CHAPTER 3

INNERVATION OF "PAINFUL" LUMBAR DISCS

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3.1 INTRODUCTION

The concept of primary discogenic pain, particular in the lumbar spine, is well accepted in the literature.[5,6,10,16,28,36,44,46,49] Damage to the intervertebral disc can produce pain, but no consensus exists on the responsible mechanisms. It seems unlikely that discogenic pain is merely generated by mechanical irritation of sensory nociceptive terminals. Chemical stimuli in a degenerated disc have been reported to play a substantial role as well. In this context, the observations of extremely high phospholipase A2 enzyme activity in herniated disc tissue are very interesting.[43] In addition, a wide variety of substances, with the ability to excite - or increase the excitability of - primary sensory neurons have been reported in the interstitial fluid of the disc. These include prostaglandin E, histamine-like substances, potassium ions, lactic acid, and several polypeptide amines.[6,33,36,49] In this respect, Weinstein et al.[49-52] emphasized the important role of the dorsal root ganglion, which is located in the intervertebral foramen and serves as warehouse for all kinds peptides. It is very likely that the dorsal root ganglion has a pain-modulating function around each motion segment.

Assessment of these data in combination with a thorough study of the anatomic pathways conducting discogenic pain seems indispensable for a better treatment of patients with low back pain.

By means of a whole-mount technique with acetylcholinesterase (AChE), a general neural marker,[3,12,13] it has been demonstrated that intervertebral discs are surrounded by a continuous network of interlacing nerve fibers. Ventrally, this network is constituted by the nerve plexus of the anterior longitudinal ligament and dorsally by the nerve plexus of the posterior longitudinal ligament. At the level of the intervertebral foramens, the anterior and posterior nerve plexuses are interconnected by branches directed medioventrally and mediodorsally, the rami communicantes, which overly the lateral border of the disc.[13] Contributions to the ventral nerve plexus are delivered by the sympathetic trunk, its rami communicantes, and the perivascular nerve plexus of segmental arteries. As early as 1850, Von Luschka had discovered that the dorsal nerve plexus is supplied by the sinuvertebral nerves.[48] Whether the sinuvertebral nerves are connected to both the spinal nerve and the sympathetic trunk or its rami communicantes, or are exclusively connected to the rami communicantes, has been discussed exclusively.[5,17,18,40,45,48,53] Although the ring of nerve fibers surrounding the intervertebral discs, including the sinuvertebral nerve, is exclusively related to structures generally considered as sympathetic, according to Groen et al.[13] this does not imply that these structures are fully sympathetic in function. Recent studies support this.[35,37] Most such nerves may have a sensory function. Furthermore the sympathetic nervous system can

interact with sensory C-fibers, sensitizing nociceptors, which in turn induce further sympathetic activity in the spinal cord.[1]
Classic histologic studies have shown the presence of nerve endings in the longitudinal ligaments and in the most superficial layers of the anulus fibrosus.[9,16,19,29,40,42] In some studies, the innervation of the disc was observed to extend as deep as the outer third of the annulus fibrosus.[5, 54] Most authors described the presence of free nerve endings in these tissues. More complex encapsulated endings were mentioned by Malinsky.[29]

Immunohistochemical studies have demonstrated the presence of small-diameter substance P (SP)-immunoreactive nerve fibers in the posterior longitudinal ligaments.[23,27] Furthermore, the presence of calcitonin gene-related peptide (CGRP)-, vasoactive intestinal peptide (VIP), and SP-immunoreactive nerve fibers has been reported in rat intervertebral discs, although restricted to their outer zone.[34] More recently, the same neuropeptides were identified in the anulus fibrosus of human intervertebral discs.[2] The longitudinal ligaments surrounding the intervertebral disc may act as a source of pain in view of their profuse innervation,[5,13,23] but, in addition, a direct nerve supply to the disc itself may be significant.

The literature thus provides conflicting data with respect to the presence of nerve fibers in the different parts of the human intervertebral discs.[2,5,6,29,54] This may be due to differences in innervation between normal and degenerated discs.

