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Brain Research 1002 (2004) 120–128
BRAIN RESEARCH
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Research report

Absence of Meissner corpuscles in the digital pads of mice lacking functional TrkB

Tatiana González-Martínez[a,1], Germana P. Germanà[b], Diego F. Monjil[a,c], Inmaculada Silos-Santiago[d], Félix de Carlos[c,e], Giovanni Germanà[b], Juan Cobo[c,e], José A. Vega[a,f,*,1]

[a] *Departamento de Morfología y Biología Celular, Facultad de Medicina, C/ Julián Clavería, s/n, 33006 Oviedo, Spain*
[b] *Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Università di Messina, Italy*
[c] *Instituto Asturiano de Odontología, Oviedo, Spain*
[d] *Department of Neurobiology, Milennium Pharmaceuticals Inc., Cambridge, MA, USA*
[e] *Departamento de Cirugía y Especialidades Médico-Quirúrgicas, Universidad de Oviedo, Oviedo, Spain*
[f] *Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, Oviedo, Spain*

Accepted 5 January 2004

Abstract

The TrkB-expressing sensory neurons seem to be involved in touch and other discriminative sensibilities. Thus, several slowly and rapidly adapting cutaneous mechanoreceptors, as well as muscle spindles, are reduced or absent in the territory of the trigeminal nerve in functionally TrkB-deficient mice. Whether this also occurs in the cutaneous or muscular territories of dorsal root ganglia has not been analyzed. Here we used immunohistochemistry and transmission-electron microscopy to analyze the impact of a mutation in the gene coding for TrkB on Meissner and Pacinian corpuscles, and muscle spindles. The animals were studied at the post-natal days 15 and 25, because at this time all the mechanoreceptors examined are fully developed. Typical Meissner's corpuscles, displaying S-100 protein immunoreactivity, were found in the digital pads of wild-type and TrkB+/− mice whereas they were absent in the TrkB−/− animals. Regarding Pacinian corpuscles, the mutation in the trkB gene does not alter either the immunohistochemical or the ultrastructural characteristics. Finally, in muscle spindles the arrangement of the intrafusal muscle fibers and nerve fibers was unchanged in the mutated animals. Nevertheless, about 10% of muscle spindles showed increased number of the intrafusal cells (between 6 and 12) and were supplied by more than one large myelinic nerve fiber. The present results strongly suggest that TrkB-expressing sensory neurons in dorsal root ganglia, like those of the trigeminal ganglion, are responsible for the development and maintenance of several rapidly adapting cutaneous mechanoreceptors, i.e. Meissner's corpuscles.

Theme: Development and regeneration
Topic: Sensory systems

Keywords: TrkB-deficient mouse; Meissner corpuscle; Pacinian corpuscle; Muscle spindle

1. Introduction

Sensory corpuscles are specialized organs formed by a central axon surrounded by differentiated Schwann-related and perineurial-derived cells, variably arranged [16,25,31,43,44]. The central axon corresponds to the extreme tip (dendritic zone) of the peripheral sensory neurons pseudo-unipolar axon. It is always coated by Schwann-related cells, and sometimes also by an external, more or less developed, capsule of perineurial origin [42]. The axonic sheaths in sensory corpuscles are continuous with the Schwann and perineurial cells of the nerve trunks, respectively [25], and share most of their immunohistochemical characteristics (see for a review Ref. [43]).

On the basis of their structure, two main subtypes of sensory corpuscles can be considered: capsulated and non-

* Corresponding author. Departamento de Morfología y Biología Celular, Facultad de Medicina, C/ Julián Clavería, s/n, 33006 Oviedo, Spain. Tel./fax: +34-98510367.
E-mail address: javega@correo.uniovi.es (J.A. Vega).
[1] Both authors contributed equally to this paper.

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doi:10.1016/j.brainres.2004.01.003

or poorly capsulated, the Pacian and Meissner's corpuscles being, respectively, the prototype [25,31,43]. Functionally, they are rapidly adapting low-threshold mechanoreceptors [17] and depend on Aα, Aβ or Aδ sensory nerve fibers, originated on large- and intermediate-sized sensory neurons [34,44].

Sensory axons are known to be essential for the development and maintenance of sensory corpuscles, and reciprocal interactions between growing sensory axons and target cells seem to initiate their morphogenesis [21,37]. In the last decade it has been established that specific subtypes of dorsal root ganglion (DRG) sensory neurons are dependent upon the Trk receptor-neurotrophin systems for development and maintenance (see for a review Ref. [11]). Mutations in the genes encoding for these proteins cause selective loss of neuronal subtypes, and of the corresponding sensory corpuscles connected to them. Thus, animals carrying these mutations have become ideal models to analyze the neuronal dependence of specific subtypes of cutaneous, hair-associated or muscular mechanoreceptors [2,4,7,8,10,12,13,24,28].

The TrkB-expressing sensory neurons, and their primary ligands, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4), seem to be involved in touch [8,18], and probably other kinds of discriminative sensitivity. They regulate the development and maintenance of several high- and low-threshold mechanoreceptors, supporting non-overlapping cutaneous sensory neurons. In this way, Carroll et al. [3] observed electrophysiologically that cutaneous slow-adapting mechanoreceptors, but not other types of cutaneous afferents, require BDNF in postnatal life, and BDNF treatment can specifically alter high-threshold mechanosensitivity [29]. Recently, enlargement of Meissner's corpuscles [24], and increase in the number of Merkel cells [2] in animals over-expressing BDNF in the skin of the territory of DRG neurons, has been found; the maturation of the periodontal Ruffini's corpuscles depends on BDNF for postnatal maturation, and NT-4 is required for the survival of D-hair receptors that innervate a subpopulation of hair cells [40,41]. Furthermore, BDNF might regulate the density of muscle spindles in the trigeminal territory, whereas its deficiency has no effect on the hind limb muscle spindles (see Ref. [9]). Also, Meissner corpuscles [13], longitudinal lanceolate nerve endings and Ruffini's corpuscles associated with mysticial pads [12,28], and Merkel cell–neurite complexes [2,14] are absent in the territory of the trigeminal nerve in functionally TrkB-deficient mice. The possible modifications in the density or in the structure of the mechanoreceptors in the dorsal root ganglion (DRG) sensory neurons territory, as a consequence of a mutation in the *trkB* gene, have not been investigated. Similar changes like those reported in the cranial territory might be expected , although differences between the trigeminal and DRG territories in the neurotrophin dependence of some mechanoreceptors, i.e. muscle spindles, have been observed [9,26].

