	1	3	5	1	3	2	0	1	0	0	0	1	3	1
		s		4,84	10,60	7,78	7,37	6,53	8,46	6,94	7,22	8,75	9,88	7,84	6,37	8,32	10,06	7,87	10,15	10,82
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	12	11	10	11	12	9	9	8	7	5	3	5	5
		0 - 15	l plantar	0	15	15	14	12	11	12	14	9	8	7	6	6	6	3	1	1
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	9	10	9	6	9	9	6	7	8	6	5	5	5	4	4	4
Prints		mm	r TS	21	21,00	21,6	21,6	21,6	21	21	22	21,3	20	21	22	21	20,6	20	21,3	21
		mm	l TS	20,6	7,6	8,3	13,3	7,6	7	7	7,6	9,6	8,3	9,6	12,6	13	13	16	16,6	18
		mm	r ITS	14,6	13,3	14,6	13	15	13,3	13,6	14,6	14,6	13,6	14,3	15	14,3	14,3	11	15	16
		mm	l ITS	15	4	6,6	6,6	5	4,6	4	4,3	4,6	5	6,3	6,6	6,3	6,3	8,6	8,6	8
	SSI (mean)			-1,85	-94,49	-73,94	-62,21	-93,97	-99,56	-98,82	-95,89	-86,38	-87,38	-78,61	-70,09	-69,45	-64,80	-31,09	-46,13	-38,18
		mm	r TS	20,3	22,3	21,6	21,3	22	22,3	21,3	22	22,6	20,6	22	20,6	20,3	22	21,3	21	22,3
		mm	l TS	21,3	6	9,6	9,3	9,3	7,6	8,6	9,6	10	8	9	9	10	10,3	11,6	12	11
		mm	r ITS	9	11	11	11,6	12,3	11	11	11,3	12,6	12	12	11,3	11,3	11	11,3	12	12,3
		mm	l ITS	10,3	3	5	5,3	5,6	4,6	4,3	5	5	4,6	5,3	5	6	5,6	6,6	8,3	6,6
		mm	r PL	21,6	25,3	25,6	26	29	29	26	28,6	28,6	25,3	30	29,3	28	29	27	29	25,3
		mm	l PL	22,3	37	37	38	38	36,6	38,6	40	36,6	36	36,3	38	38	37	36	36	37,6
	SFI (mean)			-2,60	-111,28	-92,00	-96,63	-90,42	-100,08	-100,15	-93,43	-85,81	-96,80	-93,08	-91,74	-86,12	-82,03	-77,18	-74,77	-76,00
ENG		m/s	r	71																
		m/s	l	31																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	8031		N. plantaris		r	1831		
		ml	l	1								l	12828				l	513		

RAT	V9 (B)	Tube		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,26	3,14	3,56	2,78	3,00	3,69	3,59	3,13	3,53	3,41	3,56	3,47	3,53	2,88	3,07	3,13	4,03
	3 cm	mistakes	total	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,39	5,56	4,04	4,56	3,41	4,03	3,66	5,68	4,19	4,63	4,75	4,75	3,94	3,50	4,04	4,28	3,94
	2 cm	mistakes	total	0	2	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			slip	0	2	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0
		s		3,43	7,36	5,58	5,94	4,41	5,09	6,19	8,85	5,59	5,81	5,47	6,57	4,78	3,75	6,25	4,50	4,32
	1,5 cm	mistakes	total	0	3	8	0	0	1	2	1	0	2	1	0	0	1	1	1	1
			placing	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	3	0	0	0	1	2	1	0	2	1	0	0	1	1	1	1
		s		5,34	8,33	5,22	4,62	5,78	8,03	5,81	7,28	5,81	7,44	5,75	8,24	5,59	6,47	6,32	5,44	6,94
	1 cm	mistakes	total	0	0	4	8	8	3	0	4	0	3	2	2	1	1	2	4	1
			placing	0	0	0	8	8	0	0	2	0	0	0	1	0	0	0	1	0
			slip	0	0	4	0	0	3	0	2	0	3	2	1	1	1	2	3	1
		s		10,86	15,57	7,78	6,31	7,00	8,54	5,56	10,84	6,09	7,22	6,28	8,28	7,79	6,32	7,00	8,43	7,91
	II 1 cm	mistakes	total	0	4	7	4	6	6	2	1	1	4	2	4	2	6	5	5	6
			placing	0	1	2	2	1	4	0	0	0	0	0	0	1	2	2	1	1
			slip	0	3	5	2	5	2	2	1	1	4	2	4	1	4	3	4	5
		s		6,06	10,82	9,19	6,87	6,29	6,53	6,62	7,31	6,72	8,97	7,60	7,47	7,44	8,19	8,92	8,10	7,93
	II 0,8 cm	mistakes	total	0	5	6	5	6	3	5	4	3	5	2	4	4	7	4	6	2
			placing	0	1	2	2	3	0	1	0	0	1	0	1	0	2	1	0	0
			slip	0	4	4	3	3	3	4	4	3	4	2	3	4	5	3	6	2
		s		6,20	8,75	7,53	7,50	9,50	5,75	7,60	7,85	5,97	7,29	7,09	6,81	7,34	8,94	10,16	7,75	6,84
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	11	11	11	10	11	10	7	6	6	6	6	6	5
		0 - 15	l plantar	0	13	15	14	13	11	7	6	5	4	3	3	3	3	2	2	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	9	10	8	7	8	7	6	5	5	3	5	4	4	3	3	3
Prints		mm	r TS	20,3	19	20	20	21,3	20	20,3	20,6	19,6	22,6	20,3	21,3	19	19,3	19,3	20	20,3
		mm	l TS	21	6	7,6	5,3	6	7,6	8,6	10,6	7,6	11	9	9	8	9,3	8	8,6	9
		mm	r ITS	13,6	12,6	15	12	11	10	10,6	13	11,3	14	14	10,6	11	12	11,3	11,6	11
		mm	l ITS	14	4	4	4	4,3	4,3	4	4,6	4	4,3	4,3	4,6	4,3	5	4,6	4	4
	SSI (mean)			4,34	-101,34	-95,69	-105,14	-101,08	-90,93	-85,21	-79,31	-90,56	-84,45	-84,29	-87,26	-84,64	-81,63	-88,43	-87,02	-82,05
		mm	r TS	21,6	21,6	21,6	21	22	22	20,6	21,3	22	22	21,6	22	21	23,3	21,6	21,6	22
		mm	l TS	20	6	6,3	6	6,3	7,3	7,6	7,3	7	7	8	7,6	7,3	7,6	7	7,3	7,6
		mm	r ITS	9	10	9,6	10,6	11,6	11	11,3	11,3	10	11	11	11,6	10,3	11,3	10	10,6	11
		mm	l ITS	9,3	3,6	4	4	4,3	4,3	4,3	4,6	4	4,3	6	4,3	4	5,3	4,3	4,3	4,6
		mm	r PL	27,6	30	29,6	29	29,6	30	28,6	30	29	27,6	29,3	28	31	29	27,3	29	29,6
		mm	l PL	27,6	35	36,3	35	35,6	35,6	37,6	35,6	36,6	35	38,6	37	38,6	39,6	38,6	36,3	36,3
	SFI (mean)			-12,28	-102,27	-95,76	-94,79	-97,07	-94,41	-94,01	-101,00	-95,67	-90,22	-93,43	-100,42	-97,42	-97,80	-103,96	-97,53	-92,41
ENG		m/s	r	60																
		m/s	l	32																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	9010		N. plantaris		r	1540		
		ml	l	0,5								l	6288				l	23		

RAT	V10 (B)	Tube		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		1,40	3,09	2,96	3,09	2,87	2,90	4,22	3,59	3,03	2,91	3,25	3,37	2,90	2,88	3,46	3,09	3,84