The current study was conducted to get a better understanding of the origin of primary discogenic pain in patients with severely degenerated lumbar discs. The innervation of intervertebral discs and adjacent anterior tissue was investigated by means of AChE histochemistry and neurofilament (NF90) immunohistochemistry. A possible nociceptive nature of nerve fibers was determined by SP immunocytochemistry. Preliminary results of this study have been published previously.[7,8]

3.2 MATERIALS AND METHODS

In 10 patients (age range, 24 - 51 years; mean age, 37.1 years; 6 women, 4 men), the anterior segments of one lower lumbar intervertebral disc (L3-L4, L4-L5, or L5-S1) were excised *en bloc* during anterior interbody fusion for chronic low back pain. The segments measured approximately 3×3 cm and consisted of anterior longitudinal ligament, anulus fibrosus, and nucleus pulposus tissue (Figure 3.1). All patients suffered from unremitting low back pain for several years (mean, 7 years) and had extensive disc degeneration confirmed discographically. On all operated levels an intense pain-related response had been provoked by intradiscal injection of Iopamidol (Dagra, Diemen, The Netherlands), a water-soluble, nonionic and inert contrast agent. Additional injection of 0.5 to 1 ml bupivacaine into the disc through the same needle relieved the pain for 1 to 4 hours. These discography-provocation tests were part of a prospective, protocolized study we are conducting for the selection of lumbar fusion candidates.

Two anterior disc segments were obtained during surgery for a spinal metastatic tumor in two patients (Table 3.1) and served as controls. All 12 discs were embedded in a sugar compound, Tissue Tek (Miles Laboratories, Elkhart, IN), and frozen in liquid nitrogen-cooled isobutanol.[31] Transverse cryostat sections (15 μm) were then obtained. The total number of sections obtained in every disc was 200 to 250. AChE enzyme histochemistry [21,32] and NF90 monoclonal, and in five cases also SP polyclonal immunocytochemistry (Cambridge Research Biochemicals, Northwick, Cheshire. U.K.) were performed on alternate, consecutive sections. The NF90 antibodies (Department of Physiology,

University of Leiden, Leiden, The Netherlands) are capable of detecting the phosphorylated low, medium, and high subunits.[39] For immunocytochemistry, the sections were rinsed three times in phosphate-buffered saline (pH 7.6) containing 0.1% bovine serum albumin (Sigma Chemical Company, St. Louis, Missouri), incubated overnight with the primary antisera NF (1/10,000, ascites) or SP (1/1,500) in moist chambers in phosphate-buffered saline containing 0.1% bovine serum albumin and normal goat serum (1/1000), rinsed again before incubation with the secondary, peroxidase-conjugated, antisera (DAKO, Copenhagen, Denmark) for 2 hours, and finally rinsed and incubated with the peroxidase enzyme. Controls were performed by omitting the first or second antibody for immunocytochemistry, by omitting the substrate, or by heating the section to 100 C to destroy enzyme activity I order to detect nonenzymatic localizations by the substrate or capture agent.

Figure 3.1 Disc segment obtained during anterior interbody fusion operation (patient C2810).

Table 3.1 Series of normal and degenerated discs.

Series	Gender	Level	Age (yr)	Type	AChE/ NF90	Substance P
C 2810	F	L4-L5	29	C	Y/Y	N
C 2918	M	L3-L4	51	C	Y/Y	N
C 2959	M	L4-L5	51	DD	Y/Y	N
C 3187	F	L5-S1	24	DD	Y/Y	N
C 3236	F	L5-S1	39	DD	Y/Y	N
C 3455	M	L4-L5	24	DD	Y/Y	N
C 3524	F	L4-L5	38	DD	Y/Y	N
C 3556	F	L4-L5	43	DD	Y/-	Y
C 3664	M	L5-S1	36	DD	Y/Y	Y
C 4030	F	L4-L5	33	DD	Y/Y	Y
C 4031	M	L5-S1	44	DD	Y/Y	Y
C 4035	F	L4-L5	39	DD	Y/Y	Y

AChE = acetylcholinesterase; NF90 = neurofilament; F = female; M = male; C = control disc; DD = degenerated disc; Y = staining performed; - = staining unsuccessful; N = staining not performed.

3.3 RESULTS

A variety of nerve fibers were found that, according to their diameters, could be grouped in various functional classes of nerve fibers (Table 3.2).

Perivascular small nerves

It was possible to recognize blood vessels on account of the endogenous blood-related peroxidase activity. Most vessels were surrounded by perivascular nerves, which contained both NF90-positive and AChE-positive fibers with a diameter of approximately 0.25 μm. These fibers were exclusively found in the anterior longitudinal ligament and the connective tissue in control discs as well as in degenerated discs. In the anulus fibrosus and nucleus pulposus, no blood vessels were found.