In this study we used immunohistochemistry and transmission-electron microscopy to analyze the impact of a targeted mutation in the gene coding for TrkB [20] in Meissner's corpuscles from the digital pads [1], Pacinian corpuscles from the tarsal interosseal membrane [32], and muscle spindles of the soleus muscle [7]. Previous data report the absence of Meissner corpuscles in TrkB-deficient mice in the territory of the trigeminal nerve [13], whereas the territories analyzed here are innervated by spinal nerves. On the other hand, the dependence of Pacinian corpuscles on neurotrophins is completely unknown, but nerve fibers entering them [34] could be depending on the TrkB-expressing DRG neurons, on the basis of their size range [11,38]. Finally, the dependence of hind limb muscle spindles of TrkB-expressing DRG neurons remains to be established. The animals included in this study were sampled at the post-natal days 15 and 25, because at this time Meissner and Pacinian corpuscles, as well as muscle spindles, are fully developed and can be identified structurally or immunohistochemically [1,44].

2. Material and methods

2.1. Animals and tissues

Mice with a targeted mutation in the *trkB* gene, resulting in a nonfunctional protein, were kindly provided by Dr. I. Silos-Santiago, and were bred out over the C57B1/6 background [20]. Mice were genotyped by polymerase chain reaction, and selected at 15 and 25 days. Wild-type ($n=3$ for each age-group), heterozygous ($n=5$ for 15 days; $n=3$ for 25 days) and homozygous mutant mice ($n=4$ for 15 days; $n=3$ for 25 days) were included in the study. The animals received an overdose of chloral hydrate and were perfused transcardially first with a cold solution of 0.9 sodium chloride and then with cold 4% paraformaldehyde in 0.1 M PBS, pH 7.4.

The entire hind limbs were removed and placed immediately into the same perfusion fixative and then rinsed generously in PBS. As a rule the left hind limbs were used for immunohistochemistry, whereas the right ones were used for ultrastructural analysis. In both cases the limbs were divided into segments containing the toe- and finger pads, and the tarsal joints together with the tarsal interosseal membrane (which contains a group of Pacinian corpuscles). The right soleus muscle from each animal was isolated and processed independently. The pieces used for immunohistochemistry were processed for routine paraffin embedding, sectioned at 10 μm thick at a plane perpendicular to the skin surface, and the sections mounted on gelatin-coated microscope slides. The pieces used for ultrastructural study were processed for resin embedding.

2.2. Immunohistochemistry

Rehydrated sections were rinsed in 0.05 M Tris–HCl buffer (pH 7.5) containing 1% bovine serum albumin and

0.1% Triton X-100. Then, endogenous peroxidase activity (3% H_2O_2) and nonspecific binding (25% fetal calf serum) were blocked. Sections were incubated overnight in a humid chamber at 4 °C with the following primary antibodies: (1) anti-PGP 9.5 (polyclonal raised in rabbit, used diluted 1:1000, Biogenesis); (2) anti-S100 protein (polyclonal raised in rabbit, used diluted 1:1000, Dako). These antibodies were diluted in Tris–HCl buffer (0.05 M, pH 7.5) containing 1% bovine serum albumin, 0.2% fetal calf serum and 0.1% Triton X-100. After incubation with the primary antibodies, sections were rinsed in the same buffer, and incubated with peroxidase-labeled sheep anti-rabbit IgG (Amersham) diluted 1:100, for 1 h at room temperature. Finally, sections were washed and the immunoreaction visualized using 3-3′ DAB as a chromogen. For control purposes representative sections were processed in the same way as described above using nonimmune rabbit serum instead of the primary antibodies, or omitting the primary antibodies in the incubation.

2.3. Ultrastructural study

Pieces fixed in 4% paraformaldehyde were generously washed in 0.1 M PBS, pH 7.5, post-fixed in 1% osmium tetroxide, and routinely embedded in EPON. Semithin sections (1 μm) were obtained, stained with toluidin blue and used for quantitative analysis. The ultrathin sections (600 Å) were stained with uranyl acetate and lead citrate and examined and photographed under an electron microscope JEOL-JEM-T8.

2.4. Quantitative analysis

The number of sensory corpuscles was determined directly under microscope using a 25 × objective and a 10 × oculars. The density of Meissner-like corpuscles was calculated by counting the S100 protein immunoreactive corpuscles in 20 sections per animal, 50 μm apart. The sections were perpendicular to the skin surface and included finger and toe pads. The number of Pacinian corpuscles was determined in 20 sections, 50 μm apart, of the hind limb perpendicular to the long axis containing tarsal interosseal membrane processed for S100 protein detection. Finally, muscle spindles in the soleus muscle were identified following morphological criteria and were quantified in one for every 25 semithin (1 μm) sections. Spindle equators were counted to avoid a spindle being counted twice; the results

