CONTRIBUTION OF FEMORAL AND PROXIMAL SCIATIC NERVE BRANCHES TO THE SENSORY INNERVATION OF HINDLIMB DIGITS IN THE RAT

Anna Puigdellívol-Sánchez[1], Pau Forcada-Calvet[1], Alberto Prats-Galino[1*], Carl Molander[2,3].

1. Department of Morphological Sciences, Faculty of Medicine, University of Barcelona, c/Casanova 143, 08036 Barcelona, Spain.

2. Department of Neuroscience, Nobels väg 12a, Karolinska Institutet, 171 77 Stockholm, Sweden.

3. Department of Public Health Sciences, Division of Rehabilitation Medicine, Norrbacka, Karolinska Hospital, S-171 76 Stockholm, Sweden.

*Corresponding author: Alberto Prats-Galino. Telephone number: 34-93-4021905. E-mail address: aprats@medicina.ub.es. Mail address: Department of Morphological Sciences, Faculty of Medicine, University of Barcelona, c/Casanova 143, 08036 Barcelona, Spain.

Running title: Innervation of the digits in the adult rat.

Grant sponsor: Fundació Marató TV3; Grant number: 003/97.

Grant sponsor: Swedish Medical Research Council; Grant numbers: 11566 and 13124.

ABSTRACT

The present study was performed to investigate the possibility of "aberrant" innervation of the tips of the hindlimb digits in the rat, i.e. from other sources than the main sciatic branches (tibial, peroneal, sural). Cutaneous injections of fluorescent tracers in the digits were combined with either selective nerve transections to restrict afferent routes followed by detection of labeled neurons in dorsal root ganglia (DRGs), or by a delayed application of a second tracer to afferent nerves under study to detect double labeled neurons in DRGs. The results show that the tips of the digits were represented in DRGs L3-6. The femoral nerve afferents from digits 1 and 2 projected primarily to DRG L3 and to a smaller extent to DRG L4. A small number of neurons from primarily medial digits 1 and 2, but also from lateral digits 3-5, were found to project to DRGs L4 and L5 via a proximal branch that leaves the sciatic nerve near the sciatic notch and runs distally in the posterior part of the thigh, here called the musculocutaneous nerve of the hindlimb. We also have some evidence indicating innervation of the tips of the digits from the posterior cutaneous nerve of the thigh. Aberrant innervation such as that described here might contribute to remaining and perhaps abnormal sensibility after nerve injury and is of interest for the interpretation of results in experimental studies of collateral and regenerative sprouting after such injury.

Key words**:** nerve injury, primary afferent neurons, dorsal root ganglia, cutaneous innervation, sciatic nerve.

Detailed knowledge of the sensory afferent pathways from the densely innervated tips of the digits (Leem et al., 1993) is important for the understanding of sensory functions such as tactile exploration. The dense innervation combined with the way the digits are naturally isolated from each other also makes them an attractive experimental model to study how well regenerating sensory afferents grow to their previous targets after nerve injury. Previous studies of the normal anatomy have demonstrated a topographical representation of the tips of the rat hindlimb digits in dorsal root ganglia (DRGs; Prats-Galino et al., 1999), and spinal cord dorsal horn (Molander and Grant, 1985). There is incomplete knowledge, however, about which peripheral nerves the afferents from the tips of the digits use. Standard gross anatomical textbooks of rat anatomy state that the digits are innervated by branches of the femoral and sciatic nerves (Greene, 1963; Hebel and Stromberg, 1986). More detailed information is provided by a study using electrophysiological recordings of the activity of low threshold mechanoreceptors and plasma extravasation for C-fibers, showing the cutaneous innervation territories of the saphenous and main sciatic nerve branches (Wiesenfeld-Hallin et al., 1989). There is also a study of the rat hindlimb dermatomes based on plasma extravasation after C-fiber stimulation (Takahashi et al., 1994).

The aim of this study has been to investigate the contribution of different hindlimb nerves to the sensory innervation of the tips of the digits, and to find out in which DRGs the cell bodies of the afferents in these nerves are located. We used retrograde tracers to be able to provide semiquantiative data for our ongoing studies of regeneration accuracy to the hindlimb digits and of collateral sprouting from neighboring undamaged nerves after sciatic nerve injury and repair. In particular, we were interested to know the contribution of the saphenous branches to the innervation of the tips of the digits, and to find out if there is contribution also from other sources than the saphenous and main distal sciatic branches.

MATERIALS AND METHODS

Twenty-six adult female Sprague Dawley rats (275-350 b.w) were used in the present study. All the animals have been obtained from Harlan Interfauna Iberica S.A., maintained in the Animal Care Service, Faculty of Medicine, University of Barcelona, and treated in compliance with the ethical guidelines of this Center. Anaesthesia was initiated with ether and then continued with chloral hydrate (300mg/kg.) during surgical procedures and perfusion.

Previous experiments have shown (unpublished) that labeling in contralateral DRGs does not occur after injection in digits. We therefore used bilateral injections. In some cases, one of the sides was used for the present investigation and the other for the purpose of another study not directly related to the one presented here.

Injections of tracers and surgical procedures

1. Nerves contributing to the innervation of the digits

In the first group of animals (12 rats, n=17, table 1), the sciatic nerve was transected close to the knee at the level where it divides into the tibial, peroneal, lateral sural, and sural nerves. It was then resected about 1,5 cm proximally, leaving the posterior cutaneous and the nerve to the femoral biceps muscle (musculocutaneous branches, see below) intact. In addition to sciatic nerve transection, some animals of this group received also resections of one or several other nerves (table 1): (i) the femoral nerve (1,5 cm) just distal to the inguinal ligament, including the saphenous and all cutaneous branches to the anterior aspect of the upper thigh (n=4), (ii) the posterior cutaneous nerve of the thigh (1,5 cm) near the sciatic notch (n=4), (iii) a mixed branch (1,5 cm) that leaves the sciatic nerve proximal to the posterior cutaneous, here referred to as the musculocutaneous nerve (see Discussion) at the level of the caudofemoral muscle (n=6).

