Neuronal networks involved in low back pain
Experimental studies

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ABSTRACT

Low back pain is a common cause of disability, with a lifetime prevalence of up to 80%. A lumbar disc herniation, involving a bulging disc and/or leakage of the intervertebral disc's nucleus pulposus, may be a possible cause of back and sciatic pain. Low back pain has also been associated with dysfunctional control of the paraspinal muscles. The aim of this thesis was to study the neuronal networks involved in low back pain including sciatica. In paper I, the contribution of two descending tracts, the pyramidal tract and the reticulospinal tract to the activity of motoneurones innervating one of the muscles of the erector spinae (longissimus muscle) were investigated in the cat. In papers II and III, changes in evoked neuronal activity in the ventral posterior lateral (VPL) nucleus of the contralateral thalamus were investigated following an experimental disc herniation affecting the ipsilateral 4th lumbar (L4) dorsal root ganglion (DRG) in the rat. Paper II concerns the role of mechanical compression and application of nucleus pulposus to the DRG while paper III investigates the role of two individual cell populations of nucleus pulposus, notochordal and chondrocyte-like cells. The results in paper I show that central activation from pyramidal neurones to erector spinae muscle is primarily mediated via reticulospinal neurones, while a limited proportion is also mediated via interneurones activated by pyramidal tract neurones. In paper II, opposite effects on evoked neuronal activity in the VPL were found where the mechanical compression induced a decrease in neuronal activity and nucleus pulposus had a facilitating effect. In paper III, neither of the two cell populations of nucleus pulposus induced an increase in neuronal activity resembling the increase reported previously following application onto the DRG of whole nucleus pulposus tissue. This thesis investigates some of the complex neuronal networks likely to be involved in low back pain, both directly and indirectly. Insights gained from the use of animal models will contribute to our ultimate understanding of the complicated processes that operate during the establishment and maintenance of low back pain including sciatica.

Keywords: low back pain, disc herniation, pyramidal tract, reticulospinal tract, longissimus muscle, nucleus pulposus, VPL, thalamus, rat, cat

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Ländryggssmärta drabbar cirka 80% av befolkningen någon gång i livet. Olika strukturer i ryggen såsom muskler, kotor eller intervertebraldiskar kan vara orsaken till smärtan. Vid degeneration av eller skador på en intervertebraldisk uppkommer sprickor i den yttre delen, annulus fibrosus, och genom dessa sprickor kan diskens kärna, nucleus pulposus, läcka ut och påverka närliggande nervrot och/eller dorsalrotsganglion (DRG). Ett diskläckage kan ge upphov till ischiassmärta eller ländryggssmärta. Smärtupplevelsen är ett resultat av sensorisk information från ischiasnerven eller fria nervändar i till exempel annulus fibrosus till ryggmärgen och vidare via thalamus till sensoriska hjärnbarken. Aktivering av smärtsystem är ofta kombinerat med motorisk aktivitet för att skydda en skadad kroppsdel och studier har visat påverkan på ländryggsmuskulaturen i samband med skador på intervertebraldisken.

Metod: Med elektrofysiologiska studier på sövda djur studerades dels motorisk kontroll av ländryggsmuskulaturen från hjärnbark och hjärnstam och dels effekter på det centrala nervsystemet av experimentellt frambringade diskbråck. I det första delarbetet undersöktes hur två motoriska bansystem, pyramidbanan och den retikulospinala banan, kontrollerar ländryggs-muskulaturen på katt. I det andra och tredje delarbetet var frågeställningen hur nervcellsaktiviteten i thalamus påverkas akut vid ett diskbråck och i dessa arbeten användes råttor för att studera thalamusaktiviteten efter applikation av nucleus pulposus och/eller akut tryck (delarbete II) eller av enskilda cellpopulationer av notochordala och chondrocyt-lika celler från nucleus pulposus (delarbete III) på ett DRG i ländryggen.

Resultat: Delarbete I visar att central aktivering av ländryggsmuskulatur från cortex förmedlas via retikulospinala neuron, medan en mindre del också förmedlas via spinala interneruon som i sin tur aktiveras av pyramidbanan. Delarbete II visar att vid ett akut, experimentellt framställt diskbråck påverkar det mekaniska trycket och exponeringen av nucleus pulposus thalamusaktiviteten på motsatt sätt, där ett mekaniskt tryck verkar hämmande och nucleus pulposus faciliterande. En tidigare visad ökad effekt av thalamusaktivitet efter applikation av hel nucleus pulposus på ett DRG kunde inte återupprepas med en enskild eller kombination av cellpopulationer från nucleus pulposus (delarbete III).

Diskussion: Denna avhandling visar dels hur ländryggsmuskulaturen kan kontrolleras från motoriska hjärnbarken och dels hur det centrala nervsystemet påverkas av olika komponenter i ett diskbråck. Denna kunskap är viktig för förståelsen av de komplicerade processer som sker när ländryggssmärta uppkommer och i de fall då smärtan blir bestående.

LIST OF PAPERS

This thesis is based on the following studies, which are referred to in the text by their Roman numerals.

I. Galea, MP, Hammar, I, Nilsson, E, Jankowska, E. Bilateral postsynaptic actions of pyramidal tract and reticulospinal neurons on feline erector spinae motoneurons. Journal of Neuroscience 2010; 30(3), 858-69.

II. Nilsson, E, Brisby, H, Rask, K, Hammar, I. Mechanical compression and nucleus pulposus application on dorsal root ganglia differentially modify evoked neuronal activity in the thalamus. Submitted.

III. Nilsson, E, Larsson, K, Rydevik, B, Brisby, H, Hammar, I. Evoked thalamic neuronal activity following DRG application of two nucleus pulposus derived cell populations: an experimental study in rats. Submitted.

CONTENTS

ABBREVIATIONS

ASIC3	Sodium selective acid-sensing ion channel 3
DDD	Degenerative disc disease
DRG	Dorsal root ganglion
EMG	Electromyogram
EPSP	Excitatory postsynaptic potential
FSC	Forward angle scatter
GABA	γ-aminobutyric acid
$GABA_A$	γ-aminobutyric acid type A
IPSP	Inhibitory postsynaptic potential
IVD	Intervertebral disc
LBP	Low back pain
LCN	Lateral cervical nucleus
MLF	Medial longitudinal fascicle
NP	Nucleus pulposus
PSP	Postsynaptic potential
PT	Pyramidal tract
RS	Reticulospinal
SSC	Side scatter
TNF-α	Tumour necrosis factor α
VIP	Vasoactive intestinal peptide
VPL	Ventral posterior lateral nucleus of the thalamus

BRIEF LIST OF DEFINITIONS

Thalamus	Relay structure for sensory information from the spinal cord to the cerebral cortex.
Spinothalamic tract	Ascending tract forwarding sensory information from the spinal cord to the thalamus.
Spinocervico-thalamic pathway	Ascending pathway forwarding sensory information from the spinal cord to the thalamus which relay in the lateral cervical nucleus.
Spinoreticulo-thalamic pathway	Ascending pathway forwarding sensory information from the spinal cord to the thalamus which relay in the reticular formation.
Pyramidal tract	Descending motor tract from the motor cortex to the spinal cord. Most fibres contact motoneurones indirectly via interneurones.
Reticulospinal tract	Descending motor tract from the nuclei of the reticular formation to different target cells, including motoneurones, in the spinal cord. Most fibres descend in the medial longitudinal fascicle (MLF).

1 INTRODUCTION

Low back pain (LBP), with or without radiating sciatic pain, constitute disabling musculoskeletal disorders where the pain may originate from a variety of spinal structures including ligaments, facet joints, the vertebral periosteum, the paravertebral muscles and fascia, the intervertebral discs, and the spinal nerve roots (Deyo and Weinstein 2001). Most often, the pain cannot be attributed to a specific pathology and is then termed non-specific. It has been estimated that as many as 80% of adults will experience at least one episode of acute LBP during their lifetime (Shvartzman, Weingarten et al. 1992; Andersson 1998). A systematic review (Pengel, Herbert et al. 2003) presented the natural recovery from LBP as being generally rapid with most people recovering within a month. Unfortunately, affected individuals are likely to suffer a recurrence of pain episodes within a 12-month period (Cassidy, Cote et al. 2005; Wasiak, Kim et al. 2006). The prevalence of chronic LBP is estimated to be about 23% with 11–12% of the European population being disabled by it (Airaksinen, Brox et al. 2006).

