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Review

Sympathetic sprouting in dorsal root ganglia (DRG): A recent histological finding?

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Summary. During the nineties it was described, as an original finding, the existence of afferent amyelinic nerve endings in animal dorsal root ganglia (DRG) caused by diverse experimental lesions. These works do not take into account the historical studies carried out by Ehrlich (1886), Ramón y Cajal (1890) and Dogiel (1885) among others. Ramón y Cajal (1899) confirmed the existence of these nerve endings naming them after their discoverer as "*Dogiel's arborisations*". Ramón y Cajal claims that these endings originate from fibres of sympathetic nature, something supported by later authors devoted to this topic. In any case, the same authors remarked already a possible relationship with pathological phenomena, nonetheless always referring to the frequent occasions in which the same images appeared in healthy animals. In this work we review the bibliography about the classically named "Terminal Dogiel's nests" which in modern literature have been referred to as sprouting of sympathetic axons in dorsal root ganglia likely related with sympathetically maintained pain. Furthermore, we present the finding, not described up to date, of multiple afferent amyelinic nervous endings related with the "Terminal Dogiel's nests" observed in different DRG from young adult healthy rabbits.

Key words: Dorsal root ganglion, Sympathetic nervous system, Dogiel´s arborisations, Multiple afferent amyelinic nervous endings

Introduction

The interest awakened in the study of spinal ganglion nerve endings afferent to sensitive neurones was due, as contemporary authors point out (Kim and Chung, 1991; Chung et al., 1993; McLachlan et al., 1993; Ramer and Bisby, 1997a,b; Lee et al., 1998), to the involvement of the sympathetic nervous system in neuropathic chronic pain. This sort of pain is observed in human patients as well as in experimental animals and a role for DRG is suspected.

During the last decade several authors have described the occurrence of afferent nerve endings around ganglionar cells, focusing their studies in different molecular aspects involved in their characterisation, regulation and origin. Thus, it has been observed that several factors such as NGF, IL-6 or Neurotrophin 3 (NT-3) could be responsible for this axonal growth (Ramer et al., 1998a, 1999; Jones et al., 1999; Ramer and Bisby, 1999; Zhou et al., 1999; Hu and McLachlan, 2000). Everybody admits, without doubt, that this process is invariably related to pathological phenomena, not reporting the occurrence of the same images in healthy animals, either embryos, new-borns or adults.

The lack of bibliographic references, former to these works, about the existence of these structures that, actually, were first described in the late XIX century is surprising. The aforementioned nerve endings have been observed in the affected dorsal root ganglia ending around the neurones soma in the shape of pericellular arborisations, suggesting the establishment of functional connections with them which would explain the symptoms of the chronic hyperalgesia.

In a recently published work the afferent nerve ending is described of a unique fibre that, either presents the shape of a nest or a plexus enveloping the soma or the zone of the axonal glomerulus. It is doubtful to relate these features with the aetiology of the chronic hyperalgesia in patients with nervous lesions, given that after the results published by Ringkamp et al. (1999b), this symptom appears in an earlier phase than the occurrence of the Dogiel's nests, and, besides, is not alleviated by therapeutical sympathectomy.

In the present work, we review the current knowledge about the classically denominated "*Terminal*

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Dogiel's nests" which have been referred to in modern literature as sprouting of sympathetic axons in dorsal root ganglia probably related with sympathetically maintained pain. We have also tried to reproduce, following the same technical procedure, the findings described by classical authors using as subject of research the dorsal root ganglia of healthy young adult rabbits. These animals, besides their easy manipulation, are phylogenetically closer to humans in the evolutive scale than the rodents used in experimental models described up to date.

Historical background

During our bibliographic search we were surprised that the authors to tackle this subject during the nineties, described as an original finding the presence of sympathetic sproutings stimulated by damage. Among these McLachlan et al. (1993), described, using immunohistochemical methods after sciatic nerve damage, the presence of sympathetic sproutings in the spinal ganglions that end in the soma of some neurones forming pericellular arborisations, suggesting the establishment of functional connections with them. However these authors do not refer that these structures were known in the late XIX century, when Aronson (1886) and Ehrlich (1886) described the existence of fibres afferent to the spinal and cerebrospinal ganglia with a different course and ending. The major contribution regarding this subject belongs to Santiago Ramón y Cajal, awarded the Nobel Prize in Medicine in 1906 together with Camillo Golgi. He, echoing the works using methylene-blue mentioned by Ehrlich, reports pericellular endings similar to those found elsewhere in the nervous system described by Köelliker as *"Endkörber"* (terminal baskets).

Ramón y Cajal (1890) using Golgi's impregnation in 1-15 day healthy mice, describes the presence of amyelinic ramified fibres that seem to penetrate the DRG from the anteroexternal part running from the central to the cortical area where they turn into terminal arborisations. These endings are either simple or produce a tangled varicose thread ball around the soma of a ganglionar cell under the satellite glia. He doubts about the origin of these fibres although he assures that most of the times they proceed from the "grand sympathetic". He observes twice these kinds of fibres in the tract that runs from the sympathetic ganglion in front, to the origin of the corresponding spinal pair, not being able in any case to follow one of these fibres up to a pericellular nest.

Van Gehuchten (1892) and Retzius (1894) were able to follow sympathetic nervous fibres up to the DRG, but then they left the ganglion to penetrate either the anterior or posterior root of the periferic nerve. They never doubt about Ramón y Cajal's reports and blame the failure to Golgi's method inconstancy.

G. Carl Huber (1896), from Michigan University, discussing the possible function of multipolar star-shaped cells in frogs DRG, cites Lenhossék, who suggests that these cells could be related to the sympathetic fibres in the DRG described by Ehrlich and Ramón y Cajal (Lenhossék, 1894). He also refers to Dogiel's recent observation of sympathetic endings that distribute around healthy cat ganglionar cells forming terminal nests wrapping neural somas. Dogiel observes two types of endings: type I, formed by fine and varicose little branches, probably emanated from sympathetic fibres; and type II evolved from myelinic fibres whose amyelinic endings coil around the ganglionar cells and form, under the capsule, a terminal thread ball comparable to certain periferic endings (Krause corpuscle).