Immediately after nerve resection/s, each one of the five digits was injected with 0,5 µl of one of the following tracers: 5% Fast Blue (FB, Sigma), 2% Fluoro-Gold (FG, Fluorochrome Inc.), or 5% Diamidino Yellow (DY, EMS-Polyloy). The tracer used for the different digits varied between cases. We have previously shown that similar injections of these tracers at the concentrations used give rise to similar number of labeled DRG neurons

from each digit (Puigdellívol et al., 1998b). Separate 10 µl Hamilton syringes and 26 or 25S gauge needles were used for each tracer. An operating microscope was used for optimal control of the needle and to ensure that tracer injections were made into the central parts of the tips of the digits. In a few cases where leakage occurred, the leaked tracer was aspirated with the syringe and reinjected with the aim to obtain equal injection volumes between animals.

2. Quantification of the innervation of the digits via the femoral nerve

In a second group of animals (6 rats, n=6), digit 1 was injected with 0,5 µl 5% FB and digit 2 with 0,5 µl 2% FG. After five days, the femoral nerve was transected at the level of the groin, and its proximal end exposed 30 minutes to 1 µl 5% DY contained in a polyethylene cup made from the cut-off bottom of a 1 ml Eppendorff vial. Frequent inspection ensured that the cut end of the nerve remained covered with dye during the exposure time. The tracer was then removed, the nerve cleaned, the skin sutured, and the animal allowed to recover.

3. Quantification of the innervation of the digits via the musculocutaneous branch of the sciatic nerve

In a third group of animals (6 rats, n=6), the tip of digits 1 and 2 were injected with 0,5 µl 5% FB and digits 3, 4, and 5 with 0,5 µl 2% FG. After five days, the musculocutaneous branch of the sciatic nerve was transected immediately distal to where it crosses the caudofemoral muscle, and its proximal cut end exposed to 5% DY in a plastic cup as described above. Animals were perfused five days later. The detailed course of the musculocutaneous branch of the sciatic nerve was dissected in six rats.

Fixation, tissue sectioning and fluorescent microscopy

Animals were perfused five days after the final dye application. The rats were reanaesthetised as above and a thoracotomy was performed. After an intracardial injection of 1000 IU of heparin/kg body weight, the rats were perfused through the ascending aorta with 100 ml of warm saline followed by 1000 ml cold 4% paraformaldehyde and 10% sucrose in 0.1M phosphate buffer (PB) at pH 7,40 for twenty minutes. Dorsal root ganglia L3-5 were removed in all the animals in all experimental groups. The L6 DRG was removed in all animals except for some rats in the first

group (see table 1). The L2 DRG was removed in most of the cases of the second group of animals (table 2). Spinal cord segments L2-6 were removed in the animals of the second and third groups. The DRGs and spinal cord segments were postfixed for three hours in the same fixative and then immersed in 15% sucrose in PB at 4ºC overnight. The DRGs were cut on a cryostat in 16 μm thick horizontal sections and the spinal cords in 30 μm thick longitudinal sections, thaw-mounted on gelatinized (5%) slides, and coverslipped using an antifading solution containing 1% para-phenylenediamine and 10% PBS in glycerol.

Sections were examined in an Olympus Vanox fluorescent microscope, using appropriate filter combinations (ultraviolet light filters: DM 400 dichroic mirror and UG1 excitation filter, which gives 365 nm excitation- and 420 nm emission wave lengths; and violet light filters: DM 455 dichroic mirror and BP 405 excitation filter, which gives 405 nm excitation- and 455 nm emission wave lengths; see Puigdellívol et al. (1998b) for more information on the identification of labeled neurons. Only labeled neuronal profiles with an identifiable nucleus were counted. Counting was made in all sections of the L5 and L6 DRGs in the first animal group (see above). In the remaining ganglia in this group, and in all ganglia of the second and third groups, counts were performed in every fifth DRG section. Every second consecutive section of the spinal cord was examined for labeled motoneurons. Counting was performed on the day of tissue sectioning to avoid bias from possible spread of labeling in the sections. Counts were not corrected for split cells.

In four cases, the detached skin and the corresponding parts of the feet were examined under epifluorescent illumination to estimate the spread of the tracer from the injection site.

RESULTS

Autotomy occurred in three cases after nerve injury (R131 right side; R244 bilateral). The exact time the autotomy occurred was not recorded, but the duration did not exceed 5 days prior to perfusion.

Spread of tracers

Examination of the detached skin and skinless digits in the fluorescent microscope showed that the injected tracers was restricted to the most distal part of the injected digit, including to some extent its dorsal part. Although occasional cases showed small amounts of dye also in more proximal parts of the digits than the tips, no labeling was seen proximal to the digits, nor in adjacent digits. It may also be relevant to the question of spread of dye that some cases where different tracers were used in different digits showed very few profiles with labeling from more than one digit, i.e. double- or triple-labeled profiles (see below).

Vascular spread of the tracer was suspected in cases where all DRG cells showed a very faint FB or FG labeling. The difference in labeling intensity between these cells and those labeled from axonal transport was evident and was not considered to cause any problem regarding identification. Diamidino Yellow did not show such vascular spread in any case.

