

**SCIATIC AND FEMORAL NERVE SENSORY NEURONES OCCUPY
DIFFERENT REGIONS OF THE L4 DORSAL ROOT GANGLION IN THE
ADULT RAT**

Anna Puigdellívol-Sánchez¹, Alberto Prats-Galino^{1*}, Domingo Ruano-Gil¹,
and Carl Molander²

1. Department of Morphological Sciences, Faculty of Medicine, University of
Barcelona, Spain.
2. Department of Neuroscience and Department of Surgery, Section for
Rehabilitation Medicine, Karolinska Institutet, Stockholm, Sweden

* Author for correspondence

Corresponding address:

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

Fax number: 34-93-4035260

Telephone number:34-93-4021905

E-mail: aprats@medicina.ub.es

KEY WORDS: primary sensory afferents, three-dimensional reconstructions, dorsal root ganglion somatotopy, sciatic nerve, femoral nerve, fluorescent tracers.

ABSTRACT

The topographical distribution of sciatic and femoral nerve sensory neuronal somata in the L4 dorsal root ganglion of the adult rat was mapped after retrograde tracing with one or two of the dyes Fast Blue, Fluoro-Gold, or Diamidino Yellow. The tracers were applied to the proximal transected end of either nerve alone, or from both nerves in the same animal using separate tracers. Three-dimensional reconstructions of the distribution of labelled neurones were made from serial sections of the L4 dorsal root ganglion which is the only ganglion that these two nerves share. The results showed that with little overlap, femoral nerve neurones distribute dorsally and rostrally whereas sciatic nerve neurones distribute medially and ventrally. This finding shows an example which indicates the existence of a somatotopical organisation for the representation of different peripheral nerves in dorsal root ganglia of adult animals.

A somatotopic distribution of hindlimb sensory afferents has repeatedly been described in the superficial laminae of the spinal cord of the rat [2,4,7,14,15]. Within dorsal root ganglia (DRG), a somatotopic organisation has been reported in the rat during development for hindlimb nerves [6,16,18], and in adulthood for cutaneous afferents in the frog [1,3] and cat [5], whereas no clear organisation has been demonstrated for muscle afferents or mixed nerves in adult rats [8,9,10,11,12] or pigeons [13]. The aim of the present study has been to reinvestigate whether a topographical organisation can be demonstrated in a DRG of the adult rat by retrograde tracing of sensory afferents in the sciatic and femoral nerves to the L4 DRG using different fluorescent dyes, and to present a three dimensional reconstruction of the positions of neurones labelled from each nerve inside the ganglion.

Fifteen adult Sprague Dawley rats (250-350 b.w.) were used. All the animals have been treated in compliance with the guidelines of the Faculty of Medicine, University of Barcelona. Anaesthesia was initiated with ether and then continued with chloral hydrate (30mg/100g b.w.) during surgical procedures and perfusion.

In the first group, the femoral nerve was transected in the upper thigh and the proximal end inserted into a plastic capsule containing 2% Fluoro-Gold (FG; Fluorochrome.Inc; unilateral experiments in 5 rats). After 20 minutes, capsules were removed, and the proximal nerves and surrounding tissue carefully cleaned to avoid hematogeneous spread of dye. All animals in this group were also subjected to subcutaneous injections of other tracers, which are unlikely to interfere with the results presented here and will be presented elsewhere.

In a second group, the sciatic nerve was transected at mid-thigh level and the proximal end inserted in a capsule containing 5% Fast Blue (Sigma), or 2% FG

combined with 5% Diamidino Yellow (DY, EMS Polyloy) (unilateral experiments in 2 rats, bilateral in 5 rats). Double labelling percentages and cell counts will be presented elsewhere.

In a third group of animals (n=3), the proximal end of the transected femoral nerve was inserted into a capsule containing either 2% FG (n=2) or 5% DY (n=1). The ipsilateral sciatic nerve was labelled in the same way with 5% FB. Capsules were left in situ for 20 minutes, as described above.