	3 cm	mistakes	total	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,34	5,54	4,81	4,03	4,62	5,31	5,35	4,78	4,12	4,50	4,09	3,72	4,81	3,75	5,34	5,13	4,66
	2 cm	mistakes	total	0	1	4	1	1	0	0	1	2	0	0	0	0	0	0	1	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	1	4	1	1	0	0	1	2	0	0	0	0	0	0	1	0
		s		2,41	6,47	4,25	3,50	5,29	4,62	5,41	7,94	6,56	4,78	4,50	4,22	4,37	3,88	5,56	5,07	5,94
	1,5 cm	mistakes	total	0	2	7	0	0	2	0	0	4	2	0	0	1	1	2	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	2	7	0	0	2	0	0	4	2	0	0	1	1	2	0	0
		s		4,28	6,59	5,94	4,91	5,69	9,88	6,09	6,28	9,75	6,50	5,41	5,82	7,50	5,41	7,03	5,25	5,41
	1 cm	mistakes	total	0	8	8	8	2	3	0	0	3	1	1	3	3	5	1	4	4
			placing	0	8	8	8	0	0	0	0	0	1	0	2	1	1	0	1	0
			slip	0	0	0	0	2	3	0	0	3	0	1	1	2	4	1	3	4
		s		7,50	20,18	7,38	6,50	6,84	8,04	7,69	6,72	8,12	7,16	7,10	6,79	8,84	7,22	7,28	6,94	7,18
	II 1 cm	mistakes	total	0	5	6	5	7	6	7	6	6	6	6	7	7	6	4	6	6
			placing	0	2	4	3	2	2	2	2	1	1	3	2	5	0	2	0	3
			slip	0	3	2	2	5	4	5	4	5	5	3	5	2	6	2	6	3
		s		5,13	11,14	7,84	7,09	7,72	7,56	8,56	6,97	5,91	7,47	7,15	6,59	7,81	6,78	8,51	6,94	9,44
	II 0,8 cm	mistakes	total	0	7	6	6	7	7	7	6	4	4	4	5	7	6	4	5	6
			placing	0	3	5	4	5	3	1	1	1	1	0	1	2	4	1	0	0
			slip	0	4	1	2	2	4	6	5	3	3	4	4	5	2	3	5	6
		s		7,21	9,12	7,41	8,62	8,41	9,46	7,75	9,25	6,87	6,93	6,47	7,09	7,35	8,12	7,97	7,84	8,81
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	12	10	10	9	8	7	8	8	5	5	4	6	5
		0 - 15	l plantar	0	14	15	14	13	14	12	11	10	8	8	8	5	5	3	2	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	10	7	8	7	4	5	6	6	5	5	4	5	3	3	3
Prints		mm	r TS	20	21	19,6	21,6	22	22,3	21,3	20,3	21,6	21,6	20,3	21	19,3	21	21,3	22	22
		mm	l TS	19	8	8	8	6,6	9,3	9,3	9	11	9,6	12,3	9,6	8,6	10,3	9,6	9,3	9
		mm	r ITS	12,3	13,3	10,3	16	14	14,3	13	14	12	12,3	13,3	13	13	15	12,3	15	13
		mm	l ITS	13,6	4	4,6	4	4	4	4,6	4,6	5	4,3	4,6	5	5	5	4,3	3,3	4,3
	SSI (mean)			-4,73	-92,41	-80,61	-91,84	-102,63	-89,08	-85,74	-86,42	-77,73	-85,51	-70,00	-82,25	-82,95	-79,52	-86,29	-90,66	-91,55
		mm	r TS	20,6	22	20,6	21,3	20,6	22	21,3	21,6	21,3	22	21,3	22	22,6	22,6	22	23	22,3
		mm	l TS	20,3	7,3	7,3	7	8,6	8,3	7,3	8	7	9	8,6	7,6	7	8	7	7	7,3
		mm	r ITS	8,6	11,6	10,3	11	11,3	11,6	11,6	12	10,6	11,3	12,3	11,6	11,6	12,3	11,6	12,3	11,3
		mm	l ITS	9,6	4	5	4,6	4	4,3	4,3	4	4,6	4,6	4,3	4,3	4	4,6	4	4	4
		mm	r PL	22	27,6	27,3	26,6	27,6	28,3	27,6	27,3	30	27	29,3	27	29,6	27,3	26,6	26,3	28,3
		mm	l PL	23	37,6	37,3	34,6	37	36,6	37,6	38	37	37	37,3	37,6	37,3	39,6	39	37	39
	SFI (mean)			-15,04	-102,94	-95,65	-96,10	-92,08	-91,15	-95,45	-94,84	-97,49	-97,79	-91,94	-101,92	-100,51	-99,28	-102,40	-109,24	-100,30
ENG		m/s	r	55																
		m/s	l	39																
Postmortem	Gastrocnemius	ml	r	2		Morphometry: myelinated fibres				N. ischiadicus		r	6961		N. plantaris		r	2077		
		ml	l	0,4								l	2621/0				l	0		

RAT	V11 (B)	Tube		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,35	3,10	3,22	3,47	2,97	2,90	2,85	2,69	3,28	3,41	3,22	2,94	3,03	2,47	2,75	3,58	2,56
	3 cm	mistakes	total	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,50	8,47	6,10	3,81	3,53	3,50	3,47	3,66	3,16	3,47	3,16	3, 13	3,37	3,60	2,78	3,47	3,46
	2 cm	mistakes	total	0	0	2	2	1	2	1	0	1	0	1	1	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
			slip	0	0	2	2	1	2	1	0	1	0	0	1	0	0	0	0	0
		s		2,00	5,22	4,90	5,34	3,88	4,62	4,00	4,60	4,68	3,90	5,71	3,69	4,28	3,78	3,07	5,09	3,44
	1,5 cm	mistakes	total	0	3	3	5	3	1	2	1	1	0	3	0	2	0	0	0	1
			placing	0	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	2	3	2	2	1	2	1	1	0	3	0	2	0	0	0	1
		s		2,95	7,22	5,19	8,10	7,37	6,05	5,00	5,53	4,65	3,88	5,15	4,75	4,75	4,09	4,53	4,38	4,00
	1 cm	mistakes	total	0	8	3	2	3	4	3	3	4	5	4	6	4	4	3	2	1
			placing	0	8	1	0	0	2	0	2	0	2	0	1	0	1	2	1	0
			slip	0	0	2	2	3	2	3	1	4	3	4	5	4	3	1	1	1
		s		4,65	7,78	8,07	6,18	5,84	7,35	5,06	9,31	7,10	6,28	5,53	6,93	6,34	6,53	6,69	7,62	5,35
	II 1 cm	mistakes	total	0	7	8	7	7	7	7	6	7	7	7	7	8	5	7	7	6
			placing	0	5	3	3	4	2	3	4	3	1	5	3	2	4	1	4	3
			slip	0	2	5	4	3	5	4	2	4	6	2	4	6	1	6	3	3
		s		4,17	8,50	5,53	5,22	6,06	6,56	7,81	7,25	7,13	7,66	7,10	6,81	7,75	5,72	7,97	7,10	5,88
	II 0,8 cm	mistakes	total	0	7	8	8	8	6	7	8	7	8	7	7	5	5	7	6	5
			placing	0	6	5	1	3	2	4	5	4	4	3	4	2	1	1	6	3
			slip	0	1	3	7	5	4	3	3	3	4	4	3	3	4	6	0	2
		s		3,96	7,97	5,71	7,40	7,37	7,00	5,47	5,97	5,97	8,22	7,09	6,47	8,16	7,25	9,03	6,72	5,56
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	11	11	12	11	11	10	11	9	9	4	5	5	6	4	6	6
		0 - 15	l plantar	0	12	14	15	11	10	10	10	7	8	5	4	4	5	2	3	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	7	8	7	7	6	5	5	5	5	4	4	3	4	4	6	4
Prints		mm	r TS	21	20,6	21,3	21,6	20,3	21,3	21,3	20	20,3	20,6	20,6	20,3	21,3	20	21	19,6	20
		mm	l TS	20,3	6	6	12,6	9	10,3	10	10,6	11	10	9,6	11	9	9	9	8,3	10
		mm	r ITS	13,3	14	13,3	11,3	14,3	14,3	13,6	11,3	12,6	12,6	14,3	15,3	12,6	9,6	13,3	11,6	11,3