Both animal and human models have been used to study the neuronal pathways involved in development and maintenance of low back pain. This thesis deals with changes in the transmission of nociceptive information in the peripheral and central nervous system as well as motor control, both of which might be activated by events related to low back pain. Physiological responses to a noxious stimulus may involve both afferent and efferent fibres in ascending and descending neuronal systems. To understand the possible role of neuronal networks involved in low back pain, input to the lumbar longissimus muscle motoneurones in a cat model was investigated (paper I). We also investigated acute changes in ascending activity in evoked thalamic responses in a recently developed rat model after experimental intervertebral disc hernia (papers II and III).

2 BACKGROUND

2.1 The Spine

The human vertebral column consists of 7 cervical, 12 thoracic, 5 lumbar, and 5 sacral vertebrae (joined together into the sacrum) and, most caudal, the coccyx of 3–5 very small vertebrae. The number of vertebrae—and thereby also the number of intervertebral discs—differs slightly between species. The cat has 7 cervical vertebrae, but 13 thoracic and 7 lumbar vertebrae, 3 sacral (the sacrum), and 22 or 23 caudal vertebrae in the tail (Reighard 1949) whereas the rat has 7 cervical, 13 thoracic, 6 lumbar, 4 sacral, and 27–30 caudal tail vertebrae (Greene 1959).

2.1.1 The Intervertebral disc

Anatomy and function

The intervertebral discs (IVDs) are positioned in-between the vertebral bodies in the spinal column (Figure 1). Their role is mechanical—to bear and/or transmit loads arising from body weight and muscle activity. The IVD consists of three anatomical structures, vertebral endplates, annulus fibrosus, and nucleus pulposus (Raj 2008). The vertebral endplates are thin hyaline cartilage layers at the borders of the disc facing the superior and inferior vertebral bodies. The annulus fibrosus is the fibrous cartilage outer part of the disc, consisting of concentric lamellae of collagen fibres oriented in parallel with elastin fibres in-between. The annulus fibrosus surrounds the inner gelatinous core of the disc, referred to as the nucleus pulposus, which is composed of a more irregular proteoglycan-rich matrix.

Nucleus pulposus

Nucleus pulposus is the IVD's cell-poor inner core tissue consisting of at least two cell populations, chondrocyte-like cells and notochordal cells (Chelberg, Banks et al. 1995). The chondrocyte-like cells are small and round cells, resembling cells found in articular cartilage, whereas notochordal cells are large and highly vacuolated (Trout, Buckwalter et al. 1982; Trout, Buckwalter et al. 1982; Maldonado and Oegema 1992; Errington, Puustjarvi et al. 1998). The proportions of cells in nucleus pulposus are species-specific (Guilak, Ting-Beall et al. 1999; Poiraudeau, Monteiro et al. 1999; Gan, Ducheyne et al. 2003). While notochordal cells are still present after skeletal maturity, for example in rats (Hunter, Matyas et al. 2004), the composition of human nucleus pulposus changes with age so that the large notochordal cells

decrease in number and the smaller chondrocyte-like cells increase (Trout, Buckwalter et al. 1982; Trout, Buckwalter et al. 1982; Hunter, Matyas et al. 2003; Cao, Guilak et al. 2007; Guehring, Urban et al. 2008). It has previously been proposed that notochordal cells disappear entirely within the first three decades of life (Trout, Buckwalter et al. 1982; Trout, Buckwalter et al. 1982; Pazzaglia, Salisbury et al. 1989), but there is now increasing evidence that some cells of the notochordal population in nucleus pulposus are preserved in adulthood (Choi, Cohn et al. 2008; Gilson, Dreger et al. 2010; Risbud, Schaer et al. 2010; Weiler, Nerlich et al. 2010). Ichimura et al. (Ichimura, Tsuji et al. 1991) suggested that the smaller cells in nucleus pulposus are a heterogeneous population, and recently Kim et al. (Kim, Deasy et al. 2009) described the presence of a population of small notochordal cells with chondrocytic phenotype in rabbit nucleus pulposus. While the fate of notochordal cells have been debated, chondrocyte-like cells have been found in nucleus pulposus from both elderly humans (Trout, Buckwalter et al. 1982) and rats (Hunter, Matyas et al. 2004).

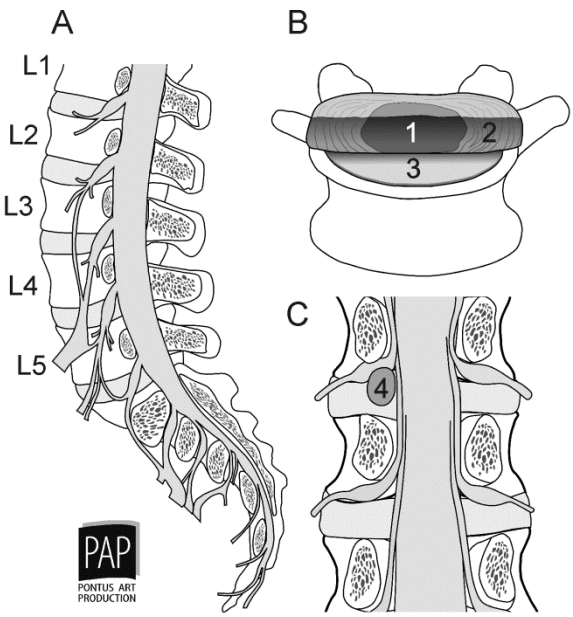

Figure 1. Anatomy of the spine. A. The lumbar (L) vertebrae 1–5. B. The intervertebral disc with 1) nucleus pulposus, 2) annulus fibrosus, and 3) vertebral endplate. C. A disc hernia compressing the dorsal root ganglion (DRG) (4).

Extracellular matrix of the intervertebral disc

The IVD consists of a large amount of extracellular matrix, which makes up approximately 99% of the total volume of the disc, interspersed with a small number of cells (Roberts, Evans et al. 2006). The proteoglycan and water content of the matrix is largely conserved across animal species (Beckstein, Sen et al. 2008). Proteoglycans are negatively charged molecules, and their hydrophilic nature draws water by osmosis into the disc, thus giving the load-bearing characteristics of the IVD (Martin, Boxell et al. 2002).

Innervation of the intervertebral disc

In the healthy spine, only the outer part of the annulus fibrosus of the IVD is innervated by nerve fibres and blood vessels (Malinsky 1959; Gronblad, Weinstein et al. 1991). The meningeal branch of the spinal nerve, better known as the sinuvertebral nerve, innervates the outer posterior and posteriolateral part of the disc (Bogduk, Tynan et al. 1981; Bogduk 1983) while the anterior part is supplied by nerves arising from the sympathetic plexus (Peng, Wu et al. 2005). Nociceptive neuronal markers such as substance P, calcitonin gene-related peptide (CGRP), and vasoactive intestinal polypeptide (VIP) immunoreactive nerve fibres have been found in the outer layers of human annulus fibrosus (Konttinen, Gronblad et al. 1990). A small number of mechanoreceptors are also present, most commonly having a morphology similar to that of Golgi tendon organs, but a few Ruffini receptors and even fewer Pacinian corpuscles have been reported in bovine discs (Roberts, Eisenstein et al. 1995).