Five days later after dye application, the rats were reanaesthetised and a thoracotomy was performed. After an intracardial injection of 1000IU of heparin/kg b.w., the rats were perfused through the ascending aorta with 100ml of saline and then with 1000ml 4% paraformaldehyde and 10% sucrose in phosphate buffer (PB; pH 7,4) for twenty minutes. The ganglia L2-6 were removed, postfixed for 3h in the same perfusion solution, and immersed in 15% sucrose in PB overnight. All ganglia were carefully oriented before embedding (Tissue-Tek[®], Miles), so that the original orientation in the rat could be reconstructed. The DRG's were cut on a cryostat, in either 10µm (L4-5), or 16µm (L2, L3 and L6) thick longitudinal sections which were all mounted on chrom-alum gelatinised (5%) slides and then coverslipped with an antifading solution containing 1% paraphenyldiamine and 10% PBS in glycerol. Slides were examined in an Olympus Vanox fluorescent microscope, using ultraviolet light filters (DM 400 dichroic mirror and UG1 excitation filter, which gives a 365 nm excitation, 420 nm emission wave length) and violet light filters (DM 455 dichroic mirror and BP 405 excitation filter, which gives a 405 nm excitation, 455 nm emission wave length). The distribution of labelled neuronal profiles was mapped from photographs and drawings of the serial sections.

To further improve illustration, one of the cases with combined femoral and sciatic nerve tracing was randomly selected for computerised three-dimensional reconstruction. The computer analysis consists in three steps: (i) digitalisation of labelled cell positions and ganglionic tissue contours using a digitising tablet (Digicad) connected to an image analysis system (IMCO 10, Kontron), (ii) 3-D surface reconstruction from aligned serial sections by triangularisation using a specially developed software [11], and finally (iii) generation of a shaded 3D model from the resulting triangular facets using 3D Studio Max 1.2 (Kinetix) software. Original data files were transformed to VRML format and then converted by the Crossroad File Format Converter into Autocad DXF format, which could be imported into 3D Studio Max. The opacity of the ganglionic surface was diminished up to a 30% to allow visualisation of the spheres representing neurones inside. The model was reconstructed in dorsal, rostral and caudal views and has a final resolution in the Z-plane of 30 μm and about 90 to 100 μm in the X and Y planes. The graphic output TIF format files, with different views of the ganglia, were finally processed by a Powerpoint 97 program (Microsoft) and printed in a Kodak Digital Science 8650 PS Color Printer.

Retrogradely labelled sensory neurones were found in ganglia L4-6 from the sciatic nerve and in ganglia L2-4 from the femoral nerve. No clear topographical arrangement of labelled sciatic or femoral neurones was obvious by observing individual sections in any of these ganglia, except for ganglion L4. In this ganglion, most femoral nerve neurones were found in its dorsal and rostral parts (Fig. 1A). In more ventral parts of the ganglion, the number of labelled femoral nerve neurones became gradually reduced and distributed only in its rostralateral part (Fig. 1B), leaving only a small number of neurones in its most ventral part (Fig. 1C). Sciatic

nerve neurones were found mainly medially and ventrally and clearly outnumbered the femoral nerve neurones.

Although slight inter-individual variations were found, the different distribution of labelled sciatic and femoral nerve neuronal somata was a consistent finding in the both the single nerve experiments and the cases where both the femoral and sciatic nerves were labelled in the same animal. Furthermore, the latter cases supplemented the results from the single nerve experiments by showing that there were few overlapping neurones in the boundary zone. The topographical arrangement is exemplified by a computerised 3D reconstruction of the distribution (Fig. 2 A.B.C).

This study demonstrates that femoral and sciatic afferent neurones have different distributions in the L4 DRG, the main DRG that these two nerves share [8]. Several previous studies have indicated the possibility of a somatotopical arrangement in DRG's. Neurones supplying hindlimb muscles in the rat were reported to show a tendency to cluster in groups which supply individual muscles [9,11]. Following retrograde labelling of nerves in immature rats, a rostrocaudal organisation of neuronal somata within DRG's was described at lumbar [6,16] and cervical [17] levels. In adult rats, however, no clear somatotopical organisation in the DRG could be observed after tracing from intercostal nerves [19] or hindlimb digits [7]. Following retrograde tracing from peripheral structures and the spinal cord in adult cats, however, DRG's were found to be organised in proximally to distal elongated slabs or columns [5]. Neuronal somata located along the lateral border of the ganglion were connected to rostral root filaments, whereas those distributed along the medial border were connected to caudal root filaments. This principle seems to

correspond well to the finding in the present study, where the more rostrally represented femoral nerve occupied rostralateral parts of the L4 DRG.

It has been suggested that the difficulty to demonstrate a somatotopic organisation in the DRG in adult rats may partly be due to technical factors, including spatial orientation and relations of the DRG to its surroundings [18]. Although no special measure to preserve the spatial relationship to the surroundings of the DRG was taken in this study, special care was taken to preserve the dorsoventral and rostrocaudal orientation of the ganglia when embedding in the sectioning medium before sectioning. This was made by taking into account that the DRG's are not uniformly spherical, having a wider region caudally which projects in the medial direction and that the dorsal root exits laterally to the main mass of the ganglion.