Myelinated bundles of nerve fibers

Myelinated bundles of nerve fibers (ACgE- and NF90-positive) were found in the ligament and the transitional area from ligament to anulus. In all control and degenerated discs, these thick, myelinated bundles were present. They penetrated only the most superficial layers of the anulus fibrosus and were composed of several fibers. The diameters of these bundles varied between 15 and 25 μm (Figure 3.2).

Small free nerve fibers

In all discs, degenerated as well as control, the anterior longitudinal ligament and the outer parts of the anulus fibrosus contained free nerve fibers (AChE- and NF90-positive) with a diameter ranging between 0.25 and 2.5 μm (Figure 3.3). Several of these small free nerve fibers were clustered together in the more superficial layers of the anulus, becoming solitary as they traveled inward.

In 8 of 10 degenerated discs (C 3187, C 3236, C 3455, C 3524, C 3556, C 4031, and C 4035), solitary free nerve fibers of this diameter could be found in the inner areas of the disc - namely, deeper than the outer third of the anulus fibrosus.

In two of these degenerated discs (C 3455, and C 4035), free nerve fibers with a diameter of 0.25 μm were discernible in the periphery of the nucleus pulposus as well (Figure 3.4).

In two discs (C 3455, and C 3556), fine solitary fibers with a varicose-like appearance and of a caliber below 1 μm were abundant in parts of the anterior longitudinal ligament; they lacked a network-like configuration, however.

Mechanoreceptors

Receptors with the morphology of Pacinian corpuscles and Golgi tendon organs were detected in four degenerated discs (C 3187, C 3236, C 3455, and C 3524; Figure 3.5). They were seen laterally and medially in the connective tissue between the anulus fibrosus

and the anterior longitudinal ligament or in the interlamellar spaces at the periphery of the anulus fibrosis. The smallest diameter of both mechanoreceptors was 20 μm and their length exceeded 230 μm, whereas the orientation of the longitudinal axis of these receptors was generally parallel to the direction of the anular fibers. Pacician corpuscles had large perineural capsules consisting of several layers. The NF90- and AChE-positive fibers had a diameter of 2.5 to 3.5 μm at the entrance of the central part f the Pacinian-like endings. The Golgi tendon organs were in general cylindric with or without a thin capsule.

Substance P-positive fibers

In all five discs that had been immunocytochemically stained for SP (C3556, C3664, C4030, C4031, and C4035), positive fibers were present, although sporadically (Figure 3.6). SP-positive axons were always single and detected only in the anterior longitudinal ligament as well as in the outer zone of the anulus fibrosus. Their diameter ranged from 0.25 to 2.5 μm. No SP reactivity was noted in perivascular endings.

Unspecific and specific staining reactivity

Omitting the substrate for AChE gave negative results in the ligament, anulus, and nucleus. Immunocytochemical controls showed absence of the diaminobenzidine reaction product if the first or second antibody was omitted. Because endogenous peroxidase activity was found in larger blood vessels on the surface of the anterior longitudinal ligament, in the normal procedures it was inhibited by 3% H_2O_2 before incubation. In the anulus fibrosus and nucleus pulposus, aspecific staining was sometimes found at cracks and edges.

Table 3.2 Survey of nerve structures present in the various parts of the intervertebral disc.

	Perivascular small nerves		Myelinated large-caliber		Small free nerve fibers		Mechano-receptors	
	C	DD	C	DD	C	DD	C	DD
ALL	+	+	+	+	+	+	-	-
Transitional zone between ALL and AF	+	+	+	+	+	+	-	+ (4/10)
Outer zone AF (outer 1/3)	-	-	+	+	+	+	-	+ (1/10)
Inner zone AF (inner 2/3)	-	-	-	-	-	+ (8/10)	-	-
Nucleus pulposus	-	-	-	-	-	+ (2/10)	-	-

C = control discs (n = 2); DD = Degenerated discs (n = 10); + = presence of nerve fibers; - = absence of nerve fibers; ALL = anterior longitudinal ligament; AF = anulus fibrosus; 1/10, 2/10, 4/10, 8/10 = 1, 2, 4, or 8 of 10 discs (if ratio is not given, it means the finding is seen in all discs).