2.1.2 Muscles of the spine

Anatomy and function

There are numerous muscles surrounding the vertebral column and most of them are extensor muscles (Martini 2003) (Figure 2). The paraspinal muscles are responsible for movements of the vertebral column. The m. erector spinae, which consists of the m. spinalis, m. longissimus, and m. iliocostalis, is the large extensor of the vertebral column and the m. multifidus, m. semispinalis, m. rotatores, mm. interspinales, and mm. intertransversarii produce both extension and rotation of the vertebral column. The m. latissimus dorsi is a superficial back extensor muscle extending the vertebral column and it also acts as a stabilizer of the shoulders. In the lumbar spine, the large m. quadratus lumborum is the vertebral column flexor muscle.

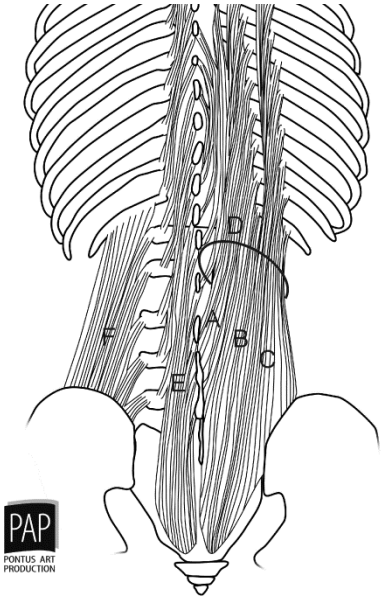

Figure 2. Anatomy of the lumbar spine. The extensors A) m. spinalis, B) m. longissimus ,C) m. iliocostalis of D) the m. erector spinae, and E) m. multifidus and the flexor F) m. quadratus lumborum.

Nociceptive sensory pathways

Afferent nociceptive fibres (Aδ- and C-fibres) mediate information about intense thermal, mechanical, or chemical stimuli applied to free nerve endings (nociceptors) in the periphery via dorsal roots toward the dorsal horn of the spinal cord (Bessou and Perl 1969; Lynn 1977). In the lower back, Aδ- and C-fibres are found in ligaments, facet joints, the vertebral periosteum, the paravertebral muscles and fascia, the intervertebral discs, and spinal nerve roots (Deyo and Weinstein 2001).

The Aδ-fibres are thin, lightly myelinated and slowly conducting fibres while the C-fibres are unmyelinated and the most slowly conducting fibres. The cell bodies are located in the dorsal root ganglion (DRG) and the nociceptive primary afferent fibres terminate mainly in the superficial laminae I and II of the dorsal horn but also in the deeper laminae, mainly in lamina V (Rexed 1952).

The thalamus is the main relay structure for sensory information from the spinal cord to the cerebral cortex and the information reaching the right thalamus mainly originates from left side of the body and vice versa (Brodal 1981). Several ascending tracts forward nociceptive information from the spinal cord to the thalamus. The spinothalamic tract is the most direct pathway (Carstens and Trevino 1978) but there are also several indirect pathways conveying information to the thalamus via one or more relay

nuclei. The spinocervico-thalamic tract relays in the lateral cervical nucleus (LCN) (Craig and Burton 1979), and the spinoreticulo-thalamic tract relays in the reticular formation (Peschanski and Besson 1984).

The thalamus consists of several distinct nuclei (Dekaban 1953) and on the whole the principal anatomy of the thalamus appears to be the same in most mammalian species (Davidson 1965; Brodal 1981). The ventral posterior lateral (VPL) thalamic nucleus is a part of the ventrobasal complex and is generally considered to relay both noxious and non-noxious information from the body and limbs (Peschanski, Mantyh et al. 1983; Craig, Bushnell et al. 1994; Willis, Zhang et al. 2001; Gauriau and Bernard 2002), and recordings from the VPL in rats have revealed an acute increase in mean firing rates, in the number of responsive neurones, and in total response counts with gradually increasing nociceptive stimulation applied to the plantar surface (Zhang, Wang et al. 2011).

Supraspinal control of spinal muscles

There are several descending neuronal systems originating in the cortex and brainstem mediating motor activity via motoneurones. The work in this thesis has focussed on two of these systems, the corticospinal and reticulospinal descending tract neurones and their contribution to the control of paraspinal muscle motoneurones.

The pyramidal tract (PT) includes nerve fibres which descend in the pyramis of the medulla oblongata (Brodal 1981). The fibres originate in the cerebral cortex and most of them continue to the spinal cord, making up the corticospinal tract. The majority of pyramidal tract fibres cross and descend on the contralateral side of the spinal cord and only a minority descend ipsilaterally. This is the situation for all mammalian species although the proportions of crossed and uncrossed fibres vary between them (Nyberg-Hansen and Brodal 1963; Armand and Kuypers 1980; Brosamle and Schwab 1997; Lacroix, Havton et al. 2004). In contrast to primates, pyramidal tract fibres in cats do not have monosynaptic connections with motoneurones but evoke excitatory and inhibitory postsynaptic potentials (PSPs) in them via interneurones or propriospinal neurones (Lemon 2008).

Reticulospinal (RS) fibres originate from several nuclei in the reticular formation of the brain stem (Torvik and Brodal 1957). Most fibres, for example those from the gigantocellular nucleus descend through the medial longitudinal fascicle (MLF) and target different neurones in the spinal cord (Brodal 1981; Robbins, Pfaff et al. 1992; Matsuyama, Mori et al. 1999). Reticulospinal neurones can be co-activated by pyramidal tract neurones from both hemispheres (He and Wu 1985; Matsuyama and Drew 1997; Kably and Drew 1998) and the reticulospinal tract is a bilaterally organized system where a single axon can innervate both sides of the cord (Matsuyama,

Mori et al. 1999; Jankowska, Hammar et al. 2003; Davidson and Buford 2004; Schepens and Drew 2006). The MLF contains not only reticulospinal tract fibres but also other tract axons; as all of these but the reticulospinal fibres terminate at cervical or thoracic segments, stimulation applied within the MLF can be used to activate reticulospinal axons descending to lumbosacral segments (Holstege and Kuypers 1982; Mitani, Ito et al. 1988).

Postural adjustments have been shown to depend on both corticospinal and reticulospinal neurones (Massion 1992; Massion 1994) and trunk muscles to receive greater bilateral drive compared to the distal limb muscles (Carr, Shepherd et al. 1985; Marsden, Farmer et al. 1999). Previous investigations have focussed primarily on how pyramidal tract and reticulospinal neurones affect neck motoneurones (Wilson and Yoshida 1968; Alstermark, Pinter et al. 1985) and how reticulospinal neurones affect back motoneurones in thoracic segments (Peterson, Pitts et al. 1979) while actions on back motoneurones in lumbar segments have been less investigated.

2.2 Low back pain

Aging of the IVD

The lumbar IVDs undergo extensive changes with age, i.e. loss of structure in all three anatomical parts (Buckwalter 1995). Major changes occur in the endplate in the first decades of life and they precede changes in the nucleus pulposus and annulus fibrosus later in life (Boos, Weissbach et al. 2002). Changes include an increased number and extent of clefts and tears of the annulus fibrosus as well as loss of demarcation between the annulus fibrosus and nucleus pulposus. Decline in disc nutrition, loss of proteoglycan organization and concentration due to loss in cell amount and density, and increased degradative enzyme activity relative to matrix synthesis may lead to the loss of disc structure and function.

In addition to the age-related changes found in the IVD, structural changes can also occur following a trauma—or simply appear for some undefined reason. Whatever the cause is, changes in the IVD may be too destructive and might therefore cause low back pain (LBP) (Raj 2008), either through direct pathological changes of the IVD known as degenerative disc disease (DDD) or as a consequence of DDD when the structural changes cause the disc to bulge or protrude in a disc hernia affecting adjacent nerves, often causing both rhizopathy and LBP (Boden, Davis et al. 1990).

Degenerative disc disease is a pathological state that may or may not be present together with LBP. The aetiology of the disease process is not fully understood, although histopathological observations indicate that degenerative changes in the nucleus pulposus and in the surrounding annulus

fibrosus may occur early on in the disease process. While healthy nucleus pulposus contains no nerve endings (Derby, Kim et al. 2005), they have been found in the nucleus pulposus in patients with chronic LBP (Freemont, Peacock et al. 1997). The ingrowth of nerve endings has been suggested to be the patho-anatomical correlate to the dull chronic ache by the mechanical loading of the spine that is experienced by patients with chronic LBP and which is sometimes referred to as discogenic pain (Brisby 2006).