Distribution of labeled neuronal profiles in DRGs after nerve resection/s

After resection of the sciatic nerve and injection in digits 1-5, labeled neuronal profiles were present in DRGs L3-5 (table 1)**.** Injection of one dye in digits 1 and 2 (and of a different dye in digits 3-5) showed that DRGs L3-4 contained labeled neuronal profiles from digits 1 and 2, but not from digits 3-5. The L5 and L6 DRGs contained very few labeled cells from any digit after sciatic nerve transection. When resection of the femoral nerve was added to the sciatic resection, labeled profiles from each one of the digits were present in DRGs L5 (7-45 profiles) and L6 (0-22 profiles), with no labeling in DRGs L3 and L4. When a resection of the posterior cutaneous nerve of the thigh was added, similar numbers were seen in L5, but now almost no labeled neuronal profiles were seen in L6 (table 1).

Animals that underwent resection of the sciatic nerve near the knee, and of its more proximal musculocutaneous branch, without resection of the posterior cutaneous nerve, showed only occasional (1-3) labeled neuronal profiles in the L5 and L6 DRGs, except for one case that showed seven labeled neuronal profiles in L6 (table 1).

A small number of multilabeled neuronal profiles were seen after injection of different tracers in different digits in DRG L5 in three cases: double labeling in R142R (2 profiles) and R200L (7 profiles), and triple labeling in R231R (3 profiles).

Resection of all the nerves mentioned above, i.e. the sciatic nerve, the musculocutaneous branch and the posterior cutaneous nerve resulted in only one or two labeled profiles in the L5 and in L6 DRGs. Only one case did not show labeled neurons in these DRGs (table 1).

Contribution of the femoral nerve to the sensory innervation of the digits

The distribution of labeled profiles found after injection of FB in digit 1 and of FG in digit 2, followed by application of DY to the cut femoral nerve is shown in table 2. Fast Blue and FG were found mainly in DRGs L3-5 (mean number of counted profiles: digit 1, 16,0 in L3, 70,3 in L4, 28,6 in L5; digit 2, 9,5 in L3, 42,8 in L4, 28,6 in L5). Less than five FB and FG labeled profiles were found in DRG L6. The DY labeled femoral afferents distributed mainly in L3 (mean number 546,8), with fewer counted profiles in L4 (309,1) and L2 (128,5). Considerable interanimal variation in the number of neuronal profiles was found after injection in a particular digit and after nerve labeling.

In the L3 DRG, DY was present in 89,1% of the FB profiles, and in 90,8 % of the FG profiles (table 2). In the L4 DRG, DY was present in 12,2% of the FB profiles and in 9,8% of the FG profiles (table 2). In this group, one single FB/FG double-labeled cell was found (DRG L4; R205L).

Examination of the spinal cord revealed the presence of 131-206 DY labeled motoneuronal profiles (mean $177,6 \pm 28,7$) when counted in every second consecutive longitudinal section. The rostral and caudal parts of the spinal cord examined were devoid of motoneurones.

Contribution of the musculocutaneous branch of the sciatic nerve to the sensory innervation of the digits

Injections of FB in digits 1 and 2, and of FG in digits 3-5, followed by application of DY to the cut musculocutaneous nerve resulted in the following distribution of tracers in lumbar DRGs (table 3): FB in L3-6, FG in L4-6, and DY in L4-6. The DY distributed mainly in L5 (mean number 271,1). Fewer profiles were counted in L6 (49,0) and L4 (17,6).

Double-labeled profiles (FB/DY or FG/DY) were found in the L5 and L6 DRGs, but not in the L4 DRG. In the L5 DRG, DY was present in 2,4% of the FB profiles, and in 5,5% of the FG profiles. In the L6 DRG, DY was present in 9,2% of the FB profiles, and in 20,5% of the FG profiles (table 3). Multilabeled neuronal profiles (1-4 profiles/case) containing two different dyes injected in digits were seen in DRG L4 (R206R) and DRG L5 (R203R, R204R, R206R, R208R). In three of these cases (R203R, R206R and R208R), up two four profiles in DRGs L5 or L6 contained also DY from the musculocutaneous nerve.

Examination of the spinal cord revealed the presence of 100-167 DY labeled motoneuronal profiles (mean 133,2 ± 28,5) when counted in every second consecutive longitudinal section. The rostral and caudal parts of the cord examined were devoid of motoneurones.

Course of the musculocutaneous branch of the sciatic nerve

Photographs of the topographical relations of the musculocutaneous nerve are shown in fig.1. The sciatic nerve gives off the posterior cutaneous nerve of the thigh (PC) just caudal to the gluteus medius muscle. The PC runs lateral to the caudofemoral muscle and medial to the accessory head of the semitendinosus muscle and then becomes subcutaneous and continues towards the popliteal fossa (not shown). Just distal to where the PC nerve leaves the main sciatic nerve, a small branch which we call the musculocutaneous (MC) leaves the sciatic. It first runs in a caudal direction and then towards the popliteal fossa. It passes medial to the caudofemoral muscle and gives off multiple smaller branches to the medial side of the femoral biceps muscle. The first of these branches goes directly to the medial part of the muscle. The following two branches divide before they enter the belly more distally. The fourth branch

continues distally towards the popliteal fossa, where it divides**.** One of the terminal branches enters the deep aspect of the belly of the semimembranosus muscle, while the other enters the adipose tissue of the popliteal fossa. In two of the five cases examined, it was possible to discern that the latter branch became subcutaneous, crossed the PC nerve, and appeared to terminate in the medial skin of the knee. In two other cases, this branch appeared to anastomose with the PC nerve. The PC and MC nerves appeared to join veins in the adipose tissue of the popliteal fossa, which further distally become the small and lateral saphenous veins. It was not possible, however, to follow the MC or PC branches further distally than the level of the knee.