		mm	l ITS	13	3,6	3,3	4,6	4,6	4,6	5	5	4,3	4,6	4,6	4,6	4,3	4	5	4,6	4,3
	SSI (mean)			-12,18	-98,45	-95,65	-72,59	-87,20	-78,87	-85,73	-74,72	-77,09	-83,42	-86,01	-78,61	-88,18	-80,29	-87,70	-83,22	-76,55
		mm	r TS	21,6	20	20,6	21,3	21	21,6	21,6	21	20,3	21,6	22	21,3	22,3	21	22	21,6	22
		mm	l TS	20	7,6	7,6	8	7	7	8	7,3	8,6	7,3	6,6	7	7	7	7,3	7	7,6
		mm	r ITS	9,6	10,6	11,3	10,6	10,5	11	10,3	10,3	11	11,6	10	12	11,3	11,3	11	11,3	12
		mm	l ITS	9	4,3	4,3	4	4,3	4,6	4,6	5	5	5	4,3	4	4	4	4,3	4	4,6
		mm	r PL	21,3	29,3	28,6	29,6	30,5	30	30	30,6	30	30	32,3	31	30,3	30,6	30,3	28,3	31
		mm	l PL	21,3	36,6	35	36	39,6	37,3	39	37,6	39,6	40	39,3	40	40	40	37,6	39,3	38,6
	SFI (mean)			-17,01	-88,88	-91,31	-92,48	-99,60	-99,31	-94,12	-90,95	-85,86	-95,63	-95,06	-103,67	-100,52	-97,67	-96,94	-101,22	-95,35
ENG		m/s	r	47																
		m/s	l	26																
Postmortem	Gastrocnemius	ml	r	2,3		Morphometry: myelinated fibres				N. ischiadicus		r	8351		N. plantaris		r	2056		
		ml	l	0,3								l	2928				l	0		

RAT	V12 (B)	Tube		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,07	2,91	2,88	2,75	3,62	3,03	2,75	3,10	3,03	3,46	3,28	3,68	3,19	3,31	3,53	3,13	2,97
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,56	3,90	4,32	4,40	3,91	5,68	3,59	3,66	4,12	4,50	4,28	4,69	4,00	4,37	3,84	4,75	3,72
	2 cm	mistakes	total	0	2	2	0	2	1	0	0	0	0	2	1	0	0	1	1	0
			placing	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0
			slip	0	2	2	0	1	1	0	0	0	0	0	1	0	0	1	1	0
		s		2,44	6,22	4,41	4,54	6,41	5,56	4,21	4,12	4,47	4,50	8,41	5,60	4,75	4,43	6,47	7,90	3,94
	1,5 cm	mistakes	total	0	2	2	8	4	2	1	1	0	0	2	0	0	0	1	2	1
			placing	0	0	0	8	1	1	0	1	0	0	0	0	0	0	0	0	0
			slip	0	2	2	0	3	1	1	0	0	0	2	0	0	0	1	2	1
		s		2,04	9,06	5,25	10,22	6,91	6,44	5,44	7,37	5,65	4,50	8,90	5,00	4,47	5,35	6,16	6,13	4,94
	1 cm	mistakes	total	0	8	5	3	0	4	2	1	0	3	3	5	4	4	4	3	2
			placing	0	8	0	2	0	0	0	0	0	2	1	1	1	2	1	0	0
			slip	0	0	5	1	0	4	2	1	0	1	2	4	3	2	3	3	2
		s		4,52	10,13	7,06	7,22	6,28	9,15	8,12	6,16	5,60	9,81	7,72	9,24	9,57	6,88	8,19	7,69	7,03
	II 1 cm	mistakes	total	0	7	8	6	5	4	3	6	6	3	7	7	7	1	6	6	6
			placing	0	4	4	2	1	1	1	4	2	0	2	4	1	0	0	1	0
			slip	0	3	4	4	4	3	2	2	4	3	5	3	6	1	6	5	6
		s		4,44	8,03	8,28	6,62	7,40	7,88	6,81	7,37	7,28	6,56	6,34	9,28	7,63	6,81	8,32	8,87	9,03
	II 0,8 cm	mistakes	total	0	7	7	7	5	3	1	4	4	3	3	3	3	3	4	4	2
			placing	0	6	1	5	0	0	0	1	0	2	1	0	0	1	1	0	0
			slip	0	1	6	2	5	3	1	3	4	1	2	3	3	2	3	4	2
		s		4,27	9,31	7,75	8,25	7,71	7,25	5,91	13,81	7,66	6,97	6,53	6,24	6,31	7,28	6,66	8,13	6,75
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	11	11	12	11	10	10	10	10	9	9	7	6	8	5
		0 - 15	l plantar	0	15	14	13	10	10	11	10	9	9	7	8	6	5	4	6	4
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	8	9	9	9	8	7	9	7	6	6	5	5	5	5	6	5
Prints		mm	r TS	19	19,6	20,6	20,3	20	20,3	20,3	20,3	20	20	19,6	18,6	19,3	20	19,6	19,6	20
		mm	l TS	20,3	6	7,6	10,6	11	11,3	8,3	10,3	10	8,6	9,3	9,3	8,3	9	10	9	10,3
		mm	r ITS	13,6	15	15	15	15	14,3	14	14	14	15	14,3	15	14,6	14,3	15,3	14,3	14,6
		mm	l ITS	12,6	3,6	4	4,6	4,6	5,3	4	4,6	4,3	5	4,6	5	4,6	4,6	5	4	5
	SSI (mean)			-0,36	-103,04	-94,41	-77,93	-76,74	-73,71	-92,94	-78,31	-79,10	-86,20	-88,00	-77,12	-88,13	-89,44	-78,71	-86,46	-79,76
		mm	r TS	19,3	22,5	22	21,6	21,6	21	21	21,5	21	20,6	20,6	21,6	20	21,6	21,6	21	20
		mm	l TS	21	7	7	8	8,3	8,3	9	8	8,6	7	7,3	9	7,3	6	7,6	7,6	7,3
		mm	r ITS	10	13,5	12	11,3	11	10,6	11	11	11,3	10,6	10,6	12	11,6	11,6	11,6	11	10,6
		mm	l ITS	10	5	5	5	5,3	4,6	5	4,6	5,3	4,6	5,3	4,6	4,6	4	5,3	4,3	4,6
		mm	r PL	20,3	27	23,6	26	26,6	15	26,3	13,5	27,6	26	25,6	27,3	27	27,3	28	25,6	25
		mm	l PL	18,6	39	35,3	36	35,3	38	37	37	38,6	37,6	37,6	39,6	39,6	40	39	39	39,6
	SFI (mean)			-5,22	-106,98	-102,47	-90,61	-92,45	-98,22	-96,61	-93,49	-91,66	-102,97	-99,48	-100,48	-99,83	-113,14	-98,43	-98,58	-102,16
ENG		m/s	r	54																
		m/s	l	37																
Postmortem	Gastrocnemius	ml	r	2,4		Morphometry: myelinated fibres				N. ischiadicus		r	8698		N. plantaris		r	1995		
		ml	l	0,6								l	1582				l	0		

RAT	V13 (B)	Tube		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,28	4,44	3,62	3,59	2,97	3,22	3,82	3,09	3,22	3,12	3,56	3,35	2,78	3,50	3,03	3,34	3,72
	3 cm	mistakes	total	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,06	4,50	5,03	6,05	3,37	4,47	4,40	4,10	4,56	4,00	3,31	3,94	3,87	3,34	4,00	3,43	4,53
	2 cm	mistakes	total	0	8	3	0	3	0	3	1	0	0	0	1	0	0	0	0	0
			placing	0	8	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
			slip	0	0	3	0	3	0	2	0	0	0	0	1	0	0	0	0	0
		s		3,46	6,81	5,82	5,23	5,75	4,43	4,59	5,03	5,00	4,47	4,72	4,91	5,81	4,37	4,35	4,91	5,84
	1,5 cm	mistakes	total	0	6	2	8	1	0	6	3	1	1	0	0	2	0	0	0	0
			placing	0	5	1	8	1	0	3	1	0	0	0	0	0	0	0	0	0