Disc hernia-related pain such as LBP and sciatica was first described by Mixter and Barr (Mixter and Barr 1934), who suggested that it arises as a result of the mechanical compression that a ruptured disc exerts on adjacent nerves. While mechanical compression is still presumed to be important (Smyth and Wright 1958; Rydevik, Brown et al. 1984; Hu and Xing 1998), it has been revealed that disc rupture leading to subsequent leakage of nucleus pulposus can contribute to the experience of disc hernia-related pain by affecting adjacent nerves (McCarron, Wimpee et al. 1987; Byrod, Rydevik et al. 1998; Yabuki, Kikuchi et al. 1998; Murata, Rydevik et al. 2005) and possibly also by inducing nerve ingrowth into the disc, as recently suggested by Inoue et al. (Inoue, Ohtori et al. 2006).

Neuronal networks involved in low back pain

Changes in neuronal networks that are believed to be involved in low back pain have been observed. Patients suffering from back pain often show an augmented central pain processing, apparent as an increased pain experience and more extensive cortical activation in response to painful pressure onto the back compared to healthy subjects (Giesecke, Gracely et al. 2004). Flor et al. (Flor, Braun et al. 1997) demonstrated an expansion and shift in the somatosensory cortex area representing the lower back in low back pain patients. As a consequence, such changes might result in lower excitability in the motor cortex to evoke facilitation or inhibition of erector spinae muscles (Strutton, Theodorou et al. 2005), altered motor co-ordination of trunk muscles (Mientjes and Frank 1999; van Dieen, Cholewicki et al. 2003; van Dieen, Selen et al. 2003), and deficits in postural control (Radebold, Cholewicki et al. 2001) as observed in low back pain patients. Individuals with recurrent back pain often show a reduced (Danneels, Coorevits et al. 2002) or delayed activity (Hodges and Richardson 1996; Leinonen, Kankaanpaa et al. 2001; MacDonald, Moseley et al. 2009) in deep muscles. Kaigle et al. (Kaigle, Wessberg et al. 1998) recorded electromyogram (EMG) activity in the erector spinae muscles during flexion-extension in chronic low back pain patients and found that their ability to flex and extend the trunk was limited compared to healthy subjects.

Morphological changes in muscles in low back pain patients

Morphological changes in the paraspinal muscles have been observed in low back pain patients. Histological investigations have shown changes in distribution of muscle fibre type and in reduction of muscle size (Hides, Stokes et al. 1994; Mannion 1999; Mannion, Kaser et al. 2000) as well as infiltration of fat into the muscles (Mengiardi, Schmid et al. 2006). Similar histological changes have also been found in paraspinal muscles after intervertebral disc herniation (Dangaria and Naesh 1998; Yoshihara, Shirai et al. 2001).

2.3 Experimental studies of low back pain and disc herniation

Some models are available in which one can experimentally induce low back pain in healthy human subjects or in laboratory animals. Experimentally induced pain lasting for minutes or hours in healthy subjects, or days or weeks in animals, is obviously not the same as chronic pain that may last for months or years in patients. However, because of the many confounders affecting clinical research, such experimental models are nonetheless used frequently as a valuable complement.

Experimentally induced low back pain

In healthy human subjects, dull, acute deep back pain can be induced by an intramuscular injection of hypertonic saline. This experimentally induced back pain has been shown to alter patterns of voluntary movement (Arendt-Nielsen, Graven-Nielsen et al. 1996; Zedka, Prochazka et al. 1999) similar to that observed in low back pain patients (Radebold, Cholewicki et al. 2001; van Dieen, Cholewicki et al. 2003; Nelson-Wong, Alex et al. 2012) and to result in an absence of muscle relaxation in back muscles at full flexion (Zedka, Prochazka et al. 1999), as also observed in low back pain patients (Kaigle, Wessberg et al. 1998). Furthermore, alterations of the recruitment pattern of back muscles observed in low back pain patients (Silfies, Squillante et al. 2005) are also observed after experimentally induced low back pain (Hodges, Moseley et al. 2003). The advantage of experimentally inducing low back pain is that both pain intensity and location can be controlled and, through selective injection of hypertonic saline into a chosen muscle, the effect of the specific origin of pain on e.g. motor patterns or motor control can be determined. Hypertonic saline can also be injected into the lumbar intervertebral ligaments (Sinclair, Feindel et al. 1948). The difficulty lies in accessing the spinal ligaments, but when injected with high

accuracy by e.g. using radiography, a localized back pain can be induced. It has been suggested that injection of hypertonic saline into the interspinal ligament induces a more intense low back pain of longer duration than when injected into the back muscles (Tsao, Tucker et al. 2010).

Animal models for low back pain

There are few experimental animal models for the pathology of low back pain. The main limitation of most of the experimental models in use is the unknown outcome regarding whether or not they will actually induce low back pain. One example is the experimentally induced disc herniation model in the rat (Olmarker, Iwabuchi et al. 1998). The model include one or both of nucleus pulposus leakage and mechanical compression to a lumbar DRG and the animals have been shown to develop a lowered threshold to mechanical stimuli to the hind paw on the herniated side over time (Omarker and Myers 1998) These changes can generally be regarded as induced radiculopathy, since similar changes develop in models of neuropathic pain—including sciatic nerve ligation (Kim and Chung 1992) and crush injuries (Bester, Beggs et al. 2000). However, whether or not low back pain can be induced by the disc herniation model is not known but more general changes in behaviour (such as less moving around) have been observed (Olmarker, Storkson et al. 2002).

Some animal models have been used for investigation of intact neuronal networks that might be involved in low back pain. Miyagi et al. (Miyagi, Ishikawa et al. 2011) found that injecting paraformaldehyde into the rat multifidus muscle altered gait parameters and Indahl et al. (Indahl, Kaigle et al. 1995) found myoelectric activity in the multifidus muscle following electrical stimulation of porcine annulus fibrosus. A porcine disc damage model in which the L3–L4 intervertebral disc is lesioned results in a deranged disc (Kaigle, Holm et al. 1997) and induces atrophy of the multifidus muscle (Hodges, Holm et al. 2006) within days. A subsequent study in which Hodges et al. (Hodges, Galea et al. 2009) stimulated the porcine motor cortex following this intervertebral disc damage resulted in rapid changes in motor cortex excitability, which were most prominent in the area representing the paraspinal muscles at the injured segment.

However, most of the animal research is performed to study very specific parts of the neuronal networks that might be involved in low back pain. In order to study neuronal effects resulting from a disc herniation, changes in cellular electrical properties in the DRG (Takebayashi, Cavanaugh et al. 2001; Chen, Cavanaugh et al. 2004; Kallakuri, Takebayashi et al. 2005) and spinal cord (Anzai, Hamba et al. 2002; Cuellar, Montesano et al. 2004) have been investigated following application of nucleus pulposus to a nerve root. However, there have been few observations on changes in thalamic firing in

relation to low back pain. In a previous study by Brisby and Hammar (Brisby and Hammar 2007), it was found that application of nucleus pulposus to the L4 DRG induced an increase in evoked neuronal activity within minutes in the rat VPL. The study was, however, comprised of acute experiments and effects of a longer exposure time to nucleus pulposus and subsequent mechanical compression were not investigated.

3 AIMS

3.1 General aims

The general aim of this thesis was to perform experimental studies of neuronal networks that may be involved in low back pain, including ascending and descending tracts and their target neurones.

3.2 Specific aims

More specifically, the aims were to investigate:

1. the contribution of pyramidal tract and reticulospinal neurones to the control of erector spinae motoneurones.
2. whether the corticospinal actions on erector spinae motoneurones are relayed by reticulospinal neurones.
3. the effects on evoked thalamic neuronal activity following light mechanical compression and application of nucleus pulposus to the DRG.
4. whether a previous application of nucleus pulposus would sensitise the DRG to mechanical compression or a second exposure.
5. effects on evoked thalamic neuronal activity following application of two nucleus pulposus-derived cell populations to the DRG.