Distal to the emergence of the MC branch, the sciatic nerve runs towards the knee between the biceps muscle and the femur. At this level, the lateral sural nerve leaves the sciatic nerve and runs laterally towards the middle part of the biceps**,** crosses the muscle and innervates the lateral and posterior skin of the knee region. Finally, the sciatic nerve divides into the tibial, peroneal and sural nerves.

DISCUSSION

The main sensory innervation of the digits is assumed to be derived from the terminal branches of the sciatic and femoral nerves (Greene, 1963; Hebel and Stromberg, 1986). The results of this study indicate the existence of contribution also from a small proximal branch of the sciatic nerve, here called the musculocutaneous nerve, and possibly the posterior cutaneous nerve. Furthermore, the results of this study provide data on the ganglionic routes taken by femoral, posterior cutaneous and musculocutaneous afferents from the digits.

For several reasons described earlier (Prats-Galino et al., 1999) we were unable to use counting methods that would allow estimation of the exact number of labeled neurons. The numbers given here should therefore be accepted as semiquantitative.

A considerable variation in the number of labeled neurons from the digits and nerves was found between animals. It could be due to technical factors such as difficulties to perform identical injection in the digits in different animals, leakage of tracer out of the injected digit (although special measures were taken to reduce this factor, see Methods), and variable subcutaneous spread inside the digit. It may also be due to true inter-animal differences in the number of cells in individual DRGs (Ygge et al., 1981; Avendaño and Lagares, 1996)

Examination of the injected skin and subcutaneous tissue did not show spread of labeling to adjacent digits. We can not exclude a small amount of spread between digits, however, as several cases showed a small number of DRG neurons which contained different tracers injected in two or more adjacent digits. This explanation is weakened, however, by the observation that multilabeled neurons were found almost only in DRGs L5-L6. Even though the material is small and the use of the same tracer in several digits in many cases might have obstructed detection of double labeling, it is reasonable to assume that multilabeling would have been more common in DRGs L3 and L4 if spread were responsible. An alternative explanation would be that a few DRG neurons normally innervate more than one digit.

Resection of the sciatic nerve in the thigh and injection of FG in digits 1 and 2 resulted in labeled neurons in DRGs L3-6. As retrograde tracing from the femoral nerve at the groin level resulted in labeling only in DRGs L2-4 (see also Swett and Woolf, 1985; Puigdellívol et al., 1998a), it is reasonable to assume that the femoral nerve contributed to the innervation of these digits only via DRGs L3-4. Furthermore, our finding that about 90% of the neurons retrogradly

labeled from digits 1 and 2 in DRG L3 were labeled also from the femoral nerve whereas the corresponding numbers for L4 were 12,2% and 9,8%, respectively, indicates that the main contribution of the femoral nerve to digits 1 and 2 is routed via the L3 and to a lesser extent via the L4 DRG. This is in agreement with previous findings after antidromic stimulation of the saphenous branch of the femoral nerve followed by mapping of the distribution of extravasated Evans Blue, indicating that at least thin calibre afferents in this nerve innervate only digits 1 and 2 and only the proximal phalanx of digit 3 (Wiesenfeld-Hallin et al., 1989).

The single labeled neurons observed in DRGs L3-6 after injection in digits 1 and 2 must have taken other routes than the femoral, at least at the level of the thigh. It is conceivable that most of these afferents traveled in distal sciatic branches such as the tibial or peroneal and, although less likely, the sural. The finding that no labeled afferents were present in the L4 DRG after sciatic nerve transection and injection in digits 3-5, indicate that all afferents in the L4 DRG run in the sciatic nerve, at least at the thigh level. Otherwise, the detailed contribution of the sciatic branches to the innervation of the digits was not investigated here. Finally, some of the single labeled afferents from digits 1 and 2 in DRGs L3-4 may have escaped labeling from the application of DY to the femoral nerve for technical reasons, since only 95% of all afferents in a transected nerve become labeled in the DRG after application of fluorescent tracers such as DY to the proximal end (Puigdellívol et al., *in press* in J Neurosci Methods).

The finding of residual labeled profiles in the L5 and L6 DRGs after combined resection of the main sciatic and the femoral nerve followed by injection in all digits, indicates the existence of alternative pathways. The finding that almost all such labeled neurons disappeared after resection of also a proximal branch of the sciatic, here called the musculocutaneous branch, indicates that afferents in this nerve reach the distal phalanges of the digits. Additional routes may be present, however, as a few labeled profiles remained in the L5 and L6 DRGs after transection of all three nerves and the posterior cutaneous. As discussed above, such afferents may enter distal branches of the transected nerves and then switch more proximally to other routes that escaped transection.

The majority of the afferents in the musculocutaneous nerve were represented in the L5 DRG, together with the major part of the sciatic afferents. While the sciatic nerve follows in close relationship with the femur towards the knee, the musculocutaneous nerve continues caudally and follows a deep course towards the popliteal fossa where it becomes

more superficial and was observed to anastomose with the posterior cutaneous nerve of the thigh in some cases. The posterior cutaneous nerve runs medial and posterior in the proximal hindlimb (Swett and Woolf, 1985; Doubell et al., 1997), and has a level of origin similar to the musculocutaneous nerve.

We think that the musculocutaneous nerve of the hindlimb is an appropriate name for the proximal branch of the sciatic nerve that innervates the digits, as described above. It contains not only sensory afferents but also efferents to several muscles including the biceps and therefore shares some homology with the musculocutaneous nerve of the forelimb. A previous reports on the innervation of the femoral biceps muscle described a "single branch of the sciatic nerve which divides into two twigs within the muscle belly" (Manzano and McComas, 1988). In our material, at least three subdivisions of the musculocutaneous nerve are directed to the biceps. The second and third branches consistently bifurcate before entering the muscle belly. It is possible that differences in innervation pattern between strains of rats explain this inconsistency.