			slip	0	1	1	0	0	0	3	2	1	1	0	0	2	0	0	0	0
		s		4,22	10,04	6,28	7,09	7,22	4,09	5,81	6,32	5,50	7,97	6,75	5,37	6,06	5,78	4,96	5,56	6,16
	1 cm	mistakes	total	0	8	8	8	5	8	8	4	7	2	5	1	5	1	2	3	4
			placing	0	8	8	8	0	8	8	4	6	0	1	0	4	0	0	3	3
			slip	0	0	0	0	5	0	0	0	1	2	4	1	1	1	2	0	1
		s		5,69	8,50	8,53	11,41	6,62	8,78	5,94	9,28	7,78	6,60	8,84	6,59	6,60	6,69	7,31	7,57	8,66
	II 1 cm	mistakes	total	0	6	6	6	3	3	5	4	5	7	6	2	4	0	0	3	3
			placing	0	6	4	2	0	0	4	1	2	3	2	0	1	0	0	1	0
			slip	0	0	2	4	3	3	1	3	3	4	4	2	3	0	0	2	3
		s		7,31	10,22	9,97	7,75	6,53	8,37	8,122	7,91	9,74	9,63	7,38	8,09	7,09	6,19	6,66	7,62	7,53
	II 0,8 cm	mistakes	total	0	4	3	6	3	4	3	4	5	6	4	5	3	2	6	2	4
			placing	0	4	0	3	0	3	1	3	1	4	1	1	3	1	2	0	0
			slip	0	0	3	3	3	1	2	1	4	2	3	4	0	1	4	2	4
		s		6,06	7,19	8,83	8,81	7,69	9,34	8,65	7,74	8,46	6,22	7,69	6,47	8,69	7,00	6,21	8,16	8,09
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	11	11	10	9	11	11	9	7	8	5	4	5	6	5	3
		0 - 15	l plantar	0	15	13	13	13	13	13	8	5	4	4	6	9	8	8	9	5
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	10	9	8	7	8	6	7	4	4	3	3	4	4	4	3	3
Prints		mm	r TS	18,6	19,6	19,6	19,6	21	20	20,6	19	19	19,3	20,6	20,3	20,3	20	20	20	20
		mm	l TS	19,3	8	17	14,3	11,3	12	11,6	9,3	7,6	9,3	8	8,3	8,6	7,6	9	8	8
		mm	r ITS	13	15	14	13,6	15	14,3	15	14,3	14	14,3	15,3	15,6	15	14	14,6	15,6	15
		mm	l ITS	13	3,6	5,6	6,6	5,6	5	5	4,6	4	4,3	0,6	4	4	4	4,3	5	4,3
	SSI (mean)			3,17	-85,50	-38,68	-51,43	-74,38	-69,67	-75,32	-79,12	-90,95	-82,42	-91,41	-91,44	-89,99	-93,11	-83,92	-94,09	-87,92
		mm	r TS	20,3	21,3	21	20,3	21,3	21	20,6	22	20	22	21,6	19,6	22	22	22,3	20,3	20,6
		mm	l TS	20,6	9	8	8	6,3	8	7	7,3	6,6	7,6	6,3	7	7,6	7,6	6	6,3	8
		mm	r ITS	9,3	10	10	10,6	10	10	10	9,6	10	10,6	9,6	10,3	10	11	10,3	10	10,3
		mm	l ITS	11	4	6	5	4,3	4,6	4,3	4,6	4,3	4,3	4,6	3,6	5	5	4,3	4,6	4,6
		mm	r PL	26,3	28,3	29,5	30,3	38	30	30	27,3	31,3	30	26	31,3	27,3	29,3	30	28,3	30,3
		mm	l PL	26,6	36,3	33	37	28,6	40	39	39	39,3	40,3	39,3	37,3	38,6	40	38,6	38,6	38,3
	SFI (mean)			0,10	-81,68	-88,61	-91,25	-95,14	-94,92	-95,47	-100,14	-99,29	-98,42	-105,00	-96,85	-99,43	-99,62	-103,11	-100,57	-91,07
ENG		m/s	r	45																
		m/s	l	27																
Postmortem	Gastrocnemius	ml	r	2,3		Morphometry: myelinated fibres				N. ischiadicus		r	8228		N. plantaris		r	1934		
		ml	l	0,4								l	2565				l	0		

RAT	V14 (B)	Tube		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,28	3,25	3,06	3,12	2,47	2,44	2,84	2,72	2,88	3,03	2,60	3,53	2,94	2,69	2,91	3,40	3,62
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,91	4,25	4,97	4,05	4,54	3,53	3,94	3,31	3,81	4,03	3,49	4,19	3,62	4,19	4,00	3,88	5,16
	2 cm	mistakes	total	0	4	2	1	0	0	1	0	1	0	1	2	1	0	0	0	0
			placing	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	2	2	1	0	0	1	0	1	0	1	2	1	0	0	0	0
		s		3,19	6,72	5,22	4,99	4,57	3,97	3,72	5,19	6,75	6,00	7,59	8,40	6,41	3,81	5,31	4,44	3,75
	1,5 cm	mistakes	total	0	0	4	3	3	2	2	0	0	3	0	2	0	1	0	0	0
			placing	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
			slip	0	0	3	3	3	2	2	0	0	3	0	1	0	1	0	0	0
		s		3,79	6,34	9,53	7,38	6,00	5,32	8,28	5,03	5,56	8,06	7,25	6,37	5,09	5,91	4,37	6,07	4,97
	1 cm	mistakes	total	0	2	5	5	3	6	4	4	4	1	1	5	6	6	1	2	4
			placing	0	0	3	1	1	2	3	3	1	0	0	1	2	2	0	0	1
			slip	0	2	2	4	2	4	1	1	3	1	1	4	4	4	1	2	3
		s		4,82	7,53	8,50	7,54	7,65	8,94	8,56	6,90	9,03	7,29	10,00	8,79	7,53	8,60	7,09	6,68	10,19
	II 1 cm	mistakes	total	0	6	8	6	7	8	8	6	7	7	7	7	7	8	8	8	7
			placing	0	5	6	1	5	6	7	5	7	4	3	7	2	5	1	8	4
			slip	0	1	2	5	2	2	1	1	0	3	4	0	5	3	7	0	3
		s		5,94	9,47	11,47	7,28	6,59	8,59	9,47	8,81	9,94	7,75	9,43	11,28	8,41	8,54	8,78	11,16	11,87
	II 0,8 cm	mistakes	total	0	7	6	7	6	6	8	6	8	8	7	8	6	6	6	7	8
			placing	0	7	5	7	6	5	7	5	7	6	5	2	2	2	2	5	3
			slip	0	0	1	0	0	1	1	1	1	2	2	6	4	4	4	2	5
		s		6,00	7,19	9,53	7,47	9,15	8,59	8,91	10,75	11,54	10,60	12,51	10,65	8,53	9,41	10,25	8,81	12,62
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	12	10	12	10	9	8	8	8	6	6	8	5	6	6
		0 - 15	l plantar	0	15	15	12	11	12	8	10	6	6	7	6	5	5	5	5	5
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	9	10	9	10	8	6	8	6	3	7	5	5	7	4	6	5
Prints		mm	r TS	21,3	19,6	20,6	20,6	19,6	21	21,6	19,3	19	20,3	20,3	19,6	20,3	20	20,3	19,6	20,3
		mm	l TS	21	9,3	12,6	11	11	10,3	11,6	8,3	8,6	9	8,6	8,3	8	8	9,5	8	9,6
		mm	r ITS	15,3	14,6	13,3	11,6	14,3	14,6	14,6	14,3	13,3	15	14,6	15,3	14,6	15	15,3	12,6	13
		mm	l ITS	13,6	4,3	6	5,3	5	5,6	5,6	4,3	5	4	5	4,3	4,3	4,6	5	5	5
	SSI (mean)			-8,87	-85,62	-66,02	-72,18	-72,54	-78,87	-76,42	-89,12	-86,44	-90,68	-88,50	-90,96	-89,66	-92,23	-84,94	-87,04	-81,43
		mm	r TS	20,6	20,6	19,6	20,3	20,3	19,3	21	20,3	20,3	21,3	20	18,3	20,3	19,6	20	19	19,3
		mm	l TS	20,3	6,6	8,6	7,6	7,3	7,6	8	7,6	9	8,3	7,3	7	6,6	7	7,3	7	8
		mm	r ITS	8,6	11,3	9	10,6	10	9,6	10,6	9,6	11	10	10,6	11,3	10,3	10,6	10,3	9,6	10,3