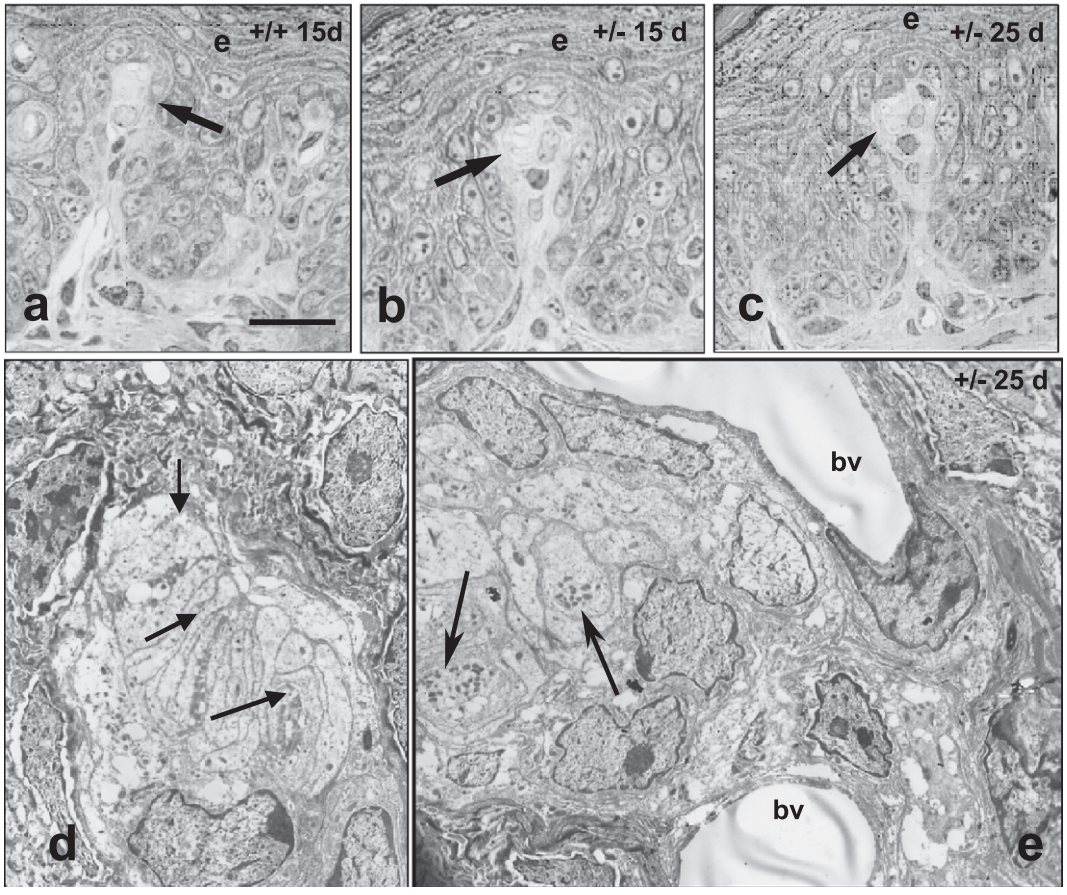

Fig. 1. Meissner corpuscles were regularly found in the dermal papillae of TrkB+/+ and TrkB+/− mice, but never in the TrkB−/− ones (a–c, arrows). Ultrastructurally (d,e), Meissner corpuscles showed a typical morphology, with pale lamellae from the lamellar cells, whose nuclei are at the periphery of the corpuscles, alternating with flattened axon (arrows). No evidences of corpuscular capsulation were observed. bv: blood vessels; e: epidermis. Scale bar = 25 μm. Original magnification in (d) and (e): 2.500 ×.

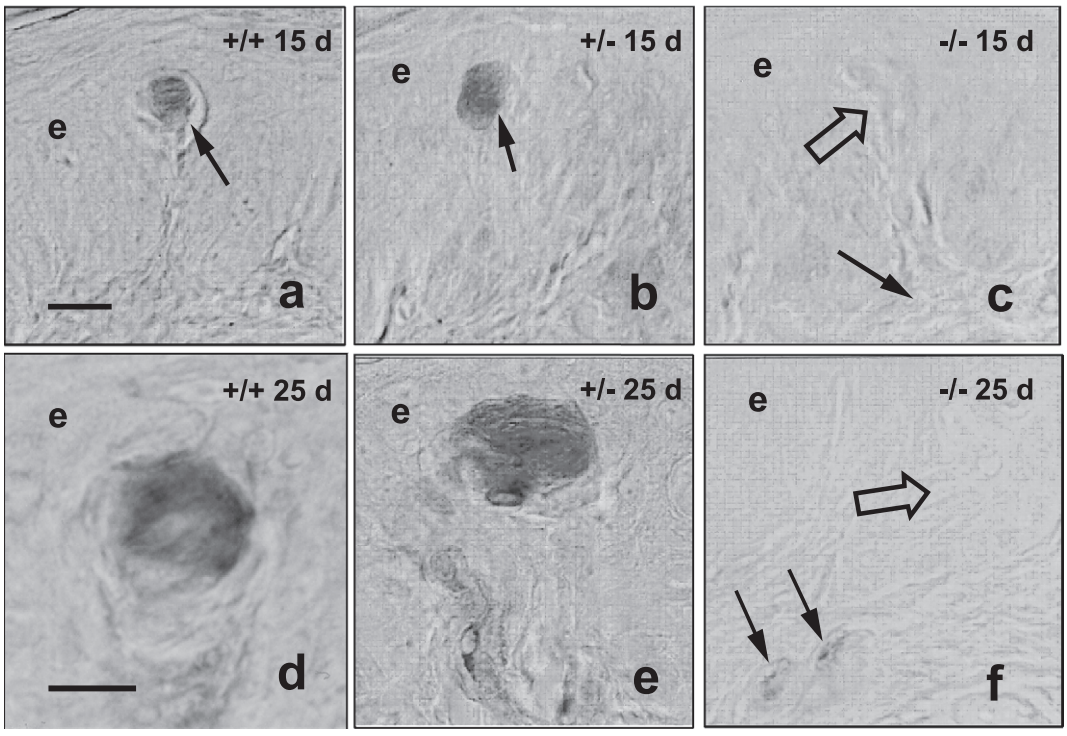

Fig. 2. Meissner corpuscles, identified by the expression of S100 protein immunoreactivity, were found in TrkB+/+ (a,d, arrow) and TrkB+/− (b,e, arrow) mice but never in the TrkB −/− ones (c,f) in which the dermal papillae were regularly empty (open arrows). However, in these animals nerve fibers displaying S100 protein immunoreactivity were detected in the cutaneous plexuses (arrows in c and f). e: epidermis. Scale bar = 10 (a–c) and 25 (d–f) μm.

were compared between wild-type and mutant muscles using analysis of variance and a Student–Newman–Keuls test.