The numbers of motoneurones in the femoral nerve reported here corresponds well with earlier findings (Brushart, 1990). We were unable to find any previous reports on the number of motoneurones in the nerve we call the hindlimb musculocutaneous nerve, innervating the femoral biceps muscle and perhaps parts of the semimembranosus muscle.

The dense innervation of the tips of the digits and their natural separation by the interdigital spaces offers an attractive model for studies of regeneration accuracy after nerve injury. In this model, a retrograde tracer is first injected in the tip of a digit. After a few days to allow the tracer to reach the cell bodies in the DRGs, the peripheral nerve that harbours the afferents is injured and then allowed to regenerate. After regeneration, a second tracer is injected into the formerly injected digit and is then axonally transported to the DRG cell bodies. The proportion of single- and double-labeled neurons in the DRGs is an indication of how well the regenerating afferents reach their former innervation targets. For this model, a description of the nerves that contribute to the sensory innervation of the tips of the digits and the DRGs involved forms important background data. Our results demonstrate that not only the femoral and main distal sciatic branches but also proximal branches of the sciatic nerve contribute to the innervation of the digits. This indicates that resection of the femoral and main distal sciatic branches is insufficient for complete

denervation of the foot. The significance of this "residual innervation" for cell counts in studies of regeneration accuracy as described above or for the sensory function in the normal rat is uncertain at present. For studies of regeneration accuracy, it may be indicated to perform transection of also proximal sciatic branches prior to tracing to remove remaining uninjured fibers. From a general point of view, it can not be excluded that activity in such residual uninjured fibers may give rise to increased postsynaptic responses in dorsal horn neurons sensitized by the adjacent nerve injury and thus contribute to hyperalgesia and/or allodynia in neuropathic pain states. Furthermore, the existence of such residual fibers should be kept in mind in studies of functional recovery after nerve injury as they may contribute to signs of reinnervation, perhaps by distal collateral sprouting as has been described for uninjured adjacent nerve afferents in rats (Kinnman and Aldskogius, 1986; Wiesenfeld-Hallin et al., 1988, 1989; Kinnman et al, 1992; Kinnman and Wiesenfeld-Hallin, 1993) and also suggested to occur in man (Aszmann et al., 1996; Healy et al., 1996).

ACKNOWLEDGMENTS

We thank Mrs. Dolors Fuster for excellent technical assistance. This project was supported with the grants of the Fundació Marató TV3, project No 003/97 and the Swedish Medical Research Council, project No.11566 and 13124.

LITERATURE CITED

Aszmann OC, Muse V, Dellon AL. 1996. Evidence in support of collateral sprouting after sensory nerve resection. Ann Plastic Surg 37:520-525.

Avendaño C, Lagares A. 1996. Stereological analysis of the numerical distribution of neurons in dorsal root ganglia C4-T2 in adult macaque monkeys. Somatosens Motor Res 13:59-66.

Brushart TM. 1990. Preferential motor reinnervation: a sequential double labeling study. Restor Neurol Neurosci 1:281-287.

Doubell TP, Mannion RJ, Woolf CJ. 1997. Intact sciatic myelinated primary afferent terminals collaterally sprout in the adult rat dorsal horn following section of a neighbouring peripheral nerve. J Comp Neurol 380:95-104.

Greene ECh. 1963. Anatomy of the rat. New York and London: Hafner Publishing Co.

Healy C, LeQuesne PM, Lynn B. 1996. Collateral sprouting of cutaneous nerves in man. Brain 119:2063-2072.

Hebel R, Stromberg MW.1986. Anatomy and embryology of the laboratory rat. Wörthsee: BioMed Verlag.

Kinnman E, Aldskogius H. 1986. Collateral sprouting of sensory axons in the glabrous skin of the hindpaw after chronic sciatic nerve lesion in adult and neonatal rats: a morphological study. Brain Res 377:73-82.

Kinnman E, Wiesenfeld-Hallin Z. 1993. Time course and characteristics of the capacity of sensory nerves to reinnervate skin territories outside their normal innervation zones. Somatosens Motor Res 10:445-454.

Kinnman E, Aldskogius H, Johansson O, Wiesenfeld-Hallin Z. 1992. Collateral reinnervation and expansive regenerative reinnervation by sensory axons into “foreign” denervated skin: an immunohistochemical study in the rat. Exp Brain Res 91:61-72.

Leem JW, Willis WD, Chung JM. 1993. Cutaneous sensory receptors in the rat foot. J Neurophysiol 69:1684-1699.

Manzano G, McComas AJ. 1988. Longitudinal structure and innervation of two mammalian hindlimb muscles. Muscle & Nerve 11:1115-1122.

Molander C, Grant G. 1985. Cutaneous projections from the rat hindlimb foot to the substantia gelatinosa of the spinal cord studied by transganglionic transport of WGA-HRP conjugate. J Comp Neurol 237:476-484.

Prats-Galino A, Puigdellívol-Sánchez A, Ruano-Gil D, Molander C. 1999. The representations of hindlimb digits in rat dorsal root ganglia, J Comp Neurol 408:137-145.

Puigdellívol-Sánchez A, Prats-Galino A, Ruano-Gil D, Molander C. 1998a. Sciatic and femoral nerve sensory neurons occupy different regions of the L4 dorsal root ganglion in the adult rat. Neurosci Lett 251:169-172.