		mm	l ITS	10	4,3	5	4,3	4,6	5	5	4,6	4,6	4,6	5	4,6	4,6	4	4	4	4,3
		mm	r PL	27,6	27,3	25	26,6	27	29,6	29,3	30	29,3	29	30,3	26,3	27,3	27	29	29,6	30
		mm	l PL	22,3	38,3	39	40,3	38	38	39	40	40,6	40,3	39	40	40	39,6	39	39,6	38,6
	SFI (mean)			2,98	-99,55	-93,11	-100,22	-96,17	-91,53	-91,20	-96,44	-90,01	-97,27	-93,27	-104,45	-105,43	-105,73	-95,15	-90,81	-89,92
ENG		m/s	r	57																
		m/s	l	30																
Postmortem	Gastrocnemius	ml	r	2,3		Morphometry: myelinated fibres				N. ischiadicus		r	7990		N. plantaris		r	1048		
		ml	l	0,4								l	2169				l	0		

RAT	V17 (C)	T-muscle		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,53	4,28	3,06	2,90	4,31	3,82	3,72	2,88	3,47	3,15	5,59	2,87	2,69	3,06	2,81	2,81	3,00
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,81	4,31	5,43	6,84	6,81	5,43	4,22	4,72	4,75	4,06	5,37	3,71	3,46	4,22	3,34	3,65	3,62
	2 cm	mistakes	total	0	8	0	1	0	0	0	0	0	0	2	0	1	0	0	0	0
			placing	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	0	0
		s		2,82	5,66	7,03	5,59	6,33	5,38	5,31	6,25	5,31	4,25	7,09	5,40	4,78	3,81	4,13	3,28	4,97
	1,5 cm	mistakes	total	0	1	0	0	4	1	0	0	0	0	0	0	1	0	1	0	1
			placing	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	1	0	0	3	1	0	0	0	0	0	0	1	0	1	0	1
		s		2,78	5,32	6,60	7,65	6,00	5,96	6,44	4,87	4,66	5,50	5,94	5,18	5,82	6,60	5,00	4,66	5,09
	1 cm	mistakes	total	0	7	2	5	4	4	4	2	0	0	3	0	2	2	1	2	1
			placing	0	1	0	4	1	3	2	1	0	0	0	0	1	0	0	0	0
			slip	0	6	2	1	3	1	2	1	0	0	3	0	1	2	1	2	1
		s		4,16	6,82	6,19	8,09	8,56	8,03	9,93	8,34	4,90	7,35	8,09	5,53	6,67	9,69	6,40	9,97	5,81
	II 1 cm	mistakes	total	0	7	8	7	7	7	7	7	7	7	7	4	5	7	6	8	7
			placing	0	7	7	3	5	3	1	2	5	4	3	1	2	5	3	3	2
			slip	0	0	1	4	2	4	6	5	2	3	4	3	3	2	3	5	5
		s		3,97	10,66	8,81	9,34	10,12	9,97	10,75	8,72	9,53	10,25	10,93	7,06	8,53	8,06	8,97	9,59	8,81
	II 0,8 cm	mistakes	total	0	6	7	6	7	5	7	7	5	7	5	7	7	7	5	6	6
			placing	0	5	4	5	5	4	1	2	2	4	1	3	1	6	1	2	0
			slip	0	1	3	1	2	1	6	5	3	3	4	4	6	1	4	4	6
		s		4,90	10,81	7,53	6,88	15,22	7,79	9,99	11,13	8,13	9,81	9,07	9,56	9,69	9,50	8,43	6,44	6,90
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	10	10	8	10	9	6	8	8	6	6	6	5	7	6	6	6
		0 - 15	l plantar	0	14	11	11	10	10	7	9	9	9	7	7	5	4	4	4	4
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	8	9	6	7	7	5	6	7	5	5	7	5	6	5	5	5
Prints		mm	r TS	20	20,3	19,6	19,6	19,3	20,3	20,6	19,3	19,6	20,3	20	20,3	22	20	21,6	21	21,3
		mm	l TS	20	7	11,3	11	10,6	9,6	9,6	8	9	10	9,3	9	9,3	11	13	10,3	11,5
		mm	r ITS	9,6	11,3	9,6	9,6	10,6	10,6	11,6	9	10	9	10,6	9,6	11,6	12	11	12,3	10
		mm	l ITS	13,6	3,3	4	4	4,6	4,3	4,6	4,3	4,6	5	4,6	4,6	4,6	5,3	5	4,6	4,6
	SSI (mean)			-0,53	-95,52	-65,42	-68,28	-68,99	-79,60	-78,85	-79,58	-74,92	-66,08	-79,72	-78,95	-82,49	-74,58	-65,34	-72,92	-63,71
		mm	r TS	19	21,3	20	20,6	22	20	21	20,6	22,3	22,3	20,3	21,3	22	20,6	22	21	23
		mm	l TS	18,3	8	9,3	7	7,6	7,3	6	7	7,3	7,6	6,3	7	8	8	9	8	8,6
		mm	r ITS	8,3	9	8	10	10	9,3	9,6	9,3	11	10,3	8,6	10	11	9	11,3	9,3	9,6
		mm	l ITS	8,6	4,3	4,3	4,3	5	4	4,6	4,6	4,3	5	5	4,6	4	4,3	4	4,6	5
		mm	r PL	23	25,3	24,3	26,3	26	26	27	25	30,3	28	25	26,3	27	25,3	25,6	25	25,6
		mm	l PL	22,3	36	35	37	37,3	37,6	38	37	37	39	37	38	38,3	38	37	37,6	38,3
	SFI (mean)			-7,92	-98,59	-97,70	-98,86	-98,99	-98,36	-100,28	-102,26	-97,40	-99,35	-106,73	-102,22	-100,30	-98,16	-96,97	-98,43	-97,60
ENG		m/s	r	53																
		m/s	l	33																
Postmortem	Gastrocnemius	ml	r	2,1		Morphometry: myelinated fibres				N. ischiadicus		r	8766		N. plantaris		r	1992		
		ml	l	0,6								l	0				l	0		

RAT	V18 (C)	T-muscle		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,50	4,09	2,85	3,07	3,16	3,10	3,09	3,21	3,41	3,50	3,16	4,06	2,59	2,78	2,75	3,38	2,85
	3 cm	mistakes	total	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,19	6,28	4,19	4,50	6,09	4,97	4,97	4,88	4,79	4,97	5,72	4,56	3,68	4,87	3,75	3,44	4,25
	2 cm	mistakes	total	0	3	2	0	1	0	0	0	1	0	0	0	0	0	1	0	0
			placing	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	2	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0
		s		2,82	7,28	7,69	4,16	6,68	4,97	4,37	4,88	5,37	6,75	5,25	5,88	4,22	4,72	4,65	3,94	4,09
	1,5 cm	mistakes	total	0	2	3	2	1	4	1	1	1	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0
			slip	0	2	3	2	1	2	0	1	1	0	0	0	0	0	0	0	0
		s		4,10	6,40	5,41	6,02	5,72	7,69	5,88	5,10	6,25	5,31	6,32	6,12	5,53	5,31	5,03	5,63	4,69
	1 cm	mistakes	total	0	6	4	2	0	5	3	2	2	1	1	0	2	1	1	3	1
			placing	0	4	0	1	0	4	1	1	1	0	0	0	0	0	0	1	0
			slip	0	2	4	1	0	1	2	1	1	1	1	0	2	1	1	2	1
		s		4,50	8,47	7,48	8,66	7,47	8,47	8,31	6,69	7,91	6,53	10,46	8,84	8,78	8,15	7,75	7,53	7,37
	II 1 cm	mistakes	total	0	8	6	7	5	6	5	6	7	8	8	7	8	8	8	8	8
			placing	0	0	4	4	4	6	3	2	5	2	6	4	5	8	8	8	6