3. Results

3.1. Meissner's corpuscles

Meissner corpuscles, identified on the basis of their morphology (Fig. 1a–c) or the expression of S100 protein immunoreactivity (Fig. 2), were localized in the dermal papillae of the TrkB+/+ and TrkB+/− mice, whereas they were always absent in the TrkB −/− animals (Figs. 1 and 2). However, nerve fibres immunoreactive for S100 protein and PGP 9.5 were found in all groups of animals arranged forming superficial and deep plexuses (data not shown). No significant variations in the density of these corpuscles were found between +/+ and +/− animals or between 15- and 25-day-old mice, although a light reduction (of about 8% at 15 days and of 10% at 25 days) was observed in TrkB+/− mice in relation to the TrkB+/+ ones (Table 1). Also, the size of the corpuscles was similar among different groups of animals.

Ultrastructurally, Meissner corpuscles of TrkB+/− mice (Fig. 1d and e) were identical to those of the wild-type (data not shown). In these corpuscles the main characteristic was the sinuous course of the axon passing through the lamellae of the so-called lamellar cells, whose nuclei were placed at the periphery. The axon innervating Meissner corpuscles was typically single and contains neurofilaments and numerous mitochondria. Occasionally, clear vesicles near the surface of the axon resembling synaptic vesicles were

Table 1
Number of Meissner's (finger and toe pads), Pacinian corpuscles (tarsal interosseal membrane), and of muscle spindles (soleus muscle) in wild-type and trkB-mutated mice

	TrkB+/+	TrkB+/−	TrkB −/−
Meissner corpuscles			
15 days	35.3 ± 4.8 (31–42)	32.6 ± 5.1 (27–40)	0 ± 0
25 days	34.1 ± 6.8 (26–45)	30.4 ± 5.8 (27–33)	0 ± 0
Pacinian corpuscles			
15 days	17.2 ± 0.8 (12–19)	15.5 ± 1.6 (13–18)	19.1 ± 1.9 (14–23)
25 days	18.5 ± 1.7 (16–21)	21.6 ± 1.6 (16–26)	18.2 ± 1.7 (17–21)
Muscle spindles			
15 days	12.3 ± 0.6 (9–14)	11.8 ± 0.4 (9–13)	12.0 ± 0.4 (10–14)
25 days	11.9 ± 0.5 (8–14)	12.1 ± 0.6 (10–14)	12.0 ± 0.5 (10–14)

Values represent mean ± standard error of the mean. The numbers in parentheses corresponds to the range of corpuscles and muscle spindles.

observed. The lamellae were electron lucent, contained scarce filaments and in some segments were invested with basal lamina. No evidences of a continuous capsule isolating the Meissner corpuscles were observed in either longitudinal (Fig. 1d) or transversal (Fig. 1e) sections, although a discontinuous capsule-like border of fibroblasts was found at the periphery.

3.2. Pacinian corpuscles

In all groups of animals investigated Pacinian corpuscles were always present, and the mutation on the *trkB* gene was without effect on the structure (Fig. 3a), immunohistrochemical (Fig. 3b–d) and ultrastructural (Fig. 3e) characteristics of these corpuscles. Also, the number (Table 1) and