Puigdellívol-Sánchez A, Prats-Galino A, Ruano-Gil D, Molander C. 1998b. Efficacy of the fluorescent dyes Fast Blue, Fluoro-Gold, and Diamidino Yellow for retrograde tracing to dorsal root ganglia after subcutaneous injection. J Neurosci Methods 86:7-16.

Puigdellívol-Sánchez A, Prats-Galino A, Ruano-Gil D, Molander C. Fast Blue and Diamidino Yellow as retrograde tracers in peripheral nerves: efficacy of combined nerve injection and capsule application to transected nerves in the adult rat, *in press in* J Neurosci Methods

Swett JE, Woolf CJ. 1985. The somatotopic organization of primary afferent terminals in the superficial laminae of the dorsal horn of the rat spinal cord. J Comp Neurol 231:66-77.

Takahashi Y, Nakajima Y, Sakamoto T. 1994. Dermatome mapping in the rat hindlimb by electrical stimulation of the spinal nerves. Neurosci Lett 168:85-88.

Wiesenfeld-Hallin Z, Kinnman E, Aldskogius H. 1988. Studies of normal and expansive cutaneous innervation territories of intact and regenerating C-fibres in the hindlimb of the rat. Agents & Actions 25:260-262.

Wiesenfeld-Hallin Z, Kinnman E, Aldskogius H. 1989. Expansion of innervation territory by afferents involved in plasma extravasation after nerve regeneration in adult and neonatal rats. Exp Brain Res 76:88-96.

Ygge J, Aldskogius H, Grant G. 1981. Asymmetries and symmetries in the number of thoracic dorsal root ganglion cells. J Comp Neurol 202:365-372.

TABLES

Table 1. **Labeled profiles in DRGs after nerve resections.** Numbers of labeled neuronal profiles counted in every fifth consecutive section in L3 and L4, and in all sections in L5 and L6 DRGs, after resection of different hindlimb nerves, followed by injection in the digits: (+), <5 profiles; +, 5-9 profiles; ++, 10-19 profiles; +++, 20 or more profiles. Ganglia which were not examined are indicated by -. All digits were injected in all cases shown in this table except case R26(∗), where only digits 2-3 were injected. L2 did not contain labeled cells and is therefore not included in the table. Nerves resected: SCI (sciatic), FEM (femoral), PC (posterior cutaneous) and MC (musculocutaneous).

Case	**Digits, tracer injected**					**Nerve/s resected**				**Counted profiles in DRG**			
	1	2	3	4	5	SCI	FEM	PC	MC	L3	L4	**L5**	**L6**
R26L*		FB	FG			X				+++	(+)	+++	-
R47L	FG	FG	FG	FG	FG	X				+++	0	+++	-
R231R	FG	FG	DY	FB	FB	X				+++	+++	++	-
R103R	FG	FG	FG	FG	FG	X	X			0	0	+++	-
R130R	FG	FG	FG	FG	FG	X	X			0	0	++	+++
R131R	FG	FG	FG	FG	FG	X	X			0	0	+	+
R142R	FB	FB	FG	DY	DY	X	X			(+)	0	++	+
R244L	FB	FB	FB	FB	FB	X	X	X		0	0	++	**(+)**
R200L	FG	FG	DY	FB	FB	X		X		++	++	++	**0**
R201R	FG	FG	DY	FB	FB	X		X		+++	++	+++	**0**
R246R	FB	FB	FB	FB	FB	X		X	X	++	0	**(+)**	**0**
R246L	FB	FB	FB	FB	FB	X			X	+++	++	**(+)**	**(+)**
R201L	FG	FG	DY	FB	FB	X			X	-	+++	**(+)**	**0**
R200R	FG	FG	DY	FB	FB	X			X	-	+	**(+)**	**0**
R232R	FB	FB	FB	FB	FB	X	X		X	-	-	**(+)**	+
R232L	FB	FB	FB	FB	FB	X	X	X	X	-	-	**(+)**	**0**
R244R	FB	FB	FB	FB	FB	X	X	X	X	-	-	**0**	**0**

Table 2. Femoral nerve afferents in digits 1 and 2. Neuronal profiles counted in one of every fifth consecutive section in DRGs L3-5 after FB injection in digit 1 (FB+FB/DY), and FG injection in digit 2 (FG+FG/DY). Percentages of FB or FG labeled profiles that contain also DY, applied at the cut end of the femoral nerve. - = DRG were not examined.

Numbers of counted profiles				%femoral	
DRG Case	FB total (digit1)	FG total (digit 2)	DY total (femoral nerve)	**FB/DY** / **FB+FB/DY** (% in digit 1)	**FG/DY** / **FG+FG/DY** (% in digit 2)
L2 R56R	-	-	-	-	-
R58R	-	-	-	-	-
R135R	0	0	24	0%	0%
R203L	0	0	41	0%	0%
R204L	0	0	214	0%	0%
R205L	0	0	235	0%	0%
Mean	**0**	**0**	**128,5**	**0%**	**0%**
L3 R56R	13	3	464	100,0%	100,0%
R58R	20	5	533	78,9%	60,0%
R135R	10	23	532	90,0%	91,3%
R203L	12	7	589	100,0%	100,0%
R204L	11	16	471	72,7%	93,7%
R205L	30	3	692	93,3%	100,0%
Mean	**16,0**	**9,5**	**546,83**	**89,1%**	**90,8%**
L4 R56R	79	52	464	8,8%	5,7%
R58R	106	68	138	3,7%	1,4%
R135R	33	22	491	15,1%	18,1%
R203L	47	44	311	21,2%	11,3%
R204L	44	36	191	11,3%	19,4%
R205L	113	35	260	13,2%	2,8%
Mean	**70,3**	**42,8**	**309,1**	**12,2%**	**9,8%**
L5 R56R	16	11	0	0%	0%
R58R	6	32	0	0%	0%
R135R	14	14	0	0%	0%
R203L	31	27	0	0%	0%
R204L	41	32	0	0%	0%
R205L	64	56	0	0%	0%
Mean	**28,6**	**28,6**	**0**	**0%**	**0%**

Table 3. Musculocutaneous nerve afferents in the digits. Numbers of neuronal profiles counted in one of every fifth section in DRGs L3-6 after injection of FG in digits1 and 2, and of FB in digits 3-5. Percentages of those cells that contain also DY applied to the cut end of the musculocutaneous nerve are presented. Numbers have not been corrected for split cells.