			slip	0	8	2	3	1	0	2	4	2	6	2	3	3	0	0	0	2
		s		6,32	10,66	9,90	10,28	9,10	12,72	10,12	8,37	9,87	8,84	11,47	8,94	8,38	8,47	8,53	10,65	7,97
	II 0,8 cm	mistakes	total	0	4	4	7	5	6	6	5	8	3	8	8	8	8	8	8	8
			placing	0	4	3	3	2	5	4	0	4	1	6	4	5	8	7	8	8
			slip	0	0	1	4	3	1	2	5	4	2	2	4	3	0	1	0	0
		s		4,41	9,69	7,88	9,79	8,40	7,06	8,87	9,45	10,34	8,03	8,56	8,34	7,71	7,41	9,50	9,44	7,91
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	11	12	10	9	11	9	10	4	8	4	4	3	4	4	3	4
		0 - 15	l plantar	0	11	11	9	9	8	6	8	3	4	4	4	4	4	3	3	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	8	8	5	5	6	5	6	4	4	3	3	2	3	3	2	3
Prints		mm	r TS	19,3	18	18,6	19,3	19,6	18,6	19	21	18,3	19,6	19	19,6	20	18,6	19,6	19,6	20
		mm	l TS	21	9	11,3	11	10,6	12,3	9,6	8,6	10	11,3	9	9	14,3	12,6	13,6	13,3	14
		mm	r ITS	12,6	13	10,6	11,6	14,3	12,3	9,6	12	12	11,3	12,3	12,3	13,3	11,6	13	13,3	13,6
		mm	l ITS	12,6	4	4	5	4,6	4,6	4,3	4,3	5	5	4,3	4,3	4,6	5	5	4,3	5,3
	SSI (mean)			6,01	-78,47	-72,77	-71,50	-74,75	-63,72	-77,75	-87,08	-74,36	-71,34	-80,41	-84,35	-64,89	-59,22	-59,46	-63,19	-57,25
		mm	r TS	20,6	20,6	21	21,6	19,3	21	21,3	20,6	20,6	21	20,6	20,6	21,6	21,3	21,3	21,3	22,6
		mm	l TS	20	9	7	6,6	9	7,6	7	7,3	8	7	8	7,6	7,6	9	8,6	8,6	8,6
		mm	r ITS	8,6	9,3	10,3	10,6	10	10,3	11,3	9,6	11	11,3	10,6	10,3	10,6	10,6	10,6	10,3	11
		mm	l ITS	8,3	6,6	5,3	5	5	5,3	4,3	5	5	4,3	4	4,6	4	4,3	4,6	4,3	4,5
		mm	r PL	24,3	23,6	24,3	24,6	24	25,3	25,3	26,6	25,3	25,6	25,6	24,6	27,3	25	25,6	24,3	25,6
		mm	l PL	23	33,3	34,3	36,6	38	36	37	36,6	37	38,3	37,6	38	38,6	37,3	37,3	39	38
	SFI (mean)			-10,46	-93,06	-104,19	-104,01	-99,96	-99,15	-104,25	-99,43	-97,43	-110,39	-101,62	-97,42	-99,16	-98,28	-96,94	-101,79	-100,83
ENG		m/s	r	55																
		m/s	l	34																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	8076		N. plantaris		r	2044		
		ml	l	0,6								l	0				l	2		

RAT	V19 (C)	T-muscle		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,69	2,59	3,31	3,00	2,75	2,97	3,81	3,53	3,59	3,18	2,75	3,88	2,94	2,97	3,32	2,66	3,13
	3 cm	mistakes	total	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		s		3,13	4,98	8,41	5,47	5,59	4,63	4,16	3,72	5,94	4,78	5,13	5,19	3,47	3,97	3,16	3,53	3,84
	2 cm	mistakes	total	0	1	0	0	1	0	1	0	2	1	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	1	0	0	1	0	1	0	2	1	0	0	0	0	0	0	0
		s		3,59	6,63	5,50	4,69	6,15	4,56	4,16	5,07	6,63	5,29	5,72	5,59	4,15	4,63	4,16	4,37	3,87
	1,5 cm	mistakes	total	0	3	0	1	0	1	2	0	1	1	0	0	3	0	0	0	2
			placing	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0
			slip	0	2	0	0	0	0	2	0	1	1	0	0	2	0	0	0	2
		s		4,75	8,25	6,06	6,34	6,68	5,66	5,56	5,44	6,87	9,22	5,43	6,63	8,85	6,44	5,10	4,57	5,72
	1 cm	mistakes	total	0	3	3	6	2	4	2	4	3	4	5	1	2	3	3	3	2
			placing	0	1	3	5	1	2	0	1	1	2	2	1	0	1	0	1	0
			slip	0	2	0	1	1	2	2	3	2	2	3	0	2	2	3	2	2
		s		4,47	6,53	9,69	8,78	7,56	7,70	8,75	10,81	9,16	6,19	9,09	7,87	8,81	7,37	7,38	6,25	6,81
	II 1 cm	mistakes	total	0	8	5	5	6	6	5	5	4	5	5	6	5	4	4	5	4
			placing	0	6	1	3	4	4	1	1	0	3	3	2	5	2	3	3	0
			slip	0	2	4	2	2	2	4	4	4	2	2	4	0	2	1	2	4
		s		7,53	7,78	7,75	7,41	7,12	7,37	5,69	7,34	7,28	6,97	10,15	8,15	6,44	6,43	5,96	7,75	6,96
	II 0,8 cm	mistakes	total	0	3	8	5	5	6	4	5	5	5	7	7	6	3	3	7	6
			placing	0	1	5	3	4	3	3	3	4	3	2	3	4	2	3	2	0
			slip	0	2	3	2	1	3	1	2	1	2	5	4	2	1	0	5	6
		s		7,31	8,50	8,41	11,13	7,90	7,26	8,90	6,81	7,87	7,84	10,41	9,00	8,72	6,10	5,91	6,56	7,78
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	11	11	11	10	9	7	10	6	11	6	7	7	6	6	6	6
		0 - 15	l plantar	0	15	15	13	12	8	10	7	7	5	5	4	3	4	5	4	4
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	7	7	6	6	5	6	6	7	6	3	4	5	5	5	5	5
Prints		mm	r TS	20,3	19	18,6	18,6	20	17,6	20	20	19,6	19,6	19,3	19	19	19,3	21	20,3	20,3
		mm	l TS	19	10,3	10	10	11	10	8,3	11,6	9	11,6	13	11,3	11	10,3	11,3	10	9
		mm	r ITS	14,6	14	13,6	14	15	13	14,6	15	15	14,6	13,3	13,6	14	13,3	14,6	15	14,3
		mm	l ITS	12	4,6	4,6	5	4,6	4,6	3,6	4,6	4,6	4,3	5	4,6	4,3	4,3	4,6	4,6	4,5
	SSI (mean)			-19,48	-81,19	-78,02	-79,07	-77,81	-74,18	-92,09	-78,88	-86,72	-75,24	-62,51	-70,67	-74,01	-78,26	-78,10	-79,32	-82,94
		mm	r TS	20	21,3	21	22,3	22	22	20,3	21,3	20,3	20,3	21	20	20,6	22	22	21,6	21,3
		mm	l TS	19,6		7		7,3	7,6	8,3	7	8,6	8,6	8	7,6	7,6	8	8,3	8,3	8,6
		mm	r ITS	9	9,3	8,6	10	10	9,3	10,3	11	10,3	10,3	9,3	10,3	12	12,3	10,6	10,3	9
		mm	l ITS	9,4		4		4,3	4,5	4,3	4	5	4,6	4,3	4,3	4	4	4,3	4,5	4,3
		mm	r PL	25,3	26	27	27	31,6	29	29	25	29,3	27	28	26,6	27	26	24,6	26,6	27
		mm	l PL	24,6		37		35,6	36	38,6	34	36,3	37,6	37,3	37,6	36,6	36,6	37,3	37,3	37,3
	SFI (mean)			-11,80	n.c.	-102,79	n.c.	-98,24	-94,15	-95,17	-109,08	-86,29	-93,02	-96,94	-96,68	-97,94	-100,48	-101,12	-97,13	-87,91
ENG		m/s	r	61																
		m/s	l	36																