Taken together, the results of the single and combined nerve labelling experiments in this study indicate an example of a somatotopical arrangement in a DRG. Whether this example is an exception or a general phenomenon remains to be demonstrated. Further experiments are being prepared to demonstrate a possible somatotopic arrangement of neuronal somata in DRG projecting to other lumbosacral nerves.

We thank Mrs. Dolors Fuster for excellent technical assistance. This project was supported with the grants of the Secretaría de Estado de Universidades e Investigación, Programa Sectorial de Promoción General del Conocimiento (PM96-0059, the Fundació Marató TV3, project No 003/97 and the Swedish Medical Research Council, project No.11566. We thank Dr. Josep M^a Estañol and professor Oriol Bachs for their collaboration in printing the pictures and professor Hakan Aldskogius for valuable comments.

- [1] Baker, R.E., Matesz, K., Corner, M.A. and Szekely, G., Peripheral reinnervation patterns and dorsal root ganglion topography in skin-grafted frogs: a behavioural and histological examination, *Dev. Neurosci.*, 4 (1981) 134-41.
- [2] Cervero, F. and Connell, L.A., Fine afferent fibers from viscera do not terminate in the substantia gelatinosa of the thoracic spinal cord, *Brain Res.*, 294 (1984) 370-374.
- [3] Corner, M.A., Veltman, W.A., Baker, R.E. and Van de Nes, J., Topography of cutaneous spinal ganglion cells in the frog (*Rana esculenta*), *Brain Res.*, 156 (1978) 151-6.
- [4] Grant, G., Projection of primary sensory neurons studied by transganglionic methods: somatotopy and target-related organization, *Brain Res. Bull.*, 30 (1993) 199-208.
- [5] Kausz, M. and Rethelyi, M., Lamellar arrangement of neuronal somata in the dorsal root ganglion of the cat, *Somatosens. Res.*, 2 (1985) 193-204.
- [6] Mirnics, K. and Koerber, H.R., Prenatal development of rat primary afferent fibers: I. Peripheral projections, *J. Comp. Neurol.*, 355 (1995) 589-600.
- [7] Molander, C. and Grant, G., 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 (1985) 476-84.
- [8] Molander, C. and Grant, G., Laminar distribution and somatotopic organization of primary afferent fibres from hindlimb nerves in the dorsal horn. A study by transganglionic transport of horseradish peroxidase in the rat, *Neuroscience*, 19 (1986) 297-312.

- [9] Peyronnard, J.M., Charron, L.F., Lavoie, J. and Messier, J.P., Motor, sympathetic and sensory innervation of rat skeletal muscles, *Brain Res.*, 373 (1986) 288-302.
- [10] Peyronnard, J.M., Charron, L.F., Lavoie, J., Messier, J.P. and Dubreuil, M., Carbonic anhydrase and horseradish peroxidase: double labelling of rat dorsal root ganglion neurons innervating motor and sensory peripheral nerves, *Anat. Embryol.*, 177 (1988) 353-359.
- [11] Peyronnard, J.M., Messier, J.P., Dubreuil, M., Charron, L.F. and Lebel, F., Three-dimensional computer-aided analysis of the intraganglionic topography of primary muscle afferent neurons in the rat, *Anat. Rec.*, 227 (1990) 405-417.
- [12] Prats Galino, A., Costa Llobet, C., Arroyo Guijarro, J. and Ruano Gil, D., The facial motor nucleus of the dog. I. Cytoarchitectonic subdivisions and cytology, *Acta Anat.*, 132 (1988) 276-279.
- [13] Schmid, H., Taylor, D.C.M. and Pierau, F.K., Tracing of sensory neurones and spinal motoneurones of the pigeon by injection of fluorescent dyes into peripheral nerves, *Cell Tissue Res.*, 323 (1983) 9-19.
- [14] Shortland, P. and Woolf, C.J., Morphology and somatotopy of the central arborizations of rapidly adapting glabrous skin afferents in the rat lumbar spinal cord, *J. Comp. Neurol.*, 329 (1993) 491-511.
- [15] Swett, J.E. and Woolf, C.J., The somatotopic organization of primary afferent terminals in the superficial laminae of the dorsal horn of the rat spinal cord, *J. Comp. Neurol.*, 231 (1985) 66-77.
- [16] Wessels, W.J.T., Evidence for a rostrocaudal organization in dorsal root ganglia during development as demonstrated by intra-uterine WGA-HRP injections into the hindlimb of rat fetuses, *Dev. Brain Res.*, 54 (1990) 273-281.