		Counted profiles			% Musculocutaneous	
DRG	Case	FG total Digits1,2	FB total Digits 3-5	DY total (nerve)	Medial digits	Lateral digits
L3	R203R	31	1	0	0%	0%
	R204R	15	0	0	0%	0%
	R205R	30	0	0	0%	0%
	R206R	25	4	1	0%	0%
	R207R	35	4	0	0%	0%
	R208R	19	0	0	0%	0%
	Mean	**25,8**	**1,5**	**0,1**	**0%**	**0%**
L4	R203R	80	84	8	0%	0%
	R204R	97	58	6	0%	0%
	R205R	98	132	4	0%	0%
	R206R	129	91	20	0%	0%
	R207R	151	178	19	0%	0%
	R208R	51	98	49	0%	0%
	Mean	**101,0**	**106,8**	**17,6**	**0%**	**0%**
L5	R203R	44	195	212	2,0%	0%
	R204R	43	236	295	1,2%	0%
	R205R	70	248	253	0,4%	1,4%
	R206R	98	235	330	2,4%	5,7%
	R207R	33	152	236	4,4%	17,5%
	R208R	22	115	301	4,1%	8,3%
	Mean	**51,6**	**196,8**	**271,1**	**2,4%**	**5,5%**
L6	R203R	1,00	15	23	6,2%	50,0%
	R204R	0,00	8	61	38,4%	0,0%
	R205R	4,00	17	119	10,5%	20,0%
	R206R	4,00	12	16	0,0%	20,0%
	R207R	4,00	11	12	0,0%	33,3%
	R208R	1,00	5	3	0,0%	0,0%
	Mean	**2,33**	**11,3**	**39,0**	**9,2%**	**20,5%**

FIGURE LEGENDS

Abbreviations.

ad: adipose tissue, bf: femoral biceps muscle, cf: caudofemoral muscle, gm: gluteus maximus muscle, gn: gastrocnemius muscle, ls: lateral sural nerve, mc: musculocutaneous branch of the sciatic nerve, p: peroneal nerve, pc:posterior cutaneous nerve of the thigh, s: sural nerve, sc: sciatic nerve, sm: semimembranosus muscle, sph: small saphenous vein, t: tibial nerve, vlq: lateral vastus of the quadriceps muscle.

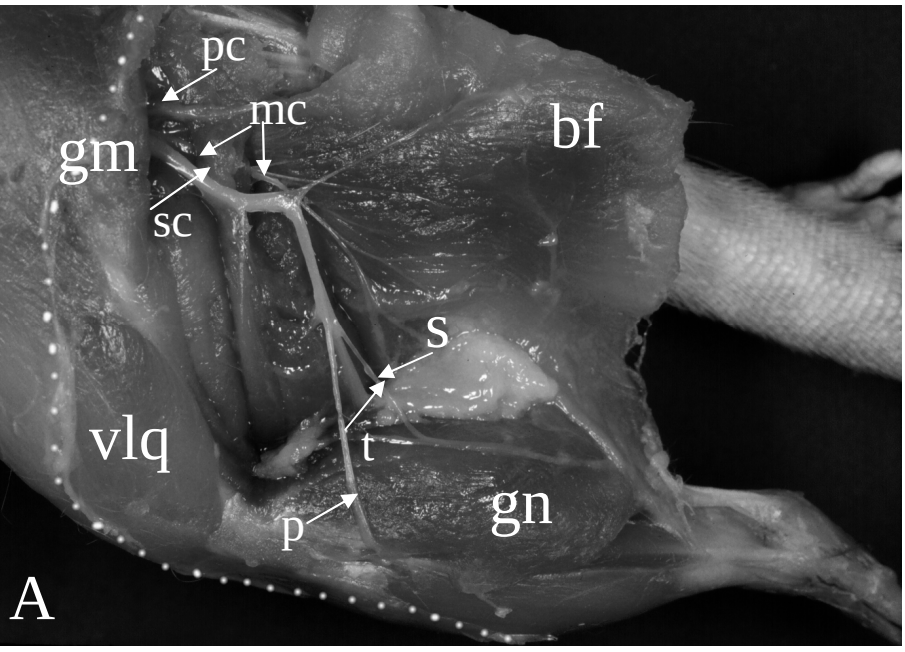
Figure 1.