Postmortem	Gastrocnemius	ml	r	2,1		Morphometry: myelinated fibres				N. ischiadicus		r	7305		N. plantaris		r	1765		
		ml	l	0,6								l	885				l	0		

RAT	V20 (C)	T-muscle		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,91	3,56	3,56	3,22	2,68	2,81	3,00	3,19	3,69	3,59	3,31	3,03	2,75	2,94	3,38	2,75	3,19
	3 cm	mistakes	total	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,65	5,84	5,19	5,91	4,13	4,46	4,69	3,84	4,69	3,50	4,78	3,63	4,56	3,44	3,28	3,59	3,34
	2 cm	mistakes	total	0	1	7	1	1	1	0	0	0	1	1	1	0	0	0	0	0
			placing	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	1	4	1	1	1	0	0	0	1	1	1	0	0	0	0	0
		s		3,56	5,60	6,09	5,53	4,34	5,35	4,72	4,22	6,44	6,53	4,75	4,57	3,68	4,16	3,72	3,46	4,07
	1,5 cm	mistakes	total	0	4	7	2	0	0	0	2	1	1	0	0	0	0	0	0	0
			placing	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	3	7	1	0	0	0	2	1	1	0	0	0	0	0	0	0
		s		4,60	6,66	7,82	7,59	5,56	5,12	6,12	6,22	5,69	4,97	5,59	4,62	4,25	5,06	4,60	4,25	5,41
	1 cm	mistakes	total	0	6	3	3	3	4	1	5	3	1	4	1	0	3	2	3	4
			placing	0	1	2	2	1	2	0	0	0	0	0	0	0	0	1	1	0
			slip	0	5	1	1	2	2	1	5	3	1	4	1	0	3	1	2	4
		s		4,97	8,57	8,31	7,47	7,16	7,53	6,60	9,31	10,84	5,16	8,78	6,66	6,37	7,12	7,41	6,91	5,68
	II 1 cm	mistakes	total	0	5	7	6	4	8	4	4	7	3	5	5	5	5	5	3	4
			placing	0	2	4	4	2	2	1	1	2	1	1	2	0	0	0	0	1
			slip	0	3	3	2	2	6	3	3	5	2	4	3	5	5	5	3	3
		s		7,16	8,75	13,74	11,13	9,06	9,03	9,34	8,47	9,37	8,34	7,25	9,97	8,28	9,03	8,15	7,31	8,28
	II 0,8 cm	mistakes	total	0	6	7	5	5	8	3	4	5	4	5	2	3	4	3	2	3
			placing	0	3	4	4	1	5	0	4	3	0	3	1	1	0	1	1	0
			slip	0	3	3	1	4	3	3	0	2	4	2	1	2	4	2	1	3
		s		5,53	8,75	14,32	9,25	7,87	9,40	9,28	8,44	9,28	7,94	9,38	7,63	7,56	8,03	6,91	8,28	7,10
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	11	10	7	6	9	6	6	5	4	5	5	5	4	4	4	4
		0 - 15	l plantar	0	13	12	11	10	7	7	7	6	5	4	4	4	4	4	4	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	7	7	5	3	5	5	5	5	5	5	5	4	4	3	4	4
Prints		mm	r TS	19	19,6	19	19,3	19,6	19,6	18,6	19,3	20,3	20	18,6	19,3	20,3	20	21,3	20	18,6
		mm	l TS	21,3	12,3	12,3	11,6	12,3	10,3	10,3	12,3	9	8,6	8,6	9,3	10	11	10,6	9	9,3
		mm	r ITS	14,3	14,3	14	14,3	13,3	13,3	13,3	14	13,3	13	14	13,6	14,6	13,3	13,6	14	12,6
		mm	l ITS	13,3	4,3	5,3	4,6	6,3	5	5	6,3	4,6	5	5	5	5	5	5	4,6	5
	SSI (mean)			2,41	-65,24	-62,07	-70,73	-59,60	-77,77	-73,26	-63,10	-85,71	-85,99	-81,30	-81,64	-77,97	-72,40	-76,42	-84,17	-77,44
		mm	r TS	21	22,6	19,6	19,6	19,6	19,3	20	20,3	19,6	19	21	20,6	20,6	21	21	21	21
		mm	l TS	19,6		7,6	7,6	8	7	6,6	6,6	7	7,3	8	7,6	7,3	9,3	8	8	9
		mm	r ITS	9,3	8,6	9,6	9,6	9,3	9,3	10	10	10,3	9,3	11,3	10	10	9,6	10,3	9,3	9,6
		mm	l ITS	9,3		5	4,6	4,6	4,3	4,6	4,3	4	4,6	4,6	4,3	5	4,6	5,6	6	5
		mm	r PL	25	25	25,6	27	29	28	28	29,6	25,6	28	28	26	25,6	26	24,3	27	24,3
		mm	l PL	27,3		34,6	35,6	36	37	36	39,3	38,3	38,6	38,3	38,3	37,3	37,3	37,6	38	37,6
	SFI (mean)			-14,64	n.c.	-96,60	-93,42	-86,71	-95,24	-96,39	-98,71	-102,44	-94,93	-94,20	-99,70	-107,53	-92,22	-104,23	-92,20	-98,49
ENG		m/s	r	52																
		m/s	l	27																
Postmortem	Gastrocnemius	ml	r	2,2		Morphometry: myelinated fibres				N. ischiadicus		r	6308		N. plantaris		r	2082		
		ml	l	0,4								l	0				l	0		

RAT	V21 (C)	T-muscle		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,62	3,44	3,53	3,15	2,94	3,25	3,41	3,19	3,12	3,82	4,47	3,44	3,47	3,06	3,75	3,22	2,93
	3 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		3,40	3,81	5,56	3,54	4,56	6,03	7,00	4,78	3,53	4,00	3,78	3,69	3,88	4,85	4,71	4,13	3,94
	2 cm	mistakes	total	0	0	1	0	0	0	2	1	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	1	0	0	0	2	1	0	0	0	0	0	0	0	0	0
		s		3,38	5,13	4,34	4,69	5,38	5,91	7,63	5,25	5,10	4,69	4,95	5,72	3,56	5,18	5,41	4,50	4,85
	1,5 cm	mistakes	total	0	5	3	0	1	0	3	1	0	0	0	1	1	2	1	1	0
			placing	0	4	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0
			slip	0	1	1	0	0	0	2	1	0	0	0	1	1	2	1	1	0
		s		4,16	6,81	5,50	3,84	6,63	6,69	9,19	6,13	6,00	5,34	5,47	6,44	4,75	6,53	7,18	5,47	7,68
	1 cm	mistakes	total	0	8	3	6	5	4	5	1	2	1	1	0	2	4	3	2	3
			placing	0	8	1	3	4	1	2	0	0	0	0	0	0	2	1	0	1
			slip	0	0	2	3	1	3	3	1	2	1	1	0	2	2	2	2	2
		s		4,28	7,71	6,41	6,75	7,91	9,94	11,32	9,72	10,32	6,78	8,12	6,44	6,56	7,75	8,94	6,16	8,37
	II 1 cm	mistakes	total	0	2	6	3	4	3	6	3	5	7	6	6	4	7	7	5	3
			placing	0	2	3	1	2	0	0	0	2	1	0	1	0	0	0	2	0
			slip	0	0	3	2	2	3	6	3	3	6	6	5	4	7	7	3	3
		s		6,31	6,65	8,82	8,41	9,38	9,47	10,43	8,59	10,00	9,66	11,09	8,84	7,75	9,09	8,59	6,66	8,87
	II 0,8 cm	mistakes	total	0	1	7	4	5	3	5	4	8	6	6	5	4	5	5	6	5
			placing	0	1	4	1	2	2	0	0	3	1	2	1	0	0	0	0	2
			slip	0	0	3	3	3	1	5	4	5	5	4	4	4	5	5	6	3
		s		5,77	6,09	9,90	8,04	9,97	8,38	11,31	10,00	8,94	10,15	10,18	9,22	7,59	9,00	9,15	9,40	8,13
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	12	12	11	10	9	10	7	9	7	5	5	5	6	5	5	5