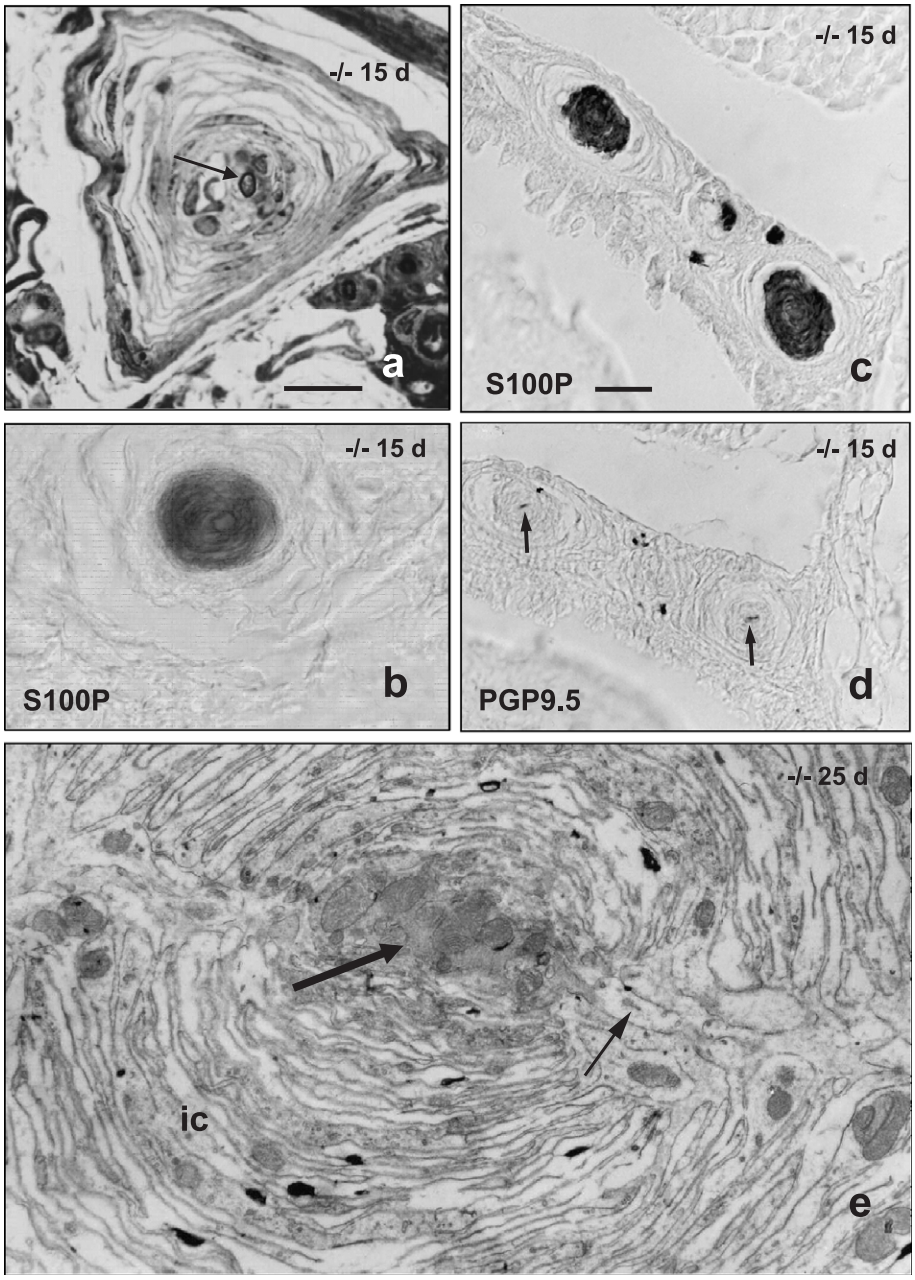

Fig. 3. Pacinian corpuscles normally develop in TrkB+/− or TrkB−/− mice, and show a completely normal structural, ultrastructural, and immunohistochemical aspect. Pacinian corpuscles were found in the tarsal interosseal membrane (a) or in the periostium (b) and were supplied by a single myelinic nerve fiber (arrow in a, preterminal segment of the corpuscle). On the other hand, corpuscles from mutated animals displayed normal patterns of immunostaining for PGP 9.5 (d, arrow) and S100 protein (b and c). At the ultrastructural level the typical terminal segment shows a central axon (thin arrow) enclosed within concentric hemilamellae of the inner core; the outer core and the capsule encircle these structures and cannot be observed in this picture. The central axon projects the so-called axonic spines (thick arrow) into the clefts formed between the two hemilamellar systems. Scale bar = 10 (c,d) and 25 (a,b) μm. Original magnification in e: 3.000 ×.

size of Pacinian corpuscles were almost identical between all groups of animals examined, suggesting that these structures are independent of TrkB-expressing sensory neurons. The three main components of the Pacinian corpuscles (i.e. the central axon, the inner core, and the outer-core capsule) were well developed, and the three main segments regarded to form the entry of the nerve fibre to the top ending (i.e. the preterminal, terminal and ultraterminal segments) were normally arranged.

The central axon, the inner core, and the outer-core capsule share the immunohistochemical characteristics of the different nerve trunk compartments [43]. Consistently, the central axon was immunoreactive for PGP 9.5 (Fig. 3d) and the inner core lamellae displayed a strong S-100 protein immunoreactivity (Fig. 3b and c).

At the ultrastructural level (Fig. 3e) the central axon contained a large amount of mitochondria clustered below the axolema, as well as intermediate filaments and scattered microtubules. Normally, it presented small protrusions, denominated axonal spines, which enter the clefts of the inner core; they were particularly abundant at the ultraterminal region (data not shown). The inner core consisted of thin cytoplasmic processes, or lamellae, from the so-called lamellar cells of the inner core. They were stacked closely on one another and separated by thin connective tissue compartments containing collagen fibrils. At the

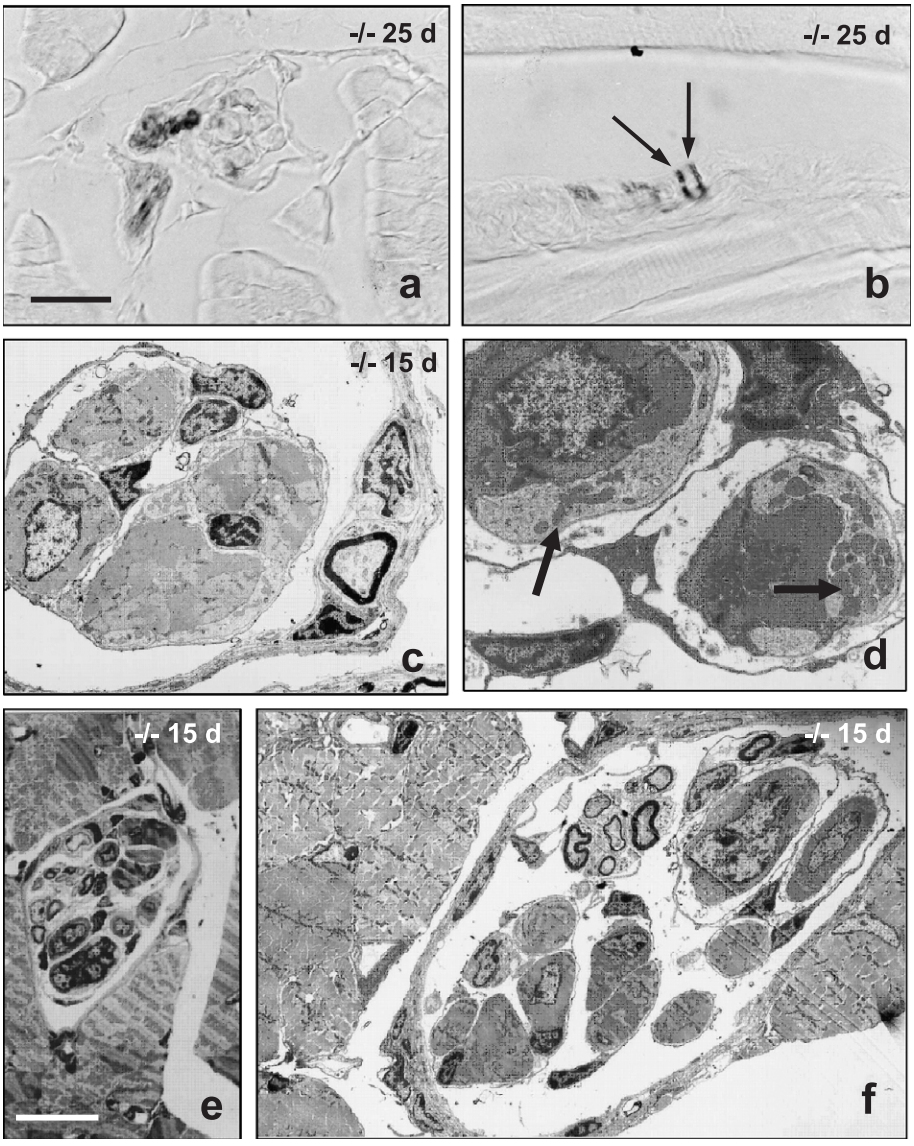

Fig. 4. Transverse (a) and longitudinal (b) sections of muscle spindles form the soleous muscle from a 25-day-old TrkB −/− mouse immunostained for PGP 9.5 (arrows) ending on intrafusal fibers forming different kinds of endings, such as anullo-spiral endings. At the ultrastructural level (c,d), both intrafusal and nerve fibres are normally arranged in the trkB-deficient animals (arrows). In the trkB −/− animals, muscle spindles receiving multiple myelinated nerve fibers, and containing an increased number of intrafusal fibers, were observed (e,f). Scale bar = 25 μm (a,b,e). Original magnification: 1000× (c,f) and 3000 (d).

terminal segment of the corpuscle (as in Fig. 3e) the lamellae do not completely encircle the axon but rather they form two bilaterally arranged symmetrical halves with a cleft dividing the inner core into two halves. The outer core and the capsule were formed by thin cell processes that completely encircle the inner core, and each layer was separated by fibrils and connective tissue (data not shown).