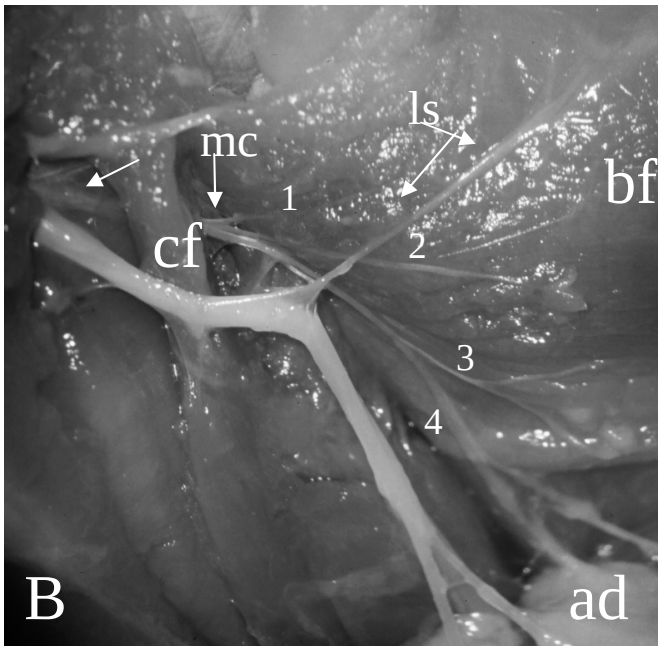
A. Lateral view of the left hindlimb. The white dots indicate the opening incision: the rostral insertion of the femoral biceps muscle has been cut along the aponeurotic layer, extending close to the gluteus maximus muscle medially and the lateral vastus of the quadriceps muscle laterally. The lateral insertion of the biceps muscle has been cut at its insertion to the tibia close to the lateral head of the gastrocnemius muscle. The origin of the posterior cutaneous nerve of the thigh, the musculocutaneous branch of the sciatic nerve, the sciatic nerve, and its main branches –the tibial, peroneal and sural nerves- are shown (small arrows).

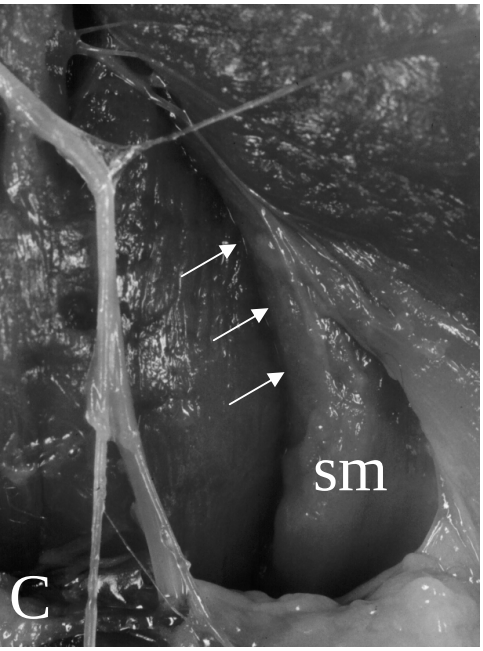
B. Detail of the course of the musculocutaneous branch of the sciatic nerve. Passing medially to the caudofemoral muscle, it gives off three branches to the biceps muscle (1, 2 and 3). The second and third branches bifurcate before entering the belly. The fourth branch (4) continues towards the adipose tissue of the popliteal fossa. The lateral sural nerve leaves the sciatic nerve before its main bifurcation, and enters the middle part of the biceps muscle (before it becomes cutaneous).

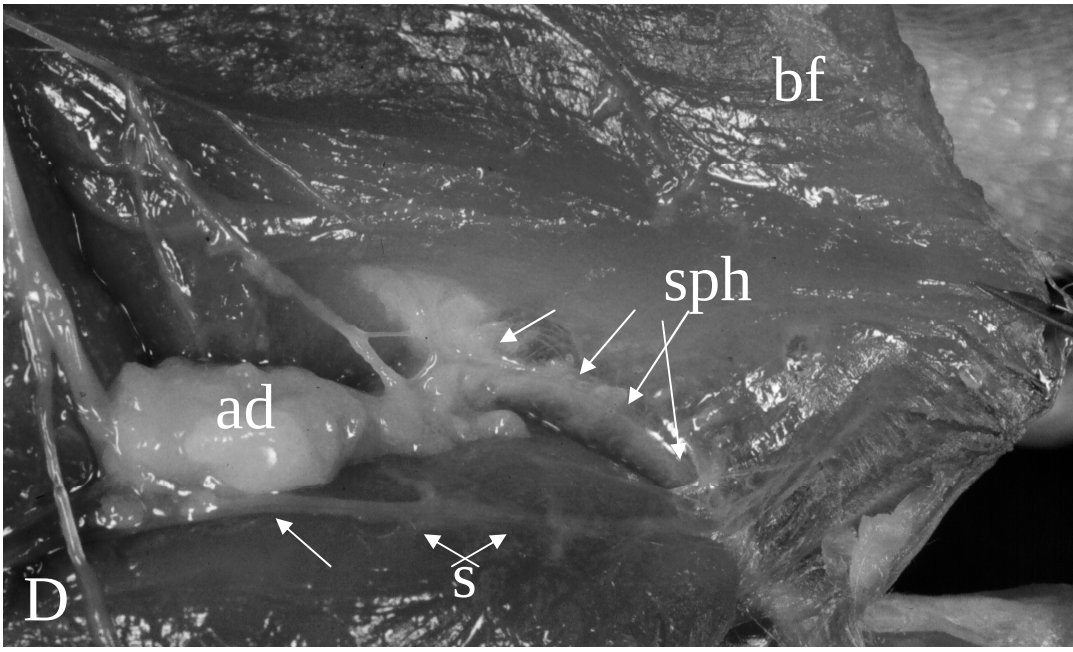
C. One of the terminal branches of the musculocutaneous nerve enters the deep aspect of the semimembranosus belly.

D. The other terminal branch appears to join blood vessels –the small saphenous vein- within the adipose tissue of the popliteal fossa and probably follows them to the posterior aspect of the ankle, where they cross the sural nerve.


pc
mc
gm
bf
sc
s
vlq
t
p
gn
A


ls
mc
bf
1
cf
2
3
4
B
ad


sm
C


bf
sph
ad
s
D