Again, Ramón y Cajal (1899), in his opus "Textura del sistema nervioso del hombre y de los vertebrados", cites Ehrlich and Aronson as the first ones who, using methylene blue, presumably saw certain pericellular arborisations around ganglionar cells, similar to those described by Arnold in sympathetic corpuscles in the frog heart. He cites Dogiel as the discoverer of these structures and names type II-like structures as "Dogiel's nests" in his honour.

Ramón y Cajal also describes the periglomerular endings, observed in methylene blue-stained ganglia by forced injection. Being the beginning of the ganglionar axon amyelinic, he supposes that its glomerular morphology has the aim of "*multiplying contact surface, either with perisomatic arborisations or with some special nervous ramification"*. He observed in many healthy animals' glomeruli, in Gasserian as well as in spinal ganglia, a kind of amyelinic fibre ball, wrapping, in a most complicated fashion, each one of the turns of the main expansion. He classifies these periglomerular endings into three types. Type I would only wrap around the glomerulus, type II would emit some fibres around part of the neural soma, and type III, mixed, would wrap around the glomerulus and the whole neuronal body.

For Ramón y Cajal, the whole glomerulus would be probably provided with a special arborisation, or at least, a particular spiral plexus, pursued or not by a perisomatic ramification. He blames the random occurrence of this phenomenon to the inconstancy of the techniques used. Regarding the fibre that engenders such plexus, he thinks that it comes from the "grand sympathetic". He never observed in its way the presence of myelinic ensheathing. Besides, in mixed arborisations, the character of the perisomatic ramification corresponds to the one previously observed in pericellular endings with Golgi's method, that seemed to be continued in fibres from the rami communicantes. To reinforce his theory about endings on the initial segment of the axon, he compares them with endings of the cerebellum nest cells on the initial segment of the Purkinje cell axon.

Apart from these three types of periglomerular endings, Ramón y Cajal describes two perisomatic sorts that agree with Dogiel's type I and II. He denominates the latter "Dogiel's nests" or "pericellular thread balls", which he never observes affecting the glomerular region.

In every edition up to the last one (Ramón y Cajal and Tello y Muñoz, 1956, published after his death) Ramón y Cajal describes repetitively these structures and states their presence in sensitive ganglia of healthy humans, where they are specially abundant, above all in the cranial sensitive nerves, attracting his attention to the fact that not every cell presents them, but only a small number of elements usually corresponding to glomerular type. These authors cite Nageotte, who observed these endings more frequently in pathological cases and exposes the idea of an aberrant sprouting process. For Ramón y Cajal and Tello the theory of its pathological aetiology would be endorsed by the following facts:

1.- Ramón y Cajal, like Nageotte, Marinesco, Rossi, Dustin, etc. has observed that such pericellular nests are very abundant in grafted or transplanted ganglia.

2.- Experimental studies by Ramón y Cajal, Marinesco and others demonstrate that it is enough to mechanically oppress a DRG, distend the corresponding nervous pair, or cause in any of them the smallest injury, to provoke the inundation of the ganglion by endings born from the injured axons, or the neurones themselves, running to the sensitive cells bodies in complicated turns in or outside the capsule.

3.- Legendre and Minot (1912) and Marinesco (1913) have provoked the formation of nests in ganglia cultured *in vitro*.

4.- Marinesco, Schäffer, Levi, Rossi, Bielschowsky, Pacheco, Achucarro, Castro, etc., remark that pericellular nests are present in high quantities, not only in spinal tabes, as Nageotte discovered, but in a good number of human nervous diseases.

It seems clear that these structures may constitute a pathological consequence easily reproducible experimentally. Nevertheless, Ramón y Cajal and Tello y Muñoz think that: *"there remains to clarify why a so curious disposition is found, from time to time and in variable proportions, in the ganglia of evidently healthy animals"*.

From this time on, every reference to these structures disappears from the medical literature and even from course books. The only reference we have found belongs to Czyba and Girod (1970), who describe these endings in the book "Cours d'histologie et embryologie" fundamentally citing Ramón y Cajal and Dogiel. Actually, most of the histology treaties do not mention this topic, and even some state that the ganglionar sensitive neurone never receives any type of ending.

In this regard, we are surprised that there is no reference to classical authors in the works written in the last years. Only Devor (1999) uses, without reference in the text, a design by Ramón y Cajal taken from the French translation by the C.S.I.C. of his book "Textura del sistema nervioso del hombre y los vertebrados", written in 1899. Surprisingly Devor shows one of Ramón y Cajal's designs (from page 428) in which no sprouting to ganglionar neurones is shown, while some pages ahead, namely 442, 444 to 446 and 448 to 451,