3.3. Muscle spindles

No differences between muscle spindles from wild-type animals and littermates carrying a mutation in the *trkB* gene were found, neither in number (Table 1) nor in their ultrastructure or innervation (Fig. 4). Muscle spindles were identified as encapsulated organs distributed irregularly within the soleous muscle. Typically, they consisted of an axial bundle of four intrafusal muscle fibres innervated by sensory and fusimotor axons (Fig. 4a–b), surrounded by the inner and outer capsule (Fig. 4c–f). Ultrastructurally, the sensory nerve fibres contained large amounts of mitochondria and encircle more or less continuously the intrafusal fibres. The motor nerve fibres formed a variable number of neuromuscular junctions at the external segment of the intrafusal muscle fibres. Typically, the axonic terminal of these junctions accumulated mitochondria and synaptic vesicles, and the muscular pole formed either small infolding or none at all. Both synaptic poles were separated by a synaptic cleft that contained a basal lamina (data not shown).

In spite of the homogeneous normality in muscle spindles from TrkB-deficient mice, in the TrkB −/− animals around 10% of muscle spindles in the soleous muscle showed an atypical structure. In these cases, a common outer capsule contained a variable number of intrafusal muscle fibres (between 6 and 13) and small nerve bundles of myelinated nerve fibres (3 to 9; Fig. 4e–f), but the pattern of innervation was largely normal.

4. Discussion

The present study was designed to investigate the effect of a mutation in the gene codifying for TrkB in several types of peripheral mechanoreceptors known to depend on subtypes of sensory neurons potentially expressing TrkB [11,38]. As a consequence of the mutation, Meissner's corpuscles in the hind limb glabrous skin were lost, whereas it had no effect on Pacinian corpuscles, and mostly innocuous on muscle spindles. However, around 10% of the analyzed muscle spindles showed increased number of intrafusal fibres and nerve fibres in trkB −/− mice. Previous studies of transgenic mice deficient on neurotrophins or their receptors, or that overexpressed neurotrophins in a target-derived manner, have demonstrated that these molecules are indirectly involved in the innervation of the skin and in the development of some sensory receptors [4,12,13,27,35], and are responsible for the development of muscle spindles (see Ref. [9]). No data are so far available about Pacinian corpuscles.

Neuronal loss in murine dorsal root ganglia deficient in TrkB is about 30–35% at the second postnatal week [20], whereas they appear without [39] or with marginal [30] deficits at births. This argues for a postnatal death of the TrkB expressing neurons, although the characterization of the type or types of sensory neurons lost in these animals has not been fully clarified. It is clear, however, that the proprioceptive neurons are not included in those missing in TrkB-deficient mice since they are required for muscle spindle development (see Ref. [33]), and these receptors are present in normal numbers and normally organized; the numbers of muscle spindles, the end organs of proprioceptive neurons, are indicators of proprioceptive neurones survival. Furthermore, it is now clear that the proprioceptive neurons in DRG depend exclusively on the TrkC-NT-3 system (see Ref. [11]).

The same seems to be true for Pacinian corpuscles which are present and normally arranged in the trkB-deficient mice. Some modification in these corpuscles could be expected since the inner core of cat and rat Pacinian corpuscles displays TrkB immunoreactivity [39]. Nevertheless, the neuronal dependence of these structures is really conflictive since they are present in animals with single or combined neurotrophins of neurotrophin-receptor deficient animals (González-Martínez et al., unpublished). So, it must be accepted that neurons lost in the trkB −/− mice must be visceral [6] or cutaneous afferents, and in this case some cutaneous mechanoreceptors must be missing. In confirming this, Meissner corpuscles were absent in mice lacking functional TrkB. These findings extend to the territory of DRG the observations in the territory of the trigeminal nerve [13]. Probably other cutaneous mechanoreceptors might be lost in the body skin, as it occurs in the head [14], but this remains to be investigated.

Since neuronal loss occurs postnatally, and Meissner corpuscles start to develop 2 days after birth [15] and maturation is not complete until the first month [1], the main emerging question is whether Meissner corpuscles do no develop or whether they degenerate postnatally, during the period of postnatal death of the TrkB-expressing sensory neurons. In no case was there evidence of degenerated Meissner corpuscles obtained from the glabrous skin of TrkB −/− mice, neither at the ultrastructural level nor by using immunohistochemistry. This suggests that Meissner corpuscles do not develop, rather they degenerate, probably because the axon of TrkB-positive sensory neurons undergoing cell death is unable to induce the formation of Meissner corpuscles [37]. Schawnn-like cells originating lamellar cells do not express S100 protein immunoreactivity in the absence of a functional axon [5] and do not accumulate to form Meissner corpuscles without axonic guidance [37].

In supporting a key role of TrkB and their physiological ligands, i.e. BDNF and NT-4, in the development of cutaneous mechanoreceptors it has been reported that

BDNF participates in the mechanical sensitivity of slowly adapting mechanoreceptors [3,29]. Also, overexpression of BDNF causes an increase in size, but not in the number of Meissner corpuscles [24]. So, it seems evident that the role of the TrkB and their ligands on Meissner corpuscles is different during development and postnatal life. In fact, it seems to regulate the acquisition and maintenance of physiological properties in adulthood [3,24,29], but it is essential for development during early postnatal life (present results).