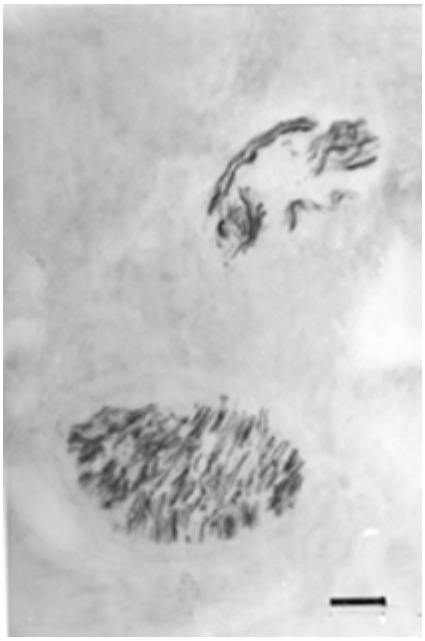
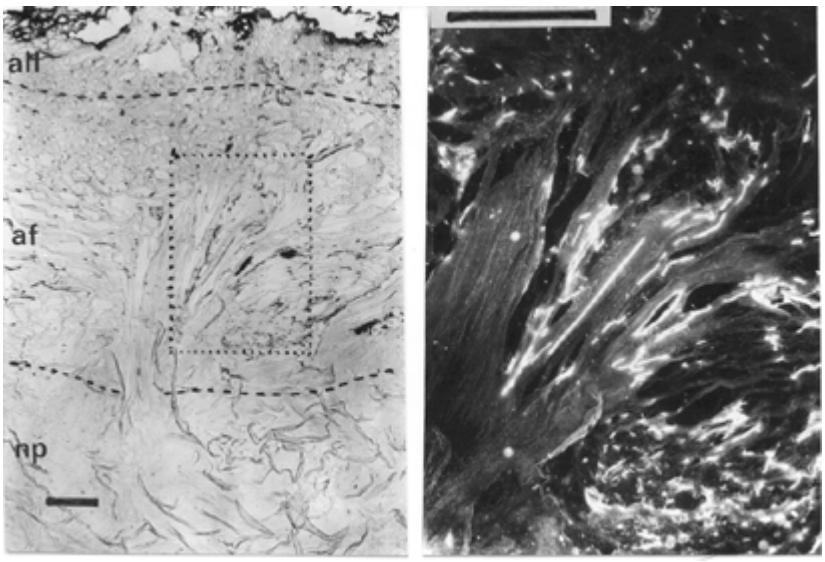


Figure 3.2 Myelinated bundles of nerve fibers in the outer zone of the anulus fibrosus. (Neurofilament (NF90) staining; bar = 5 µm.)



A B

Figure 3.3 Overview of a part of the disc, acetylcholinesterase (AchE) staining. Top contains the anterior longitudinal ligament (all), bottom reaches into the nucleus pulposus (np). **A,** AchE staining. Dotted lines indicate border between the anulus fibrosus (af), the all, and the np. B, Dark-field detail of the central of (**A**) (dotted rectangle), demostrating the ingrowth of nerve fibers in an anular cleft. The nerve fibers appear as white strands in the center of the figure. Bar = 2 µm.)

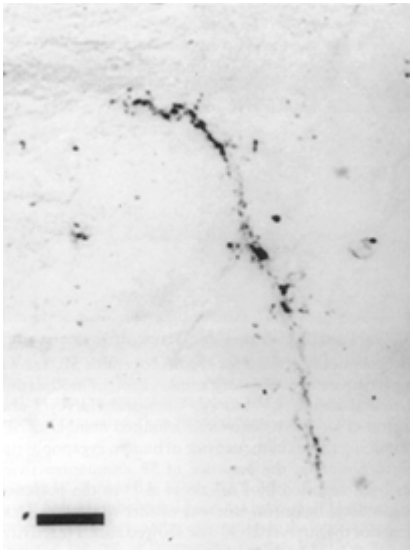


Figure 3.4 Neurofilament-positive fiber localized in the outer part of the nucleus pulposus. (Bar = 2 µm.)