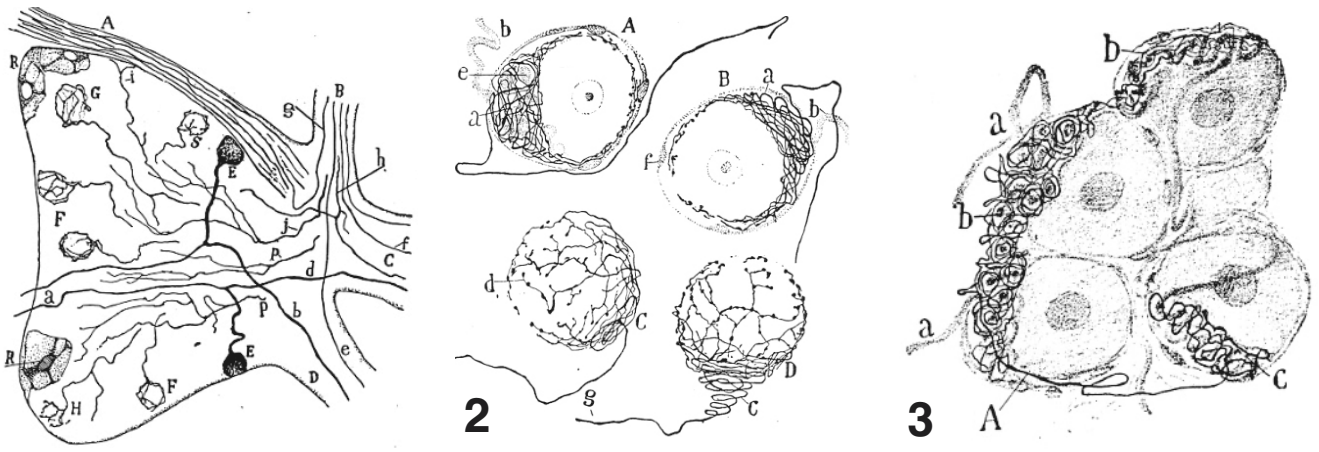


Fig. 1. Original sketch by Ramón y Cajal showing the different types of afferents to ganglia of healthy animals. F, G and H: nervous pericellular nests; P and J: fibres branching inside the ganglion; A: anterior root; B: grand sympathetic root; C: anterior ramus of the spinal pair; D: posterior ramus of the latter; E: ganglionar cells (from Ramón y Cajal, 1899, p. 365).

Fig. 2. Original sketch by Ramón y Cajal showing the mixed pericellular arborizations in the Gasserian ganglion of the cat. A and B: equatorial view of cells, to evidence the complicated periglomerular thread ball; C and D: surface view of cells in order to demonstrate the details of the perisomatic arborization. In c, an elegant spiral around the glomerulous appeared; a: periglomerular plexus; b: main expansion of the cell; e: turns of the glomerulous; f: capsule; g: non-medulated.

Fig. 3. Original sketch by Ramón y Cajal showing the periglomerular endings in the dorsal root ganglion of a cat. A: nervous fibre that constituted spirals around four different glomerouli; a: main expansions of the cells; b: gaps where the turns of the glomerulous surrounded by spiral fibres; c: first plexus formed by the afferent nervous fibre.

there are up to 8 precise designs showing each type of endings he described (Figs. 1-3).

Peripheral nerve injury triggers noradrenergic sprouting within dorsal root ganglia

Involvement of noradrenergic sprouting in neuropathic pain

Neuropathic pain is a condition that can result from disease (e.g. diabetes, herpes zoster) or trauma to nervous tissue (thalamic infarct, spinal cord or peripheral nerve injury) and that differs from "normal pain", in that it can be evoked at much lower stimulus thresholds and is often of greater severity (Ramer and Bisby, 1998b). Gagliese reports that a significant number of elderly humans experience chronic pain generally, and this remains underdiagnosed and undertreated (Gagliese and Melzack, 1997). Neuropathic pain is one form of chronic pain specially apparent in the elderly (Gibson et al., 1994), particularly in cases of postherpetic trigeminal neuralgia (Devor, 1991). It is interesting that the more painful conditions are a result of incomplete nerve injuries, which leave an intact connection to the periphery (Janig, 1985).

Presently it is believed that functional coupling of sympathetic and sensory neurones may be of major importance in pathological conditions giving a hint to the mechanism of chronic pain. This belief is based on the correlation existing between pain intensity and clinical signs of sympathetic disturbance, together with the fact that in some patients pain is alleviated by sympatholysis, sympathectomy of pharmacological sympathetic block (Hannington-Kiff, 1974; Bonica, 1990). On the other hand, diverse experimental morphological studies also show a possible connection between neuropathic pain and sympathetic sprouting to DRG (Ramer and Bisby, 1998b).

Injury to a peripheral nerve may lead to neuropathic pain which is characterised by spontaneous burning pain (causalgia), altered sensory transduction (hyperalgesia and allodynia), other unpleasant sensations (dysaesthesias, paraesthesias) and sudoripary and vasomotor autonomic disturbances (e.g. sweating and temperature changes in the skin). Burning pain has been associated with activity in injured C-fibre afferents in a human microneurography study (Cook et al., 1987). Furthermore, local treatment with a low dose of capsaicin, a C-fibre toxin, has been used to treat neuropathic pain. Allodynia (pain due to a stimulus which does not normally provoke pain) has benn suggested to be associated with activity in Aß-fibres (Campbell et al., 1988).

Increased sympathetic efferent activity (e.g. physiological or emotional stress) can aggravate the symptoms of peripheral neuropathy in patients, whereas sympathectomy may alleviate the symptoms (Loh and Nathan, 1978; Janig, 1985; Bonica, 1990). These facts suggest that the sympathetic postganglionar neurone (SPGN) contributes to symptoms occurring after nerve injury (Kinnman and Levine, 1995).

The occurrence of two types of neuropathic pain is commonly accepted (Roberts, 1986): sympathetically independent (SIP) and sympathetically maintained pain (SMP). The most effective means of relieving SMP is the removal of the sympathetic supply to the affected areas early in the course of disease. However, there has been little success in attempts to determine the mechanisms underlying SMP and the available treatments are unsatisfactory.

At present, it is unclear why partial peripheral nerve injury leads to SMP in some cases, but not in others. Initial studies, published independently in the early nineties by Chung y McLachlan, have suggested that the extent of the noradrenergic fibre sprouting in the injured nerve and dorsal root ganglion and the formation of sympathetic terminal arborisations or "baskets", following peripheral nerve injury is the key factor that determines the sympathetic dependence (Chung et al., 1993; McLachlan et al., 1993).

Although there are conflicting reports on the amount of contact between sympathetic axons and DRG neurones following nerve injury, electrophysiological evidence supports this site as a point of communication between these neuronal types (Devor et al., 1994; Michaelis et al., 1996). The sprouting phenomenon occurs after both complete and partial sciatic nerve injuries. After partial nerve injury, sprouting is well established 2 weeks postoperatively, develops with a time course similar to that of allodynia and hyperalgesia (Chung et al., 1996; Ramer and Bisby, 1997b), and lasts as long as 10 weeks following a complete nerve injury (McLachlan et al., 1993).