		0 - 15	l plantar	0	14	12	13	10	9	9	8	7	7	6	6	3	5	4	3	3
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	8	8	8	8	6	6	6	5	7	5	3	4	5	3	3	4
Prints		mm	r TS	18,6	20	20,3	20	20,3	20,3	20,6	20,6	20	20,3	20	21	21,6	21,3	22,6	20,6	23
		mm	l TS	17,6	13,6	13	10,6	10,6	12	10	11,3	9,3	10,6	12,3	12	13	10,3	10,3	11	14
		mm	r ITS	15	14,6	13,6	15	15,3	14,3	15	15	16,3	15,3	14,3	15	16	14,6	15	14,3	15
		mm	l ITS	12,6	4,3	4	4	5	4,6	5,3	4,6	4,6	5	5	4,6	5	5	5	4,6	5
	SSI (mean)			-14,76	-62,34	-66,58	-80,31	-81,26	-74,06	-78,55	-75,96	-83,13	-80,29	-68,35	-72,77	-70,63	-83,27	-90,72	-81,98	-70,85
		mm	r TS	20,3	21,6	21,3	20	20,3	20,6	20	21,6	21,3	21,6	21,3	20,3	21,6	20,6	21	22	20,6
		mm	l TS	18				7	7		7,5	7	7	6	7	7,3	7,3	9	7,6	7,6
		mm	r ITS	9	11,6	11	10,3	10,6	11,6	11,6	11,6	11	12	11,6	10,3	10,6	11,3	11	10,6	10,3
		mm	l ITS	8,6				3,6	4		4	5	3,5	4	4	4	4	4,5	4,3	5
		mm	r PL	23	25,3	27,6	26	28,6	26	30,6	28,3	31	30	28	30,3	29,6	27,3	27	27,3	27
		mm	l PL	22,6				37,3	36,5		39	37	37	38,6	39	37,3	38	39	38,6	38,3
	SFI (mean)			-21,10	n.c.	n.c.	n.c.	-107,95	-104,64	-94,5	-101,93	-100,47	-96,541	-103,56	-96,727	-98,726	-100,17	-98,433	-103,19	-99,937
ENG		m/s	r	51																
		m/s	l	34																
Postmortem	Gastrocnemius	ml	r	2,5		Morphometry: myelinated fibres				N. Ischiadicus		r	8510		N. plantaris		r	2300		
		ml	l	0,6								l	0				l	0		

RAT	V22 (C)	T-muscle		D -2	D 5	D 7	D 9	D 14	D 16	D 21	D 23	D 28	D 30	D 35	D 37	D 42	D 44	D 49	D 51	D 56
Method																				
Balancing	8 cm	mistakes	total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		s		2,16	3,09	2,66	3,38	3,78	3,93	3,69	3,97	3,03	3,44	3,87	3,00	2,78	2,56	3,62	3,19	3,18
	3 cm	mistakes	total	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		s		2,35	4,87	4,37	4,63	4,25	5,03	7,06	4,75	3,84	5,08	4,50	4,47	4,59	5,62	4,22	3,75	3,97
	2 cm	mistakes	total	0	0	0	0	2	2	1	1	0	0	0	0	0	0	0	0	0
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	0	0	2	2	1	1	0	0	0	0	0	0	0	0	0
		s		3,15	3,66	4,90	5,53	6,78	7,03	6,82	5,37	4,62	4,04	4,59	4,41	4,37	4,53	5,25	5,78	4,50
	1,5 cm	mistakes	total	0	0	2	0	0	1	2	0	0	0	1	0	3	1	0	1	1
			placing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			slip	0	0	2	0	0	1	2	0	0	0	1	0	3	1	0	1	1
		s		4,00	4,19	6,03	5,72	6,41	8,47	6,13	6,19	6,19	6,65	6,50	5,84	6,65	5,50	3,63	6,06	5,65
	1 cm	mistakes	total	0	6	5	2	4	8	3	2	2	1	0	0	0	2	2	1	2
			placing	0	2	3	1	2	6	1	0	0	0	0	0	0	0	1	0	0
			slip	0	4	2	1	2	2	2	2	2	1	0	0	0	2	1	1	2
		s		3,94	8,00	8,91	7,44	8,75	10,84	9,97	8,18	7,10	7,16	10,03	5,40	6,53	8,00	7,57	6,35	7,47
	II 1 cm	mistakes	total	0	4	7	6	6	7	7	3	6	8	8	8	2	6	5	7	7
			placing	0	3	5	6	5	2	3	1	2	4	3	3	0	3	1	2	1
			slip	0	1	2	0	1	5	4	2	4	4	5	5	2	3	4	5	6
		s		4,09	8,00	9,19	9,41	9,41	8,94	8,66	7,54	7,37	12,38	9,47	10,12	6,88	7,53	8,03	8,84	7,06
	II 0,8 cm	mistakes	total	0	2	4	6	6	6	8	7	7	8	7	7	5	6	4	6	4
			placing	0	2	1	2	3	2	2	4	4	2	3	4	0	0	1	0	2
			slip	0	0	3	4	3	4	6	3	3	6	4	3	5	6	3	6	2
		s		5,00	6,81	7,13	5,28	8,73	8,19	9,34	9,81	8,07	10,09	9,28	10,35	9,38	8,78	7,84	6,37	8,87
Nociception		0 - 2	r medial	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 2	r rest	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0 - 12	l lateral	0	10	10	10	9	9	8	6	7	8	7	9	8	7	7	6	8
		0 - 15	l plantar	0	14	13	14	13	11	12	13	13	12	9	14	11	9	7	8	5
		0 - 5	l medial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0 - 10	l dorsal	0	8	8	8	8	8	9	6	6	6	5	7	7	5	5	7	7
Prints		mm	r TS	19,6	18,3	19,6	19	20	20	19,3	19,3	20,6	20,6	20,6	20	21,3	21	21,6	21,3	21,3
		mm	l TS	19,6	10,3	7,6	7	9	10	9	7	8,3	9	7,6	8,6	8,6	9	8,3	9,3	9,6
		mm	r ITS	14	11,6	12	12,6	13	12,6	12	13,6	14	14,3	14,3	12	14,6	12,6	13,3	12,3	13,3
		mm	l ITS	13,3	4,3	3,6	4	4,6	5	5	4,3	4,3	4,6	4,3	4,3	4,3	4,6	4,3	4,6	5
	SSI (mean)			-4,76	-71,12	-87,54	-93,96	-85,69	-79,63	-81,29	-95,10	-92,64	-87,00	-90,72	-85,65	-85,55	-85,36	-96,55	-88,27	-83,71
		mm	r TS	20,3	21	20,3	22,3	21	21,6	20	22	22,3	20,6	21,6	22	22	22	21,3	22	22
		mm	l TS	19,3	6,6	7,3	7,3	7	8	8	7,6	7,3	9	7	7,3	8,3	8,6	8	8,3	9,3
		mm	r ITS	9,6	10,6	10,3	11,3	10,3	11	10	11,3	11,3	11	10,3	11	10,3	11	10,6	11,3	11,3
		mm	l ITS	8,6	4,3	4,6	4,6	4,6	4,6	4,6	5,6	4,3	5	4	4,3	4,5	4,3	4,3	4,6	6
		mm	r PL	20,6	25,3	28,3	26,3	29,6	26,6	25,3	27,6	26,3	26	26,6	28	26	26	26	25,3	25,6
		mm	l PL	22,6	36,3	36	35,3	37,6	35,3	37	34	36,3	38,3	37,6	36,3	37,6	36,3	37,3	38	36,6
	SFI (mean)			-15,30	-104,08	-91,92	-96,28	-97,95	-95,87	-99,17	-91,92	-98,60	-93,94	-101,84	-94,40	-100,72	-98,32	-100,63	-104,57	-95,96
ENG		m/s	r	51																
		m/s	l	38																
Postmortem	Gastrocnemius	ml	r	2,4		Morphometry: myelinated fibres				N. ischiadicus		r	8829		N. plantaris		r	2180		
		ml	l	0,5								l	21				l	0		

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