- [17] Wessels, W.J.T. and Marani, E., A rostrocaudal somatotopic organization in the brachial dorsal root ganglia of neonatal rats, *Clin. Neurol. Neurosurg.*, 95 (1993) S3-S11.
- [18] Wessels, W.J.T., Feirabend, H.K.P. and Marani, E., The rostrocaudal organization in the dorsal root ganglia of the rat: a consequence of plexus formation?, *Anat. Embryol.*, 190 (1994) 1-11.
- [19] Ygge, J., On the organization of the thoracic spinal ganglion and nerve in the rat, *Exp. Brain Res.*, 55 (1984) 395-401.

FIGURE CAPTIONS

Fig. 1. Photomicrographs of longitudinal sections of the L4 DRG. Rostral (r), caudal (c), medial (m) and lateral (l). Note rostral distribution of femoral neurons (FG, arrow), and caudal distribution of sciatic neurons (FB, arrowhead) in sections at dorsal level (A), and mid level (B) of the L4 DRG. In section of ventral L4 DRG (C), note sparse numbers of femoral neurons (arrows), and sciatic neurons (FB, arrowhead), occupying most of the DRG tissue at this level. Scale bar= 200 μ m in all sections.

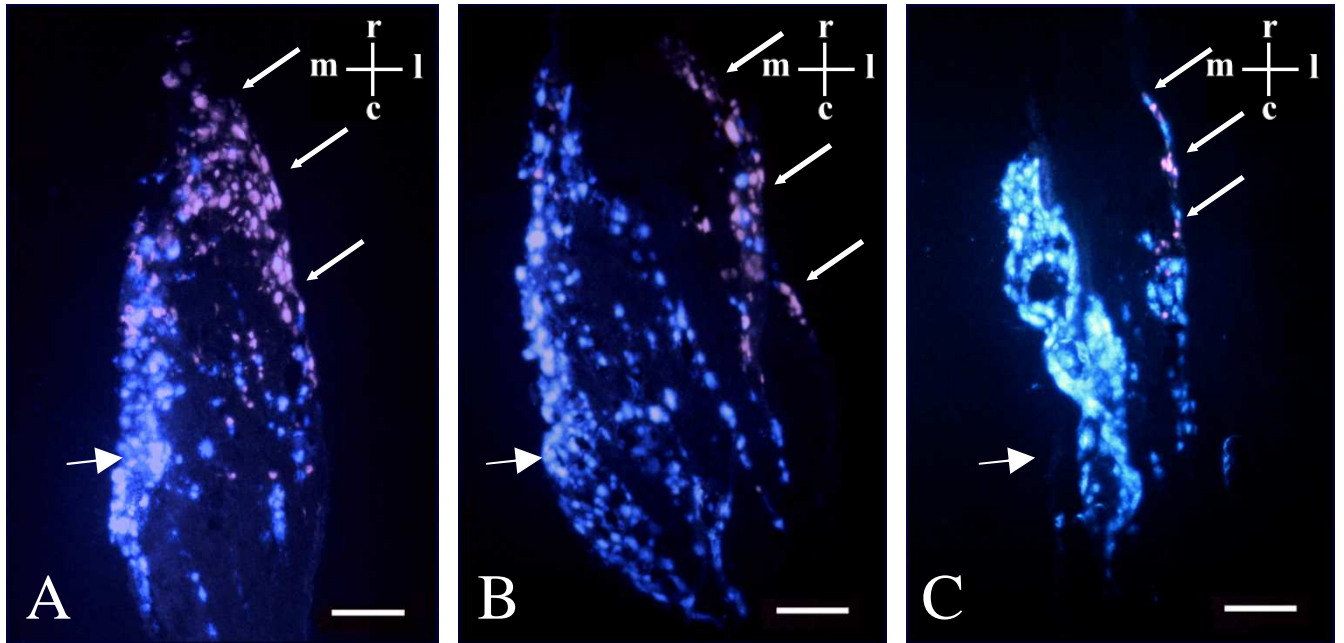


Fig 2. Three-dimensional reconstruction of L4 DRG containing femoral (red) and sciatic (blue) neurons. Dorsal view (A) rostral (B) and caudal (C) view are presented. Rostral (r), caudal (c), medial (m), lateral (l), ventral (v) and dorsal (d). Note progressively thinner ventral extension of femoral neurons from dorsal-most level in the rostral view. Scale bar= 200 μ m in all sections.