For Ramer and Bisby there is a clear relationship between the extent of sympathetic innervation of the DRG and some types of abnormal painful behaviour: specifically, there seems to be a tight relationship between thermal hyperalgesia and mechanoallodynia (which develop in both young and old animals) and sympathetic sprouting, whereas thermal allodynia and sympathetic sprouting do not always coexist (for example, in old, nerve-injured animals). Their results highlight the possibility that sympathetic sprouting in the DRG is responsible for the sympathetic generation or maintenance of pain, especially in the elderly (Ramer and Bisby, 1998b).

However, for authors like Lee et al. (1998) it is obvious that factors other than sympathetic sprouting are also involved in the generation of neuropathic pain behaviours since: (1) several models of chronic pain show pain behaviours in the absence of massive sympathetic sprouting during the first week postoperatively; and (2) sympathetic sprouting in the DRG was increased significantly in all models studied by 20 weeks postoperatively, yet neuropathic pain behaviours have receded to almost normal levels. Therefore, the significance of the long-delayed sympathetic sprouting in some pain models is not clear.

Role of sympathetic terminal baskets in physiological states

Most authors state that the only sympathetic afferents to DRG in physiological conditions innervate blood vessels and the surrounding meningeal layers (Lieberman, 1976; Belenky and Devor, 1997; Ramer et al., 1999). Reviewing the methodology of the different reports it can be seen that although some authors use normal animal tissues as controls, contralateral ganglia corresponding to non-injured nervous roots of the same animal are also considered valid controls. In both cases, the presence of sympathetic fibres (identified by TH-immunochemistry or SPG-induced autofluorescence) are considered infrequent in the spinal as well as the normal DRG. It is expressly stated that the dorsal root is largely devoid of labelled fibres, except for occasional observations of fine fibres, and although it is not clear if the physical contacts are actually functional, the contacts could only in be seen the DRG on the neuropathic side but not on the control side or in the DRG of the normal rat (Chung et al., 1993; Ramer and Bisby, 1998a). Nevertheless the same authors have reported that sympathetic innervation of the DRG in rats increased naturally with age, forming pericellular baskets mainly around large DRG neurones (Ramer and Bisby, 1998b), which implies a contradiction.

Devor, in a recent review about peculiarities of the DRG, writes that synapses in these ganglia do exist but are so rare as to be virtually non-existent (Devor, 1999). After performing co-cultures of dorsal root and sympathetic ganglia, Belenky and Devor (1997), observe some apparently specific sympathetic-sensory contacts suggesting that a functional interaction might develop between sympathetic axons and sensory neurones in vitro. In conclusion, the real functional meaning of these occasionally morphological observations does not have an explanation yet.

We have no reference about any modern author that has systematically investigated the presence of these morphological structures in different species of healthy animals; and therefore, the role that these formations might play in physiological states is completely unknown.

Experimental models

There are three main models used by the authors reviewed. Lee et al. (1998) summarise them as follows: the chronic constriction injury model (CCI) (Bennett and Xie, 1988; Ramer et al., 1997, 1998b; Ramer and Bisby, 1997b; Ma et al., 1999); (2) the partial sciatic nerve ligation injury model (PSI) (Seltzer et al., 1990); and (3) the segmental spinal nerve ligation injury method (SSI) (Kim and Chung, 1992) denominated as spinal nerve ligation by other authors (SNL) (Chung and Kang, 1987; Chung et al., 1993, 1996a; Kinnman and Levine, 1995; Jones et al., 1999; Ringkamp et al., 1999a,b; Chung and Chung, 2001). All three methods of peripheral nerve injury produced behavioural signs of both ongoing and evoked pain, with some differences in the magnitude of each pain component between them (Kim et al., 1997). These models have been combined in different ways with diverse sympathectomy proceedings either surgical or chemical in order to compare the results (Kim and Chung, 1991; Neil et al., 1991; Shir and Seltzer, 1991; Kim et al., 1993; Chung et al., 1993).

Kim et al. (1996) report that the extent of the sympathetic nerve fibre sprouting into the DRG following peripheral nerve injury is inversely related to the distance between the injury site and the DRG. They clearly show that noradrenergic nerve fibre sprouting that follows peripheral nerve in injury is more extensive in the DRG which are closer to the injury site. These models also differ in terms of time course: sprouting begins 3-4 weeks after PSI (McLachlan et al., 1993; Ramer and Bisby, 1997a), 1-2 weeks post-CCI (Ramer and Bisby, 1997b), and 2-4 weeks post-SNL (Chung et al., 1996). There are also distinct differences in the longevity of the sprouting response; PSI and CCI lead to sprouting which can last months or years, whereas following SNL, TH-positive baskets are most numerous 1 week post-SNL and they decline thereafter (Ramer and Bisby, 1999).

The study by Kinnman and Levine (1995) is probably one of the most extensive regarding number of animals used as well as different sites of injury. 86 Sprague Dawley male rats (250-300g) were kept in groups of 2 or 3. Similarly to other authors he performs a test of response to mechanical stimuli with von Frey filaments, calculating the latency in the retirement of the hind paw to a heat stimulus as well as the spontaneous pain behaviour of injured animals. Numerous possibilities were also addressed in the surgical procedures (L5 spinal nerve resection, sympathectomy at diverse levels, pre- or post-resection, capsaicin and NGF, etc…). These authors report that L5 spinal nerve resection induces an increased mechanical and thermic sensitivity together with spontaneous pain behaviours. Sensitive symptoms can be totally eliminated by cutting the dorsal root of the injured spinal nerve, indicating that activity of sensory neurones acting centrally is responsible for the increased behavioural response evoked by stimulation of the uninjured nerve evoked by the uninjured nerve territory (Kinnman and Levine, 1995).