4 MATERIAL AND METHODS

All experiments in this thesis were carried out at the Laboratory for Experimental Medicine at the University of Gothenburg, with ethical permission from the local animal ethics committee (Göteborgs djurförsöksetiska nämnd). The animals used were female and male adult cats and female adult Sprague-Dawley rats.

The experiments were carried out on anaesthetised animals with the heart rate continuously monitored via subcutaneous electrodes. During all recordings, neuromuscular transmission was blocked by intravenous injection of pancronium bromide (0.2 mg/kg/h for cats and 0.3 mg/kg for rats; Pavulon, Organon, the Netherlands) and the animals were artificially ventilated. Core body temperature was kept close to 37ºC with servo-controlled lamps. For the cat experiments, end-tidal concentration of CO_2 was kept at 4% by changing the artificial ventilation during the experiment. All experiments were terminated with a lethal dose of sodium pentobarbital intravenously (APL, Sweden).

4.1 Surgical procedures

4.1.1 Paper I

In paper I, a total of 8 anaesthetised cats were used for the experiments. Anaesthesia was induced with an intraperitoneal injection of sodium pentobarbital (40–44 mg/kg) and when motor reactions were evoked during dissection, α-chloralose was administered intravenously (in doses of 5 mg/kg, Rhône-Poulenc Santé, France) until the cat was fully relaxed and with a blood pressure of slightly below 140 mm/Hg. Usually, several doses of α-chloralose were given during the first hour of surgery and thereafter every 1–3 h to maintain the depth of anaesthesia throughout the experiment.

Catheters were inserted into the left and right cephalic veins for intravenous injections and into the right common iliac artery for blood pressure surveillance and continuous infusion of bicarbonate buffer solution with 5% glucose (1–2 ml/h/kg). A tube was inserted in the trachea and connected to a respirator. Laminectomies uncovered the spinal cord in the area of C3–C4 and Th10–Th11 for transdural recordings when positioning the stimulation electrodes in the brain stem, and for recording descending volleys. Laminectomy was also performed at L1–L4 to expose the site of intracellular recordings from motoneurones. The intermediate branches of dorsal rami of the spinal nerves innervating m. longissimus lumborum were exposed bilaterally at the L2 and L3 levels, cut, ligated, and mounted on

stimulating electrodes in a pool of paraffin oil. A craniotomy was performed over the caudal part of the cerebellum and small holes in the dura and pia were made for insertion of stimulation electrodes.

Tungsten electrodes were positioned in the left and/or right pyramids and in the left or right medial longitudinal fascicle (MLF). The original targets were at Horsley-Clarke coordinates Posterior 5 mm, Left or Right 1.4 mm, and Horizontal -10 mm for the left and right pyramids and Posterior 10 mm, Left or Right 0.5 mm, and Horizontal -5 mm for the MLF. The final positions were adjusted, however, based on the descending volleys from the spinal cord surface. The electrodes were left at depths where descending volleys were evoked at stimulus intensities of 10–20 μA or less.

Glass micropipettes with the tip broken to about 1.5 μm and filled with 2 M potassium citrate solution were used for intracellular recordings from motoneurones. The cells were tracked through small holes in the dura and pia mater at L1–L4.

4.1.2 Papers II and III

Altogether, 119 rats were used for the experiments in this thesis, 65 for paper II and 54 for paper III.

In paper II, the application of nucleus pulposus to the DRG 24 hours before the electrophysiological experiment was achieved by disc puncture surgery. The animals were anaesthetised with isoflurane (Baxter Medical AB, Sweden). Following a midline incision, the left facet joint between the fourth and fifth lumbar vertebrae was removed to expose both the L4 DRG and the intervertebral disc. A syringe connected to a 23G injection needle was used to incise the disc and inject small volumes of air until nucleus pulposus leaked out and could be applied onto the adjacent DRG. The control rats were sham-operated, i.e. subjected to the same surgical procedure except for the disc incision. Buprenorphine (Temgesic, 60 μg/kg, Shering-Plough, Belgium) was given intramuscularly as postoperative pain relief and the animals were allowed to wake up and recover in their cages for 24 hours before the acute electrophysiological experiment.

Acute electrophysiological experiments were performed in naïve rats in both paper II and III, and in paper II 24 hours after the experimental disc puncture. Anaesthesia was induced with a mixture of fentanyl (Leptanal, 272 μg/kg; Janssen-Cilag AB, Sweden) and medetomidine hydrochloride (DomitorVet, 545 μg/kg; Orion Pharma, Finland) intraperitoneally and maintained by intermittent intravenous administration of α-chloralose (dose 5 mg/kg for a total of 30 mg/kg). Atropine (0.5 mg/kg subcutaneously; Mylan AB, Sweden) was given during the preliminary dissection to limit mucus secretion in the respiratory tract.

A catheter for intravenous drug administration was inserted in the jugular vein and a tube was inserted into the trachea and connected to a respirator.

The left sciatic nerve was cut proximal to the trifurcation at knee level and mounted on a pair of silver hook stimulating electrodes in a paraffin pool created by skin flaps. The left L4 DRG was exposed in naïve rats and re-exposed in the previously operated animals in paper II. A laminectomy was performed at Th11–12 exposing the spinal cord for transdural cord dorsum records of ascending volleys. A craniotomy was done and the dura was removed for electrode insertion into the thalamus.

4.2 Electrophysiological experiments

4.2.1 Paper I

The medial branch of the dorsal ramus of the L2 and L3 spinal nerves was stimulated with constant voltage cathodal stimuli (0.2 ms duration) at intensities of 2–5 times the threshold intensity for the most sensitive fibres in the nerve as determined with cord dorsum potentials. Motoneurones innervating the m. longissimus were identified by antidromic activation following stimulation of the dissected nerves. Fibres of the reticulospinal and pyramidal tracts were activated by applying constant current cathodal stimuli (0.2 ms duration, 25–100 μA). The stimuli were applied as single stimuli (at about 3 Hz) or in trains of 2–6 stimuli at 300 or 400 Hz (delivered at about 3 Hz). Latencies and incidences of postsynaptic potentials (PSPs) evoked from pyramids and MLF were investigated in these motoneurones. To elucidate possible contributions of reticulospinal versus pyramidal tract input to motoneurones, MLF lesion and spinal cord hemisection was performed during the experiments.

Intracellular recordings were made from 50 longissimus lumborum motoneurones while stimulating the ipsi- and/or contralateral pyramidal tracts and ipsilateral or contralateral MLF. Baseline records were sampled from the motoneurones while stimulating pyramidal tract neurones and neurones of the MLF. Thereafter, an intervention was done; either a lesion was made in the MLF to prevent actions mediated by reticulospinal fibres or a spinal hemisection was made to prevent actions of both the pyramidal tract and reticulospinal tract neurones on longissimus motoneurones. The MLF fibres were transected a few mm rostral to the obex and a few mm caudal to the electrodes in the MLF and pyramids. Hemisections were performed in the 3rd cervical segment. The extents of all lesions were histologically verified after the experiments.

4.2.2 Papers II and III

The sciatic nerve was stimulated using short trains of impulses (3 stimuli, 0.2 ms duration, 400 Hz, delivered at intervals of 2 Hz) at intensities 2–50 times threshold intensity for the most sensitive fibres in the nerve. A glass micropipette with the tip broken to 2–2.5 μm and filled with 2 M NaCl was positioned in the ventral posterior lateral (VPL) nucleus of the thalamus (target measured in mm from Bregma: Posterior -2.5–3.5, Lateral 3.0, Horizontal -6) where low-intensity sciatic stimulation (2 T) only evoked scarce neuronal response while higher-intensity stimulation (20-50 T) evoked maximal responses (corresponding to Aβ and Aβ together with activation of Aδ fibres but excluding the higher-threshold and slower conducting C-fibres) (Jack 1978; Steffens, Dibaj et al. 2012).