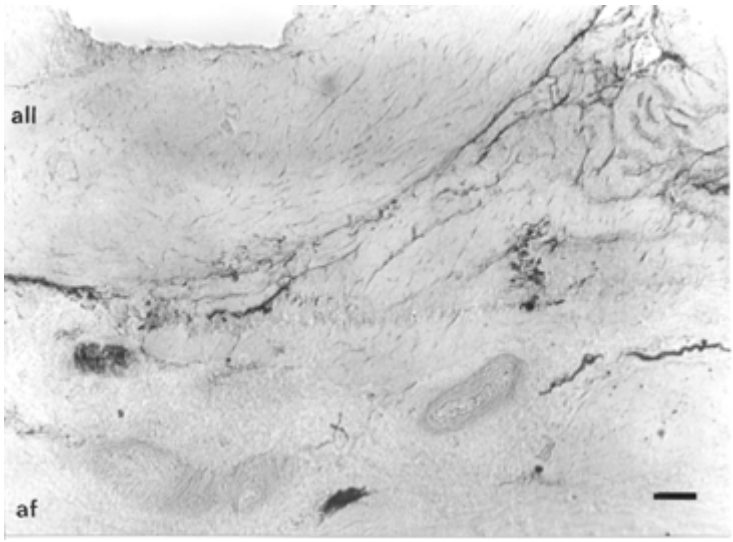


Figure 3.5 Mechanoreceptors immunoreactive to neurofilament (NF90) located between the anterior longitudinal ligament (all) and first lamella of the anulus fibrosus (af). g = Golgi tendon organ; p = Pacinian corpuscle. (Bar = 20 µm.)

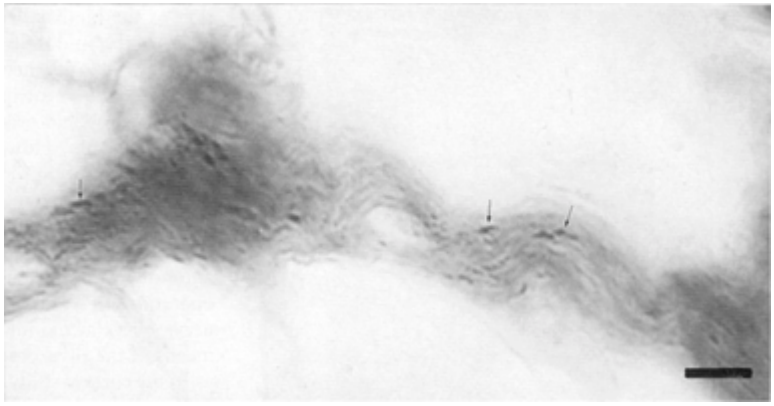


Figure 3.6 Substance P-immunoreactive fibers (arrows) in the outer zone of the anulus fibrosus. (Bar = 5 µm.)

3.4 DISCUSSION

The nerve supply of the intervertebral disc has been the subject of several studies using a variety of disc materials.[2,5,6,9,13,15,19,20,29,34,40-42,45,53,54] These materials were derived from either animal or human species and were of fetal or adult origin.

Because of this wide variety of material it is difficult to draw general conclusions. Yet, a common finding in all the studies on nondegenerated discs is that the innervation of the lumbar disc remains restricted to the outer layers of the anulus fibrosus. There are indications, however, that disc degeneration and perhaps disc injury are associated with centripetal growth of nerve fibers in the disc, which would provide a morphologic basis for true discogenic pain. However the responsible mechanism for the penetration of neural structures deeper into the disc is still poorly understood.

Yoshizawa et al.[54] investigated the innervation of the intervertebral disc in patients with low back pain using anterior sectors of lumbar discs obtained during fusion operations. It was not possible, however, to demonstrate nerve fibers in the inner half of the annulus fibrosus by means of the silver-impregnation method.

In the current study, we investigated “painful” degenerated discs obtained from patients with chronic low back pain. Therefore, a selection was made with regard to both the patient and the disc. The latter involved two inclusion criteria. In the first place, the disc had to be severely degenerated, as shown by discography, and second, injection of fluid into the nucleus pulposus had to provoke a temporary severe increase of their chronic pain. Eight of 10 degenerated discs selected in this way proved to contain nerve fibers throughout various layers of the anulus fibrosus, invading deeper than the outer third of the anulus. The control discs showed innervation only in the outer parts of the disc.

Comparison between AChE and NF90 immunocytochemistry, which both stain specifically for neural tissue, reveals a difference in sensitivity. The NF90 antibody detects only phosphorylated neurofilaments, missing nonphosphorylated ones. Therefore, fewer positive fibers are observed when NF90 is used instead of AChE staining.

However, use of NF90 antibodies provides better recognition of the fine axon structure within a nerve bundle. This can be explained by the presence of AChE activity not only in the axon but in the myelin sheat,[25,26] by which a sharp delineation of the axonal structure is blurred. In the outer anulus zone, thick, myelinated bundles, single fibers, and mechanoreceptors were found. The latter were present in the loose connective tissue between the anterior longitudinal ligament and the anulus fibrosus, and between the outer layers of the anulus proper.