Tightly ligating only the L5 or both the L5 or L6 homolateral spinal nerves in rats provokes behavioural signs of mechanical allodynia and thermal hyperalgesia on the affected hind paw lasting up to several months. A lumbar surgical sympathectomy performed after the spinal nerve ligation almost completely relieves the signs of both mechanical allodynia and heat hyperalgesia, and sympathectomy preceding spinal nerve ligations prevented the development of pain behaviours. Thus, some authors think this represents a model for SMP (Chung et al., 1993).

The species most widely used is the rat although

transgenic mice have also been used. The genetic alterations include null mutations, knockout mice for p-75 (Ramer and Bisby, 1997a) or IL-6 (Ramer et al., 1998b) or both (Walsh et al., 1999), slow Wallerian degeneration (Ramer et al., 1997; Ramer and Bisby, 1998c) and endogenous ectopic NGF overexpression (Davis et al., 1998; Ramer et al., 1998a; Walsh and Kawaja, 1998)

However, it might be of importance the use of other species since comparative studies performed by Hu and McLachlan (2000) in rats and guinea pigs demonstrate that there are interspecies differences. Axons containing noradrenaline or calcitonin gene-related peptide were visualised in DRGs and spinal roots of guinea pigs and rats. After sciatic transection in rats, varicose terminals of both types appeared around large DRG somata. These neurones were surrounded by proliferated satellite cells expressing glial fibrillary acidic protein (GFAP) and p75. This did not occur in guinea pigs. Instead, sympathetic axons grew through the DRG and centrally along the dorsal roots (which contained p75-positive glia), avoiding the DRG somata. (Hu and McLachlan, 2000).

The only studies in vitro published recently were performed by Belenky and Devor (1997) and have been already discussed.

Sympathetic nature of baskets and origin

Chung et al. (1993) measured the size of the darkest TH-positive stained fibres in the DRG, the dorsal root and the spinal nerve in normal and neuropathic rats. The width of the stained fibres was 0.6±0.3 mm (mean ± S.D.; n=58) ranging from 0.2-1.2 mm. This is the normal range for unmyelinated fibres therefore the author assumes their sympathetic nature, a point that this author confirmed by electronic microscopy (Chung et al., 1997)

The observation of the invading sympathetic axons make Ramer and Bisby (1997a) support the hypothesis that the sprouting of sympathetic axons after CCI proceed from blood vessels and dura, coinciding with the observation by McLachlan of the cathecolamine-containing axons from varicose plexuses around small diameter blood vessels (15-50 micrometers). However, after SNL they probably proceed from the injured spinal nerve (Ramer and Bisby, 1998a, 1999).

Size of ganglionar cells involved in sympathetic baskets.

Diverse authors report that the autofluorescence of TH-positive fibres wraps preferentially around large-diameter DRG cell size (McLachlan et al., 1993; Chung et al., 1993). However, baskets form transiently around a population of small-diameter DRG neurones shortly after the spinal nerve ligation developed by the Chung group, but not after sciatic transection (Chung et al., 1996). This group estimated the size of the cell bodies surrounded by the sprouting TH-positive fibres by measuring diameters of profiles of 21 such cells. The diameter of these cells ranged form 37.8-75.6 mm with an average of 50.9±10.6 mm.

Contralateral ganglion invasion

Although Chung et al. (1993) observed that sympathetic sprouting is limited to ipsilateral ganglion, and that contralateral DRG have been frequently used as negative controls, as has previously been discussed, McLachlan et al. (1993) did observe sprouting to some extent in the contralateral ganglion. A reasonable explanation would be that the target-derived factors of trophic factors from the degenerating peripheral nerve may be acting on DRG neurones and sympathetic axons in the contralateral ganglion via the circulation. This phenomenon, described lightly by some authors and underestimated by others, could cast a doubt on some models used up to date in which contralateral ganglia and not healthy animal ganglia were used as controls. Nevertheless, statistical relations could still be valid due to the higher proportion of baskets found on the neuropathic side compared with contralateral ganglia.

Electron microscopy

Several works have been published looking for evidence of synaptic contact between sympathetic fibres and DRG neurones (Davis et al., 1994; Devor et al., 1995; Chung et al., 1997). We will focus on the last one. These authors have not found any TH-IR synaptic varicosities which made a classical type of synaptic contact, with pre-and/or postsynaptic thickenings or an associated cluster of synaptic vesicles to neuronal elements. Sympathetic postganglionic fibres, as identified by electron microscopic immunostaining for tyrosine hydroxylase (TH) were all unmyelinated fibres and some of them ended as growth cones. In addition, many vesicle-containing axonal enlargements (which they refer to as synaptic varicosities) were found in the interstitial space around DRG neurones, and some were enclosed within the satellite cell capsule which surrounded the DRG soma.

They propose that these enlargements of TH-IR fibres are neurotransmitter releasing sites (synaptic varicosities) of sympathetic postganglionic fibres. This proposal is based on the following observations: (1) the shape and size of the vesicles are the same as typical synaptic vesicles; (2) TH immunostaining is dense around the cluster of small spherical vesicles; and (3) sympathectomy almost completely eliminates TH-IR profiles. In light microscopy, varicosities of beaded sympathetic fibres have been considered as synaptic terminals “en passant” type. The vesicle-containing enlargements that they observed by electron microscopy seem to represent the varicosities shown in light microscopy, thus “en passant” type sympathic varicosities. The presence of many close appositions between TH-IR synaptic varicosities and non-immunoreactive neuronal profiles suggest a strong

possibility of sympathetic and sensory interactions of the DRG. The study also suggests that not only axo-somatic interactions between sympathetic and sensory neurones but also axo-axonal interactions are possible between sympathetic fibres. They speculate that sympathetic varicosities release neurotransmitters into the vicinity of ganglion cells to influence their activity. These authors think that the fact that it takes a long time (10s) to activate DRG cells by stimulation of sympathetic nerves is consistent with such a speculation (Chung et al., 1997).