The muscle spindles of mice deficient in TrkB in the territory of DRG were found largely normal, although they are reduced in number in the territory of the masticatory muscles [9]. Muscle spindles have been reported to be present and undisturbed in most neurotrophin null mutant mice, or mice carrying mutations in Trk genes, with the exception of the TrkC-neurotrophin-3 system [7,10,18]. In fact, the development of muscle spindles is triggered by proprioceptive sensory DRG neurons throughout TrkC-NT3-dependent Ia afferents. Furthermore, muscle spindles receive secondary II fibre afferents (see Ref. [44]) whose neurotrophin dependence of II afferents is still unknown. Because no differences were encountered between wild-type and trkB-mutated mice in the distribution or density of nerve terminals on intrafusal fibres, it must be assumed that II afferents do not depend on TrkB expressing DRG neurons. Approximately 10% of the muscle spindles analyzed showed increased number of intrafusar fibres. It has been reported that after neonatal de-efferentization spindles develop almost normally [22,23] but there is an increase in the number of intrafusal fibres (see Ref. [44]). Although our data claims for a de-efferentization of some muscle spindles in the TrkB −/− mice, as far as we know no evidences exist for a reduction in the number of γ-motoneurons in neurotrophin or functionally deficient Trk mice. Moreover, the development of fusimotor neurons correlates with the presence of Ia afferents and/or spindles, and not with neurotrophins [36]. Thus the origin of this alteration must be further investigated.

5. Conclusion

Taken together, the present results strongly suggest that the effect of a mutation on the *trkB* gene on peripheral mechanoreceptors involves Meissner corpuscles, although other cutaneous or visceral afferents that were not included in this study could also be altered. This light impact might be explained at least in part by an overlapping in the expression of Trk proteins by sensory neuron since almost all postnatal trkB-expressing sensory neurons also express trkA or trkC [19].

Acknowledgements

This study was supported in part by a grant from Spanish DGICYT (CC-99-SAF-0119-CO2O2) and Fundaciò Clinic de Barcelona. T. G-M was supported by a FPI grant from Spanish DGICYT.

References

[1] M. Albuerne, J. De Lavallina, I. Esteban, F.J. Naves, I. Silos-Santiago, J.A. Vega, Development of Meissner-like and Pacinian sensory corpuscles in the mouse demonstrated with specific markers for corpuscular constituents, Anat. Rec. 258 (2000) 235–242.
[2] V.A. Botchkarev, S. Kief, R. Paus, I. Moll, Overexpression of brain-derived neurotrophic factor increases Merkel cell number in murine skin, J. Invest. Dermatol. 113 (1999) 691–692.
[3] P. Carroll, G.R. Lewin, M. Koltzenburg, K.V. Toyka, H. Thoenen, A role for BDNF in mechanosensation, Nat. Neurosci. 1 (1988) 5–6.
[4] K.M. Cronk, G.A. Wilkinson, R. Grimes, E.F. Wheeler, S. Jhaveri, B.T. Fundin, I. Silos-Santigo, L. Tessarollo, L.F. Reichardt, F.L. Rice, Diverse dependencies of developing Merkel innervation on the trkA and both full-length untruncated isoforms of trkC, Development 129 (2002) 3739–3750.
[5] M.E. del Valle, A. Cabal, J.C. Alvarez-Mendez, B. Calzada, J.J. Haro, W. Collier, J.A. Vega, Effect of denervation on lamellar cells of Meissner-like sensory corpuscles of the rat. An immunohistochemical study, Cell. Mol. Biol. (Noisy-le-Grand) 39 (1993) 801–807.
[6] J.T. Erickson, J.C. Conover, V. Borday, J. Champagnat, M. Barbacid, G. Yancopoulos, D.M. Katz, Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing, J. Neurosci. 16 (1996) 5361–5371.
[7] P. Ernfors, K.F. Lee, J. Kucera, R. Jaenisch, Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents, Cell 77 (1994) 503–512.
[8] P. Ernfors, K.F. Lee, R. Jaenisch, Mice lacking brain-derived neurotrophic factor develop with sensory deficits, Nature 368 (1994) 147–150.
[9] G. Fan, S. Copray, E.J. Huang, K. Jones, Q. Yan, J. Walro, R. Jaenisch, J. Kucera, Formation of a full complement of cranial propioceptors requires multiple neurotrophins, Dev. Dyn. 218 (2000) 359–370.
[10] I. Fariñas, K.R. Jones, C. Backus, X.Y. Wang, L.F. Reichardt, Severe sensory and sympathetic deficits in mice lacking neurotrophin-3, Nature 369 (1994) 658–661.
[11] I. Fariñas, Neurotrophin actions during the development of the peripheral nervous system, Microsc. Res. Tech. 45 (1999) 233–242.
[12] B.T. Fundin, I. Silos-Santiago, P. Ernfors, A.M. Fagan, H. Aldskogius, T.M. DeChiara, H.S. Phillips, M. Barbacid, G.D. Yancopoulos, F.L. Rice, Differential dependency of cutaneous mechanoreceptors on neurotrophins, trk receptors, and p75 LNGFR, Dev. Biol. 190 (1997) 94–116.
[13] H. Ichikawa, S. Matsuo, I. Silos-Santiago, T. Sugimoto, Developmental dependency of Meissner corpuscles on trkB but not trkA or trkC, NeuroReport 11 (2000) 259–262.
[14] H. Ichikawa, S. Matsuo, I. Silos-Santiago, M.F. Jacquin, T. Sugimoto, Developmental dependency of Merkel endings on trks in the palate, Brain Res. Mol. Brain Res. 88 (2001) 171–175.
[15] C. Ide, Development of Meissner corpuscle of mouse toe pad, Anat. Rec. 188 (1977) 49–67.
[16] A. Iggo, K.H. Andres, Morphology of cutaneous receptors, Annu. Rev. Neurosci. 5 (1982) 1–31.
[17] K.O. Johnson, The roles and functions of cutaneous mechanoreceptors, Curr. Opin. Neurobiol. 11 (2001) 455–461.
[18] K.R. Jones, I. Fariñas, C. Backus, L.F. Reichardt, Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development, Cell 76 (1994) 989–999.
[19] H. Kashiba, Y. Uchida, E. Senba, Distribution and colocalization of NGF and GDNF family ligand receptor mRNAs in dorsal root and