All experiments began by sampling a series of baseline records from the contralateral VPL while stimulating the ipsilateral sciatic nerve at above Aδ fibre thresholds. The mean number of responses evoked was set to 100%. Thereafter, the left L4 DRG was subjected to one of the interventions (see below) and any changes in the mean number of evoked responses in the VPL following the intervention were evaluated over time.

In paper II, light mechanical compression of the DRG was induced by gently dislocating the L4 DRG, and nucleus pulposus harvested from a donor rat was applied to the DRG. In the previously disc punctured rats the DRG was in this case re-exposed to nucleus pulposus. The aims in paper II were addressed in four different series of experiments (see Figure 1 in paper II). In the first series using naïve animals, records were first sampled during light mechanical compression followed by application of nucleus pulposus while the mechanical compression either remained or was removed. In the second series of acute experiments, nucleus pulposus from donor rats was applied to the L4 DRG 24 hours after either initial disc puncture or sham surgery. In the third series, light mechanical compression of the DRG was induced 24 hours after initial disc puncture or sham surgery followed by application of nucleus pulposus. In the behavioural experiments changes in mechanical withdrawal threshold were measured before and 24 hours after initial disc puncture or sham surgery.

In paper III, changes in evoked activity were investigated after exposure of the L4 DRG to a suspension containing one or both of the two cell populations derived from rat nucleus pulposus. Notochordal cells and chondrocyte-like cells were applied in different amounts, either separately or in combination. Cell suspension medium alone was used as control.

4.3 Anaesthesia

In order to carry out surgical procedures on animals, perception of pain must be completely suppressed. At the same time, a drug that inhibits the perception of pain must in some way alter the function of neuronal systems. Much of our knowledge about neuronal pathways is therefore a result of experiments in anaesthetised animals, and none of the results presented in this thesis—except for the behaviour tests in paper II, were obtained from conscious animals.

The initial surgery was undertaken after injection of either sodium pentobarbital in paper I, or of a mixture of fentanyl and medetomidine hydrochloride in papers II and III; all electrophysiological recordings were subsequently sampled during α-chloralose anaesthesia.

In paper I, sodium pentobarbital was given as a bolus dose to initiate anaesthesia. It is a barbiturate that depresses activity in the central nervous system by increasing neuronal responses to γ-aminobutyric acid (GABA) (Study and Barker 1981). In cats the half-life of sodium pentobarbital has been estimated to be 5–7 hours (Wagner, Weidler et al. 1977). The surgical procedures and preparations preceding the recordings in paper I took approximately 6–8 hours, resulting in a reduction to at least half the amount of sodium pentobarbital in the cat before recordings started.

In papers II and III, a mixture of fentanyl and medetomidine hydrochloride was given as a bolus dose to initiate the anaesthesia. Because of the mixture, it is more difficult to predict the duration of the effect, especially as medetomidine hydrochloride can be potentiated by other analgesics (www.FASS.se 2012). The half-life of medetomidine hydrochloride may be as short as one hour in cats and dogs (www.FASS.se 2012). Fentanyl, however, has a half-life of about 7 hours in humans (www.FASS.se 2012). From the experience of using this mixture in papers II and III, the deep sedative effect in rats does not last longer than an hour. Thus, the sedative effects of the mixture are likely to be markedly reduced and replaced by effects of α-chloralose at the time of the electrophysiological recordings, which were sampled after about 2 hours of initial surgery and preparation.

The anaesthetic α-chloralose, is a hypnotic agent commonly used in electrophysiological experiments. The mechanism of action is believed to be through binding to the γ-aminobutyric acid type A ($GABA_A$) receptor complex (Garrett and Gan 1998). Little is known about the pharmacokinetics of α-chloralose, but the effect is known to last for hours (Collins, Kawahara et al. 1983). Several studies have suggested that while alpha-chloralose depresses both spontaneous and evoked neuronal activity, it does so to a lesser extent than a number of other anaesthetics, in particular barbiturates

(Shimamura, Yamauchi et al. 1968; Dudley, Nelson et al. 1982; Collins, Kawahara et al. 1983; Hartell and Headley 1990), and α-chloralose has therefore been widely used for neurophysiological investigations.

4.4 Statistical analyses

In paper I, differences between samples were assessed for statistical significance using Student's t-test (for two samples assuming equal variances). In papers II and III, Kruskal-Wallis test was used to compare electrophysiological recordings at different time points between groups and paired t-test was used to compare changes in the number of evoked responses between time points within the individual groups. Behavioural test data were analysed with paired-samples t-test before and after intervention surgery for both the left and right hind paw, and for differences between groups with Mann-Whitney test. P-values less than 0.05 were considered statistically significant.

In paper I, statistical analysis was performed with parametric tests because of the estimation of normal distribution of the data. Non-parametric tests were chosen for papers II and III since the samples were small; thus, it was difficult to estimate if the data showed normal distribution.

4.5 Von Frey test

In paper II, two groups of animals were used to compare changes in mechanical paw withdrawal responses between previously disc-punctured (n = 10) and sham-operated animals (n = 10). Unfortunately, one rat in the sham-operated group did not recover from surgery (giving n = 9). The withdrawal responses were measured with von Frey filaments (North Coast Medical Inc., CA, USA) at three occasions before and one day after surgery, corresponding in time with the acute electrophysiological experiments in the study. Testing took place in a plexiglass cage with a metal-mesh floor containing 6-mm holes. After accommodation, von Frey monofilaments were applied, alternating between left and right hind paw in order of increasing stiffness starting with 3.61, followed by 3.84, 4.08, 4.17, 4.31, 4.56, 4.74, 4.93, 5.07 and 5.18 (corresponding to 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0 and 15.0 g) until the filaments bent slightly. A positive withdrawal was scored when the animal responded to two out of three stimuli presented, and the investigator was blinded to which operation the animals had been subjected to.

4.6 Cell sorting

Cell sorting of trypsin-digested nucleus pulposus by flow cytometry was performed in paper III, in the series of experiments aimed at investigating evoked thalamic activity following DRG application to the inherent cell populations of nucleus pulposus. Donor animals were adult female Sprague-Dawley rats terminated with a lethal dose of sodium pentobarbital. Nucleus pulposus was harvested from approximately 13 tail discs in each animal and pooled in cell suspension medium. Two animals were sacrificed at each cell-sorting episode.

Fluorescence-activated cell sorting (FACS) is a technique by which characteristics of single cells can be measured based on their light-scattering and diffracting properties. The side scatter (SSC) is related to granularity and the complexity of cellular organelles, including the cell membrane and nucleus, while forward angle scatter (FSC) is related to cell surface area or size. Thus, cells of different sizes or with different morphological characteristics will show different scatter patterns and can therefore be sorted into different fractions.

Nucleus pulposus contains at least two different cell populations, chondrocyte-like cells (diameter about 17–23 µm) and notochordal cells (diameter about 25–85 µm) (Trout, Buckwalter et al. 1982; Hunter, Matyas et al. 2003; Hunter, Matyas et al. 2004; Chen, Yan et al. 2006). In paper III, it was assumed that the annulus fibrosus cells are similar in size and granularity to the chondrocyte-like cells in nucleus pulposus. The FSCs for rat annulus fibrosus cells were therefore used to define the first gate for the smaller cells of nucleus pulposus while a second gate was created for the notochordal cells with a gap in-between the gates to avoid overlap between the two cell populations (Larsson, Brisby et al. 2011; Larsson, Runesson et al. 2011).