Molecular factors involved in sympathetic DRG sprouting

Wallerian degeneration

Wallerian degeneration is characterised by axon degradation and myelin clearance by activated Schwann cells and invading macrophages. These cells generate a range of growth factors and cytokines, many of which favour the growth of injured axons. Among the factors produced in the degenerating nerve we can find some that can act on sympathetic neurones and in some cases induce axonal sprouting (Ramer et al., 1997). To investigate the role of this process in the development of neuropathic pain and sympathetic sprouting in DRG, these authors have used a strain of transgenic mice (C57B1/Wld) in which Wallerian degeneration after nervous injury is impaired. The histological exam shows that the sprouting in DRG is markedly delayed in Wld mice: one week after the injury, the sympathetic fibres have invaded the ipsilateral ganglion in normal mice, whereas in Wld mice sprouting is only slightly increased three weeks after surgery. These authors conclude that Wallerian degeneration is tightly ligated to the development of pain as well as to the sprouting after chronic constriction injury, and speculate about the possibility that NGF mediates both phenomena (Ramer et al., 1997; Ramer and Bisby, 1998a).

Nerve growth factor (NGF) and neurotrophin-3 (NT3)

Many authors propose NGF as responsible for sympathetic sprouting (Herzberg et al., 1997; Ramer et al., 1997; Ramer and Bisby, 1998a). Skin NGF ectopic overexpression in transgenic mice leads to the formation of TH-immunopositive baskets in the trigeminal ganglion (Davis et al., 1994). Jones et al. (1999) think quite probable the formation of noradrenergic connections between sympathetic sprouting and neuronal bodies after exogen NGF.

There exist two hypotheses about the site of synthesis of the DRG-derived factor involved in the sprouting: the DRG itself and the retrograde transport from the periferic stump (Ramer et al., 1997, 1998a; Ramer and Bisby, 1997b). Several works detect the presence of NGF mRNA in DRG after the induction of nervous injury (Sebert and Shooter, 1993; Wells et al., 1994; Zhou et al., 1999). Besides, Zhou y Deng, describe an increase of NT3 mRNA in p75 immunoreactive satellite cells of the ipsilateral ganglion after SNL. These authors suggest that the neurotrophin receptor p75 may act as a molecule presenting neurotrophin-3 (NT3) or NGF to trigger sympathetic sprouting in DRG.

However, Jones et al. (1999), demonstrated by administrating anti-NGF antibodies that the sprouting is independent of NGF segregated at the site of periferic injury. Neither was an increase of NGF mRNA detected in contralateral ganglion and yet sympathetic baskets can still be observed (Zhou et al., 1999). It is also contradictory the fact that Kinnman and Levine (1995) describe that the perfusion of NGF onto the cut L5 spinal nerve markedly delayed the onset of increased mechanical sensitivity.

Thompson and Majithia (1998) suggest, regarding the results explained before, that there may be additional factors involved in sympathetic sprouting in DRG after axotomy.

Interleukines

Among the factors produced in the degenerating nerve there are some that can act on sympathetic neurones and, in some cases, can induce sprouting of sympathetic axons. IL-1 regulates substance P and its receptor in cultured sympathetic ganglia and stimulates the outgrowth of neurites from cultured superior cervical ganglia. IL-2 also enhances chick sympathetic neurite outgrowth and IL-6, also elevated in injured nerves, influences neurotransmitter synthesis. Leukaemia inhibitor factor (LIF) modifies protein synthesis and the sympathetic cell body (Ramer et al., 1997).

Peripheral nerve injury induces the production of IL-6 by Schwann cell (Bolin et al., 1995) and DRG neurones (Murphy et al., 1995) and it has been reported that sympathetic and sensory neurones express the IL-6 receptor gp90. In vitro experiments have shown that IL-6 and its receptor can induce neurite extensions in PC-12 cells (Satoh et al., 1988) and has been suggested to enhance regeneration of injured peripheral axons in vivo (Hirohata, 1996).

The availability of IL-6 knockout (ko) mice (Kopf et al., 1994) has allowed the further investigation of the role of IL-6 in neuropathic pain in vivo. These mice develop normally, but their responses to bacterial and viral infection as well as to tissue trauma are impaired. The Ramer group has used a well-established behavioural test and TH immunocytochemistry to examine the contribution of IL-6 to SNL induced neuropathic pain and to adrenergic sprouting in the DRG in IL-6 ko mice (Ramer et al., 1998b). They found that thermal allodynia (as assessed by measuring the latency to withdrawal from radiant heat) did not differ significantly between strains. On the other hand, in the IL-6 ko mice, mechanoallodynia (as assessed with von Frey filaments) was markedly delayed. Sympathetic invasion of the fibre tract and cell layer of the DRG, and

the formation of pericellular axonal baskets were all significantly reduced in the IL-6 knockout mice compared to the control strain. These results imply a facilitatory role for Il-6 in pain and sympathetic sprouting induced by nerve injury, and add to the growing list of roles for IL-6 in neuropathological events. For these authors the sprouting itself may arise either via a direct action of Il-6 on sympathetic neurones, or via a secondary response to tissue trauma which is impaired in IL-6 knockout mice (Kopf et al., 1994).

Leukaemia inhibitor factor (LIF)

LIF is a multifunctional cytokine and a member of the haemopoietin cytokine family defined by its interaction with the common receptor motif, gp130. Many of the gp130 cytokines, including LIF, play a role in phenotypic maturation during development of the peripheral nervous system (PNS) and promote neurite extension and morphological maturation in cultured embryonic neuroblasts (Mehler and Kessler, 1995). In the adult nervous system LIF is normally undetectable (Yamamori, 1991) but is induced at the site of peripheral nerve axotomy (Banner and Patterson, 1994) and is retrogradely transported and accumulated within the DRG in a specific population of nociceptive-specific neurones (Thompson et al., 1997).