nodose ganglion neurons of adult rats, Brain Res. Mol. Brain Res. 110 (2003) 52–62.
[20] R. Klein, R.J. Smeyne, W. Wurst, L.K. Long, B.A. Auerbach, A.L. Joyner, M. Barbacid, Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death, Cell 75 (1993) 113–122.
[21] D.M. Kopp, J.T. Traachtenberg, W.J. Thompson, Glial growth factor rescues Schwann cells of mechanoreceptors from denervation-induced apoptosis, J. Neurosci. 17 (1997) 6697–6706.
[22] J. Kucera, J. Walro, Formation of muscle spindles in the absence of motor innervation, Neurosci. Lett. 145 (1992) 47–50.
[23] J. Kucera, J. Walro, Superfluousness of motor innervation for the formation of muscle spindles in neonatal rats, Anat. Embryol. (Berl) 186 (1992) 301–309.
[24] A.M. LeMaster, R.F. Krimm, B.M. Davis, T. Noel, M.E. Forbes, J.E. Jonson, K.M. Albers, Overexpression of brain-derived neurotrophic factor enhances sensory innervation and selectively increases neuron number, J. Neurosci. 19 (1999) 5919–5931.
[25] L. Malinovsky, Sensory nerve formations in the skin and their classification, Microsc. Res. Tech. 34 (1996) 283–301.
[26] S. Matsuo, H. Ichikawa, I. Silos-Santiago, J.J. Arends, T.A. Henderson, K. Kiyomiya, M. Kurebe, M.F. Jacquin, Propioceptive afferents survive in the masseter muscle of trkC knockout mice, Neuroscience 95 (2000) 209–216.
[27] S. Matsuo, H. Ichikawa, T.A. Henderson, I. Silos-Santiago, M. Barbacid, J.J. Arends, M.F. Jacquin, TrkA modulation of developing somatosensory neurons in oro-facial tissues: tooth pulp fibers are absent in trkA knockout mice, Neuroscience 105 (2001) 747–760.
[28] S. Matsuo, H. Ichikawa, I. Silos-Santiago, K. Kiyomiya, M. Kurebe, J.J. Arends, M.F. Jacquin, Ruffini endings are absent from the periodontal ligament of trkB knockout mice, Somatosens. Motor Res. 19 (2002) 213–217.
[29] K. Miki, T. Fukuoka, A. Tokunaga, E. Kondo, Y. Dai, K. Noguchi, Differential effect of brain-derived neurotrophic factor on high-threshold mechanosensitivity in a rat neuropathic pain model, Neurosci. Lett. 278 (2000) 85–88.
[30] L. Minichiello, F. Piehl, E. Vazquez, T. Schimmang, T. Hokfelt, J. Represa, R. Klein, Differential effects of combined trk receptor mutations on dorsal root ganglion and inner ear sensory neurons, Development 121 (1995) 4067–4075.
[31] B.L. Munger, C. Ide, The structure and function of cutaneous sensory receptors, Arch. Histol. Cytol. 51 (1988) 1–34.
[32] P.B. Nava, The effects of age on murine Pacinian corpuscles, in: P. Hunuik, T. Soukup, R. Vejsada, J. Zelena (Eds.), Mechanoreceptors: Development, Structure and Function, Plenum, New York, 1988, pp. 289–294.
[33] R.A. Oakley, F.B. Lefcort, D.O. Clary, L.F. Reichardt, D. Prevette, R.W. Oppenheim, E. Frank, Neurotrophin-3 promotes the differentiation of muscle spindle afferents in the absence of peripheral targets, J. Neurosci. 17 (1997) 4262–4274.
[34] E.R. Perl, Function of dorsal root ganglion neurons: an overview, in: S.A. Scott (Ed.), Sensory Neurons: Diversity, Development and Plasticity, Oxford Univ. Press, New York, 1992, pp. 3–23.
[35] F.L. Rice, K.M. Albers, B.M. Davis, I. Silos-Sanatiago, G.A. Wilkinson, A.M. LeMaster, P. Ernfors, R.J. Smeyne, H. Aldskogius, H.S. Phillips, M. Barbacid, T.M. DeChiara, G.D. Yancopoulos, C.E. Dunne, B.T. Fundin, Differential dependency of unmyelinated and A delta epidermal and upper dermal innervation on neurotrophins, trk receptors, and p75LNGFR, Dev. Biol. 198 (1998) 57–81.
[36] T. Ringstedt, S. Copray, J. Walro, J. Kucera, Development of fusimotor innervation correlates with group Ia afferents but is independent of neurotrophin-3, Brain Res. Dev. Brain Res. 111 (1998) 295–300.
[37] R. Saxod, Ontogeny of the cutaneous sensory organs, Micros. Res. Tech. 34 (1996) 313–333.
[38] I. Silos-Santiago, A.M. Fagan, M. Garber, B. Fritzsch, M. Barbacid, Severe sensory deficits but normal CNS development in newborn mice lacking TrkB and TrkC tyrosine kinase receptors, Eur. J. Neurosci. 9 (1997) 2045–2056.
[39] B. Stark, M. Risling, T. Carlstedt, Distribution of the neurotrophin receptors p75 and TrkB in peripheral mechanoreceptors; observations on changes after injury, Exp. Brain Res. 136 (2001) 101–107.
[40] C.L. Stucky, T. DeChiara, R.M. Lindsay, G.D. Yacopoulos, M. Koltzenburg, Neurotrophin-4 is required for the survival of a subclass of hair follicle receptors, J. Neurosci. 18 (1998) 7040–7046.
[41] C.L. Stucky, J.B. Shin, G.R. Lewin, Neurotrophin-4: a survival factor for adult sensory neurons, Curr. Boil. 12 (2002) 1401–1404.
[42] J.A. Vega, M.E. Del Valle, J.J. Haro, F.J. Naves, B. Calzada, R. Uribelarrea, The inner-core, outer-core and capsule cells of the human Pacinian corpuscles: an immunohistochemical study, Eur. J. Morphol. 32 (1994) 11–18.
[43] J.A. Vega, J.J. Haro, M.E. Del Valle, Immunohistochemistry of human cutaneous Meissner and Pacinian corpuscles, Microsc. Res. Tech. 34 (1996) 351–361.
[44] J. Zelena, Nerves and Mechanoreceptors. Chapman and Hall, London, 1994.