Mechanoreceptors resembling the morphology described for Pacinian corpuscles and Ruffini endings have been considered responsible for proprioception,[29,49] and they may be involved in maintaining muscle tone and in the reflex response.[41] A nociceptive function has been assigned to structures resembling Golgi tendon organs.[41]

Malinsky[29] reported mechanoreceptors in the lateral, ventrolateral, and dorsolateral regions of the disc surface. In addition, we found these receptors in the ventromedial region. In a recent study, Roberts et al.[41] reported the presence of mechanoreceptors in 50% of discs investigated from patients with low back pain and in only 15% of those from pain-free patients with scoliosis. These findings are comparable with those in the current study. Mechanoreceptors could be found in 4 of 10 clinically pathologic and "painful" discs, but in neither of the 2 control discs.

Normally, the adult human disc is avascular. Angiogenesis associated with disc disorders is not an uncommon finding, however. Experimental studies have shown that anular lesions heal by the formation of granulation tissue containing blood vessels.[38] In experimentally injured porcine intervertebral discs, Kääpä et al.[20] even found a dense network of capillaries in the healed anular area 2 weeks to 2 moths after operation. Vascularization of the inner parts of the disc has also been reported in degenerated discs.[47] In the current study, blood vessels were found only in the anterior longitudinal ligaments. They possessed a perivascular nerve network with probably a vasomotor or vasosensory function.

To determine the possible nociceptive action of the small-caliber (Aδ and C) fibers, sections were immunohistochemically stained for the neuropeptide SP, known to participate in the sensory transmission or modulation of neural impulses.[15] Indeed, Giles and Harvey[11] and Ashton et al.[1] have discovered a limited number of SP-containing nerves in the capsule of human zygapophysial joints. Similarly, the presence of SP immunoreactivity has been reported by Korkala et al.[23] in the posterior longitudinal ligament, whereas neither the yellow ligament nor the intervertebral disc showed such a reactivity. In their material, SP-immunoreactive nerves were always found running freely in the stroma but not in the vicinity of blood vessels, although this peptide is known to act as a vasodilator in other tissues.[1,4,24] In the rat, SP, CGRP, and VIP have been identified in the outer anular fibers and supraspinous and intraspinous ligaments,[34,49] but their detection in human discs has proven difficult, as yet.[11,22,23,27] More recently, SP, CGRP, and VIP immunoreactivity was demonstrated in the outer 3 mm of the anulus fibrosus of human intervertebral discs.[2]

Our results relating the detection of SP are in agreement with those of Ashton et al.[2] In the current study, SP staining in clinically "painful" discs showed a very small amount of superficially localized SP-positive fibers, in contrast to greater abundance of AChE- and NF90-positive fibers in the same disc. McCarthy et al.[34] recommended using CGRP instead of SP because the former is more ubiquitous in the investigated nerve cells. In our opinion, however, a marked difference in the abundance of nerves immunoreactive for peptide markers of sensory nerves (e.g., CGRP and SP) or autonomic nerves (e.g., VIP) should not be considered as the most reliable method for assessing the real extent of

sensory fibers because not all the nerve fibers present would show positive staining. Therefore, the presence, but not the absence, of these markers is conclusive for the interpretation of sensory innervation.

To explain the relative lack of detectable SP immunoreactivity, it may be postulated that as with inflamed joints, a “painful” disc contains a larger amount of neurotransmitters. It has been reported that, on account of an increased local release of neurotransmitters, a weaker neuropeptide staining is found in synovium from inflamed joints with respect to normal synovium.[14,30] Thus, it is tempting to state that there is a possibility that the local release of neurotransmitters is greatest in the more degenerated central parts of these discs, leading to a weaker staining for SP immunoreactivity. Finally, the direct processing of the tissue, without prior fixation, may have contributed to the relatively weak detection of SP-immunoreactive fibers.

The relation between painful discs, neurotransmitter distribution, and neuropeptide staining needs further elaboration.

In conclusion, the data presented in this study support a neuroanatomic substrate for discogenic pain perception in patients with severely degenerated discs. However, this report does not allow conclusions to be drawn on the statements that nondegenerated discs have a less extensive innervation than painful, degenerated discs.

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