Thompson et al. (1997) examined in the adult the role of LIF in axotomy-induced sprouting of postganglionic sympathetic fibres into the dorsal root ganglia. In that study he demonstrates that exogenous application of the axotomy-associated cytokine LIF is associated with sprouting of uninjured postganglionic sympathetic neurones around sensory neurones within the dorsal root ganglion. It is likely that increased LIF expression following peripheral axotomy plays an important role in the novel sympathetic sprouting observed within sensory ganglia following peripheral nerve injury. An important observation therefore is that nerve injury itself is not, a priori, necessary for sympathetic sprouting into the DRG.

Axotomy is a potent stimulus for LIF induction. Sciatic nerve axotomy leads to LIF expression within Schwann cells in both proximal and distal stumps (Banner and Patterson, 1994; Sun and Zigmond, 1996) but not within the DRG (Curtis et al., 1994; Sun and Zigmond, 1996). LIF is, however, retrogradely transported to the DRG by sensory neurones, in particular by small-diameter nociceptive-specific neurones (Curtis et al., 1994; Thompson et al., 1997). It is unlikely that the presence of LIF within these sensory neurone cell bodies may act directly as a trophic factor for sympathetic sprouting since many TH-IR baskets in this and other studies formed around large-diameter cell profiles. Retrogradely transported LIF, or a secondary mediator, however, may be re-released into the DRG and act in a paracrine fashion, but this is yet to be demonstrated. Alternatively, LIF may act directly upon sympathetic neurones to induce sprouting. However, the process by which such expression or accumulation triggers sprouting within the DRG is unknown.

Other factors

The mechanisms by which sympathetic fibres might increase pain sensitivity and/or pain severity is not clearly known, but the ability of DRG neurones to express adrenergic receptors has been investigated, and it has been shown that some small-diameter DRG neurones up-regulate α-receptors following injury. Adrenergic α-2 receptors have been shown to be coupled to N-type calcium channels in cultures of previously axotomized rat sensory neurones which could be an evidence of sympathetic-sensory coupling (Ramer and Bisby, 1998b). Hu and McLachlan (2000) propose that the development of the baskets might be related better with changes in intraganglionar environment than with functional relations between sympathetic afferents and sensory neurones.

The expression of CGRP (calcitonin gen-related peptide) in uninjured sensory axons has also been related with the allodynia caused by CCI (Ma et al., 1999). Other factors which might be involved are oncostatin-M (OSM), cardiotrophin 1 (CT-1) and ciliary-derived neurotrophic factor (CNTF) since an observation made is that the receptor motif gp130 is the defining component of a number of closely related cytokines which also include IL6 and IL1 and could imply them in the sympathetic sprouting in the DRG (Thompson et al., 1998).

Receptors

Zhou et al. (1996) reported that sympathetic sprouts in L5 DRG were associated with glial cells which expressed the p75 low-affinity neurotrophin receptor following sciatic nerve transection, and suggested that p75 functions to present NGF or NT-3 to stimulate sprouting from the sympathetic fibres which innervate the DRG vasculature. These authors also describe an increase of NT3 mRNA in the p75-positive satellite cells of the ipsilateral ganglion following SNL (Zhou et al., 1999). The NGF-specific receptor trkA is present on sympathetic axons (Barbacid, 1994). The retrograde transport of NGF in DRG neurones also requires trkA, and although the receptor is expressed mainly in small primary afferents, it is possible that the sympathetic baskets encapsulate a subpopulation of large-diameter neurones which also express trkA (Wright and Snider, 1995).

The Ramer group have proposed a relevant role for both neurotrophin receptor p75 and trkA (Ramer et al., 1997; Ramer and Bisby, 1997b). Later work with knockout mice has demonstrated that p75 receptors enhance but are not required for sympathetic sprouting (Ramer and Bisby, 1997a).

Aportations

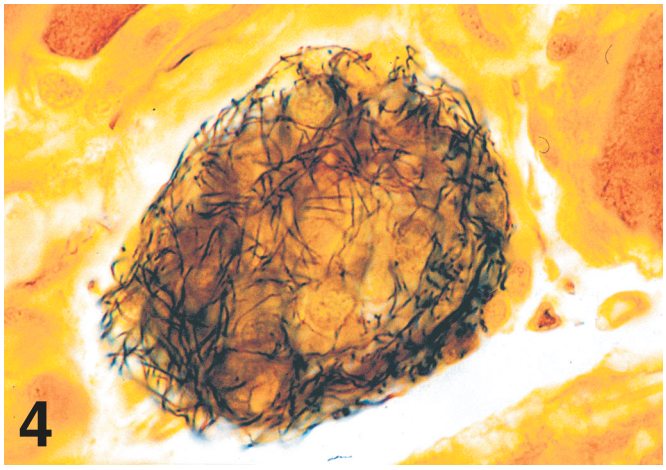
In the present work we wanted to reproduce the

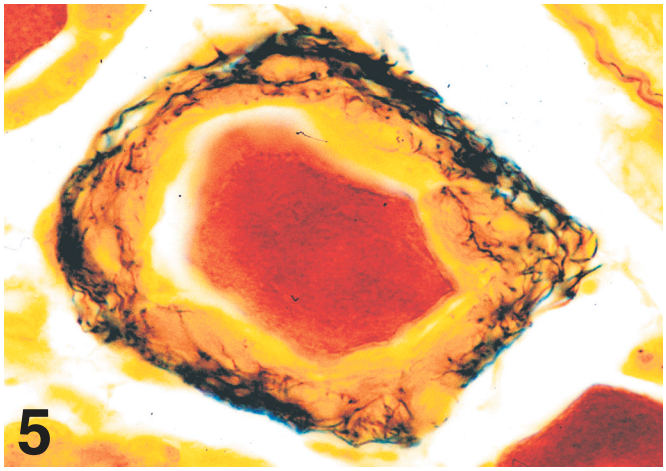
findings originally described by the classical authors following the same technical procedure as them on healthy animals with no induced lesion.