4.7 Methodological considerations regarding paper II

4.7.1 Anatomical considerations regarding the sciatic nerve

In papers II and III, the left L4 DRGs in Sprague-Dawley rats were exposed to mechanical dislocation, nucleus pulposus, or cell suspension while applying electrical stimulation of the left sciatic nerve—in order to study changes in evoked neuronal activity in the contralateral VPL mediated by this DRG. The sciatic nerve in humans contains sensory fibres foremost from the L4–S1 spinal nerves (Martini 2003), and lumbar disc hernia is most

commonly found at the level of L4–L5 and L5–S1 (Kortelainen, Puranen et al. 1985; Jonsson and Stromqvist 1996). In contrast, anatomical investigations have shown that the Sprague-Dawley rat sciatic nerve consists of components from L3–L6 spinal nerves (Asato, Butler et al. 2000). The major components are L4 and L5, which can receive up to 98% of the sciatic sensory fibres (Swett, Torigoe et al. 1991). Experimental studies in the rat have revealed that nucleus pulposus application and mechanical compression limited to a single L4 DRG can induce behavioural changes over time (Omarker and Myers 1998; Olmarker, Storkson et al. 2002; Sasaki, Sekiguchi et al. 2011) indicating an experimentally generated radiating sciatic pain induced at the L4 level.

4.7.2 Mechanical compression

Several investigators have shown that acute mechanical compression affects nerve conduction capacity, but comparisons between the results are limited as the methods of compression and choice of animal have varied greatly. However, most studies have shown negative effects in terms of nerve function following compression. Howe et al. (Howe, Loeser et al. 1977) demonstrated that light mechanical compression onto a feline DRG by a 5-g weight could produce spontaneous repetitive firing for several minutes but that the firing then ceased. The investigators also observed a reduction in evoked compound action potential, i.e. a reduction in the total number of action potentials propagating in the nerve, following the initiation of compression. Yayama et al. (Yayama, Kobayashi et al. 2010) demonstrated that a gradually increased mechanical compression applied with a pressure transducer results in a gradually reduced compound action potential in the sciatic nerve of the rabbit. Nerve conduction velocity is also negatively affected following compression. A continuous reduction in nerve conduction in the feline popliteal nerve developed into a complete block within 2–3 hours at compressions of 130–200 mmHg (Bentley and Schlapp 1943). Mechanical compressions at 200–400 mmHg of the rabbit tibial nerve resulted in a gradual decrease within hours while light compression (50 mmHg) had only minimal effects (Rydevik and Nordborg 1980).

4.7.3 Application of nucleus pulposus

There are only a few investigations regarding the neurophysiology of acute DRG exposure to nucleus pulposus. Changes in electrical activity have, however, been observed in the DRG and spinal cord in rats after application of nucleus pulposus on the same DRG or adjacent nerve root. Takebayashi et al. (Takebayashi, Cavanaugh et al. 2001) observed a gradually increased spontaneous activity over the 6 hours of investigation compared to the

control group with fat applied on the DRG. Kallakuri et al. (Kallakuri, Takebayashi et al. 2005) found a similar increase compared to naïve animals, but in contrast to Takebayashi et al. (Takebayashi, Cavanaugh et al. 2001), they did not find the responses statistically different from the control groups with animals either sham-operated or with fat applied on the DRG.

When investigating acute responses in the dorsal horn of the spinal cord after application of nucleus pulposus to the DRG or nerve root, Anzai et al. (Anzai, Hamba et al. 2002) found increased neuronal responses to noxious stimuli on the foot after individually applied either nucleus pulposus or fat while Cuellar et al. (Cuellar, Montesano et al. 2004) only observed these increased neuronal response after application of nucleus pulposus.

Acute neuronal effects observed supraspinally, i.e. in the thalamic VPL in response to sciatic nerve stimulation after nucleus pulposus application to a DRG, was previously demonstrated by Brisby and Hammar (Brisby and Hammar 2007). Interestingly, they showed an increase in evoked neuronal activity in the rat VPL minutes after application of nucleus pulposus, but with counteracting effect when fat was applied.

4.7.4 Von Frey test

The von Frey test on rodents is regarded as a standard nociceptive test (Barrot 2012) where the expected and evaluated response is paw withdrawal. It can however be debated whether the threshold response is a sensory detection response or a nociceptive withdrawal response since von Frey filaments have the disadvantage of activating low threshold mechanoreceptors as well as nociceptors (Le Bars, Gozariu et al. 2001). However, in experimental situations following surgery presumed to result in affected pain perception, withdrawal responses can be observed after stimulation with a filament that did not elicit any response either before the surgical intervention or in the control group. The lowered mechanical thresholds might therefore be considered as mechanical allodynia which has developed after the surgical intervention. Thresholds for mechanical withdrawal with von Frey filament testing in rodents can depend upon the protocol and type of filaments used (Barrot 2012). It is therefore to be expected that a variability in both baseline and detected mechanical threshold responses may occur between investigators when investigating for example experimental disc herniation in rats; for example see (Omarker and Myers 1998; Suzuki, Inoue et al. 2009; Sasaki, Sekiguchi et al. 2011).

5 RESULTS

5.1 Paper I

5.1.1 Pyramidal tract stimulation with the spinal cord intact

Stimuli applied to both the ipsilateral and the contralateral pyramidal tract evoked responses in most of the longissimus lumborum motoneurones that were recorded from. The responses were excitatory postsynaptic potentials (EPSPs) and/or inhibitory postsynaptic potentials (IPSPs). The postsynaptic potentials (PSPs) were evoked by successive stimuli in a similar way from both ipsilateral and contralateral pyramidal tracts (Figure 3). The PSPs were most often evoked after the third or the fourth stimuli in a train, and rarely after the second or first. The mean latencies were more than 4 ms for EPSPs and more than 5 ms for IPSPs from the third or fourth stimuli.

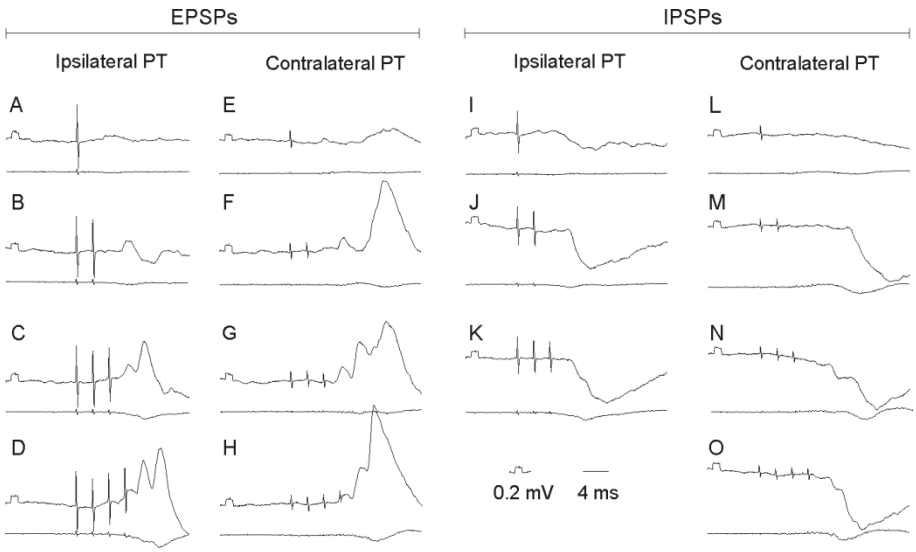

Figure 3. Examples of EPSPs (A-H) and IPSPs (I-O) evoked from the ipsilateral and contralateral pyramidal tracts (PT) stimulated at 100 μA. Upper traces are intracellular records from motoneurones with the spinal cord intact. The lower traces are simultaneously obtained cord dorsum potentials. Rectangular pulses at the beginning of the intracellular records are calibration pulses (0.2 mV). Modified from Figure 3 and Figure 5 of paper I (Galea, Hammar et al. 2010).

The mean latencies for EPSPs resemble the latencies of disynaptic EPSPs evoked in hindlimb motoneurones (Jankowska and Stecina 2007; Stecina and Jankowska 2007) but are shorter than trisynaptic EPSPs evoked by ipsilateral pyramidal tract stimulation (Edgley, Jankowska et al. 2004). The conclusion was therefore drawn that the shortest excitatory pathway between pyramidal tract neurons and back motoneurones is disynaptic or trisynaptic. The latencies of IPSPs were found to be one ms longer indicating that the earliest IPSPs are evoked from the pyramidal tract neurones via trisynaptic pathways.