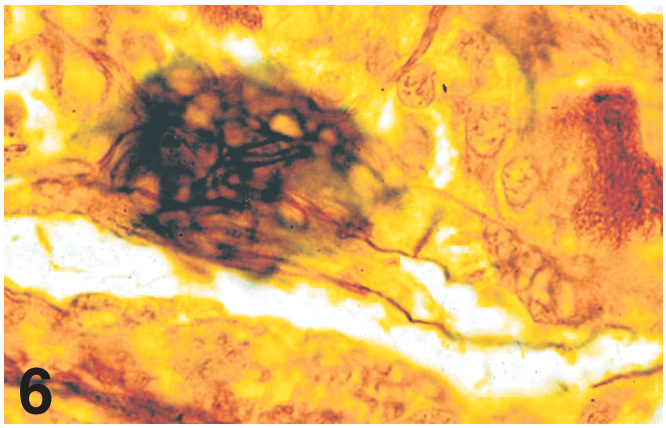
After extraction, DRG from young adult healthy rabbits were fixed in pyridine and stained, as a block, in 2% silver nitrate following "*Cajal's reduced silver*" method (Ramón y Cajal, 1920). The blocks were sliced up consecutively in 12 μm sections and observed with a Zeiss Axiophot 2 microscope.

Very fine amyelinic nervous fibres, consistent with a sympathetic nature, were randomly but frequently detected. In the final portion of these fibres we have been occasionally able to observe several dichotomic branchings finishing as a plexiform arborisation similar to a thread ball that apparently interweaves around the soma of a pseudomonopolar ganglionar cell (Fig. 4). When the cell is sliced sagittally it can be observed that the filaments that form these endings do not appear to contact the neural soma, interweaving with the satellite glia and finishing in its proximity (Fig. 5). In the first right lumbar ganglion of rabbit 5, we could detect one of the previously mentioned nests. The presence of at least five afferent fibres that integrate into a plexus can be neatly observed but it is not possible to affirm whether they anastomose or simply interlace (Figs. 6 a,b, 7).

We report, on the one hand, the finding of multiple afferent amyelinic nerve endings related to "*Dogiel's terminal nests*" observed in different dorsal root ganglia of healthy animals. On the other, we show in sagittal slices the disposition of these fibre endings among the







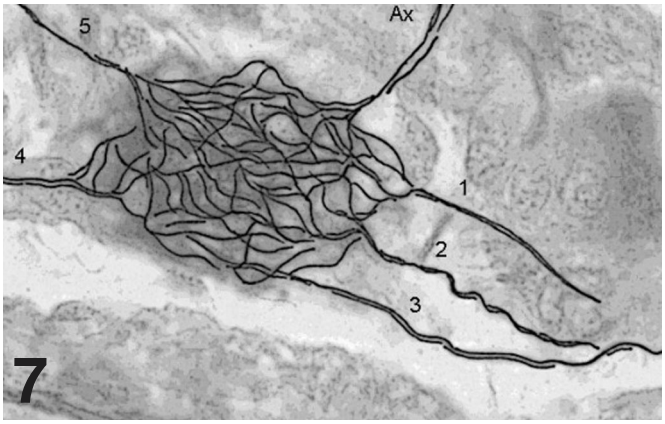


Fig. 4. Optic micrograph of a Dogiel's nest. Tangential cut of the afferent sympathetic fibres yielding an image of the three-dimensional net surrounding the satellite glia cells. Cajal's reduced silver. x 1,000

Fig. 5. Optic micrograph showing a transversal cut of the soma of a ganglionar neurone surrounded by the satellite glia cells immersed in the Dogiel's nest, whose fibres penetrate and end among them, with apparently only one termination on the neuronal soma. Cajal's reduced silver. x 1,000

Fig. 6. Optic micrographs of a Dogiel's nest in different focusing planes to observe the multiple afferent endings of the amyelinic fibres. Cajal's reduced silver. x 630

Fig. 7. Schematic representation of the observed afferent fibre endings in Figure 3, (1 to 5). Ax: ganglionar neurone axon.

cells of the satellite glia where they do not seem to contact the ganglionar cell soma. This finding would be related to recent reports in which a proliferation of satellite cells expressing GFAP and p75 has been stated associated to sympathetic sprouting in damaged dorsal root ganglia (Hu and McLachlan, 2000). Finally, we provide for photographic images with argentic impregnation of these structures, only known up to the moment by means of the, nonetheless wonderful, schematic drawings of classical authors, and we consider this method to still be of value in the range of techniques for the study of this phenomenon.

Conclusions

From all the data displayed it can be concluded that the studies performed during the last decades have only confirmed the findings of the late XIX and early XX century by the histologists of that time. So, the following issues have merely been confirmed by immunohistochemical, physiological and behavioural techniques: (1) the existence of sympathetic endings on ganglionar neurones; (2) their increase during ageing and pathological processes affecting the corresponding nervous roots; and (3) the topography of these endings around the ganglionar neurone soma, among the satellite glia. Nevertheless, it has not been possible to demonstrate what kind of structures they have synapses with, since there are no clear electronic micrographies showing classical synaptic contacts with the ganglionar neurone.

On the other hand it has not been possible to demonstrate without doubt the direct implication of these endings in neuropathic chronic pain aetiology, although it still seems a plausible hypothesis. Neither has the mechanism and kinetics of their occurrence, the sympathetic neurone responsible in each area, or what neuromediating substance would be primarily responsible for this effect been clarified. As a matter of fact, the classical bibliography lets us confirm a question of the maximum importance not addressed by modern authors: the sympathetic sprouting in DRG can also be observed in humans with a moderate frequency.

If the pathological aetiology hypothesis is to be accepted, there remains an aspect of the highest relevance without clarification, the observation of these endings in healthy animal ganglia, less frequent but demonstrated by classical authors and ourselves. As Ramón y Cajal & Tello Muñoz stated in 1956: "*there remains to clarify why a so curious disposition is found, from time to time and in variable proportions, in the ganglia of evidently healthy animals*".

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