5.1.2 MLF stimulation with the spinal cord intact

Stimuli applied to both the ipsilateral and the contralateral medial longitudinal fascicle (MLF) evoked responses in most of the longissimus lumborum motoneurones that were recorded from. In contrast to the responses evoked by pyramidal tract stimulation, EPSPs from MLF were often evoked by the first stimulus and at a mean latency of less than 1 ms, indicating a monosynaptic coupling with the motoneurones. In some cases later components followed these EPSPs. The later components showed characteristics of being evoked at least disynaptically. They could be mediated via a spinal cord interneurone but might also be evoked through direct connections between reticulospinal neurones and motoneurones if some reticulospinal neurones were activated by recurrent axon collaterals of reticulospinal tract fibres stimulated in the MLF (Matsuyama, Mori et al. 1999; Edgley, Jankowska et al. 2004). Actions of directly activated reticulospinal tract fibres would then be followed by actions of indirectly activated reticulospinal neurones. In contrast to the earliest EPSPs, the later components usually required more than a single stimulus to appear. IPSPs were also evoked by MLF stimulation; they often appeared following EPSPs and with slightly longer latencies similar to the latencies of the later components of the EPSP. No directly evoked IPSPs of MLF origin have been found in lumbar segments (Grillner, Hongo et al. 1968; Peterson, Pitts et al. 1979; Stecina and Jankowska 2007) indicating that all inhibitory responses on spinal motoneurones are mediated via inhibitory interneurons in the spinal cord.

5.1.3 Pyramidal tract stimulation after MLF lesion

A lesion of the MLF was performed to investigate the contribution of the reticulospinal neurones as relay neurones of pyramidal tract actions. The EPSPs evoked by pyramidal tract stimulation before the MLF lesion disappeared, and stimulation of either the ipsilateral or of the contralateral pyramidal tract evoked only IPSPs (Figure 4) following the lesion. The

remaining IPSPS were evoked by the 3rd–5th stimuli at latencies of above 6ms.

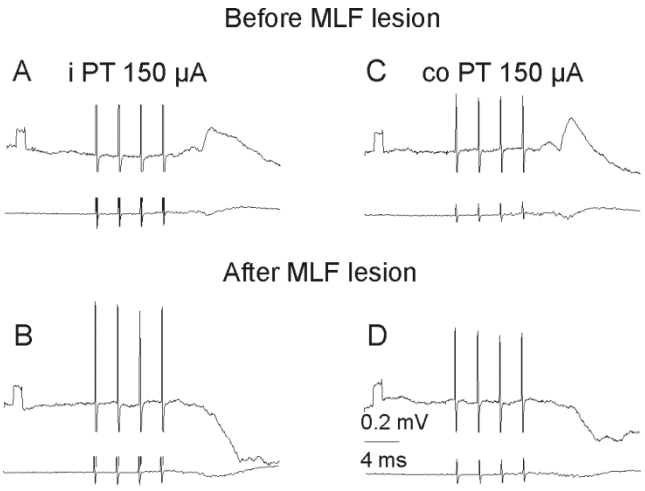

Figure 4. Effects of MLF lesion on synaptic actions evoked by pyramidal tract (PT) stimulation. Records from two motoneurones, where A and C are from before while B and D are after MLF lesion. The upper traces are intracellular records from motoneurones and the lower traces are cord dorsum potentials obtained at the same time. The rectangular pulses at the beginning of the intracellular records are calibration pulses (0.2 mV). Modified from Figure 7 of paper I (Galea, Hammar et al. 2010).

5.1.4 Pyramidal tract stimulation after lesions of the spinal cord

Lesions in the ipsilateral or contralateral white matter of the spinal cord were made to investigate the contribution of descending fibres from both the ipsilateral and contralateral pyramidal tracts and also reticulospinal neurones. The effects of spinal cord lesions on the back motoneurones following a contralateral hemisection were minor. The effects following ipsilateral hemisection were, however, more pronounced and the PSPs evoked from both the contralateral and the ipsilateral pyramidal tracts required longer trains of stimuli to reach the same amplitudes, i.e. for the same degree of effectiveness. Monosynaptic responses evoked from MLF stimulation disappeared and only small disynaptic PSPs appeared.

5.2 Papers II and III

5.2.1 Evoked thalamic responses

Light mechanical compression of the DRG resulted in a reduced number of evoked responses in the ventral posterior lateral (VPL) nucleus of the thalamus in all of the groups tested (Figure 5), but the time course differed. In naïve animals, the decrease was statistically significant within 10 minutes while compared to naïve animals, the onset of effect in both previously disc-punctured and sham-operated animals was delayed. In the group of previously disc-punctured animals, there was a statistically significant decrease following 10–20 minutes of mechanical compression while for the sham-operated group, the significant decrease developed even more slowly and was reached after 40 minutes of mechanical compression, including the first 10 minutes of the subsequent exposure to nucleus pulposus.

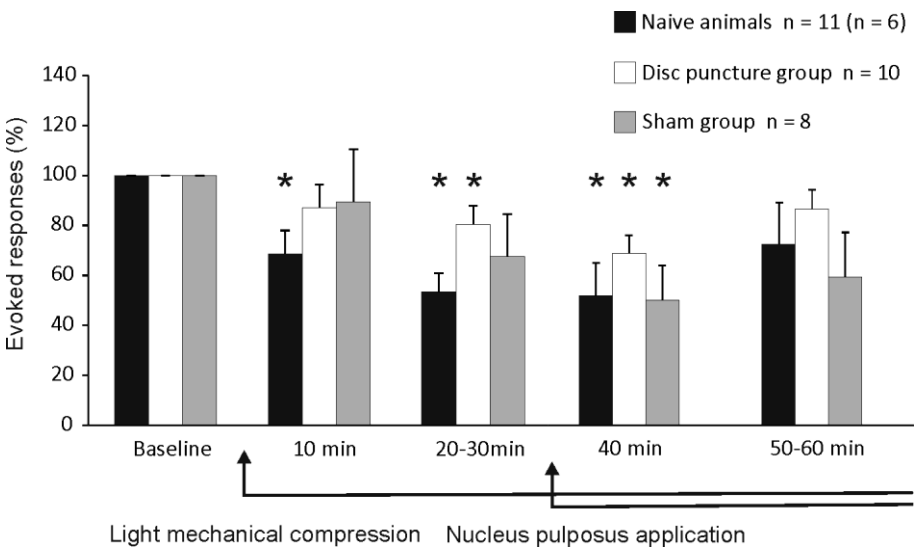

*Figure 5. Effects of mechanical compression and subsequent application of nucleus pulposus. The mean number of responses evoked in the contralateral VPL as presented as percentage of baseline records. The data presented was collected after mechanical compression in naïve or previously disc-punctured or sham-operated animals. In the naïve animal group, n =11 until the application of nucleus pulposus — when n = 6, since in 5 animals the mechanical compression was removed (data not shown, but see Figure 3 of paper II). Mean ± SEM. *p < 0.05.*

The addition of nucleus pulposus after mechanical compression resulted in an increased number of evoked responses, i.e. the compression-induced decrease was no longer significantly changed from baseline, in all the groups tested (Figure 5). The response increase occurred most rapidly in the group in which the mechanical compression was removed at the time of application of nucleus pulposus (n = 5; data not shown, but see Figure 3 in paper II).

Nucleus pulposus application without any involvement of mechanical compression failed to induce significant changes in evoked responses compared to baseline, in both previously disc-punctured animals and sham-operated animals (Figure 6). In sham-operated animals with DRG application of nucleus pulposus for the first time, the mean number of evoked responses tended to increase and was significantly different from that in animals in the disc-puncture group after 40 minutes. However, we did not observe the rapid increase after application of nucleus pulposus that was previously reported in naïve animals by Brisby and Hammar (Brisby and Hammar 2007).

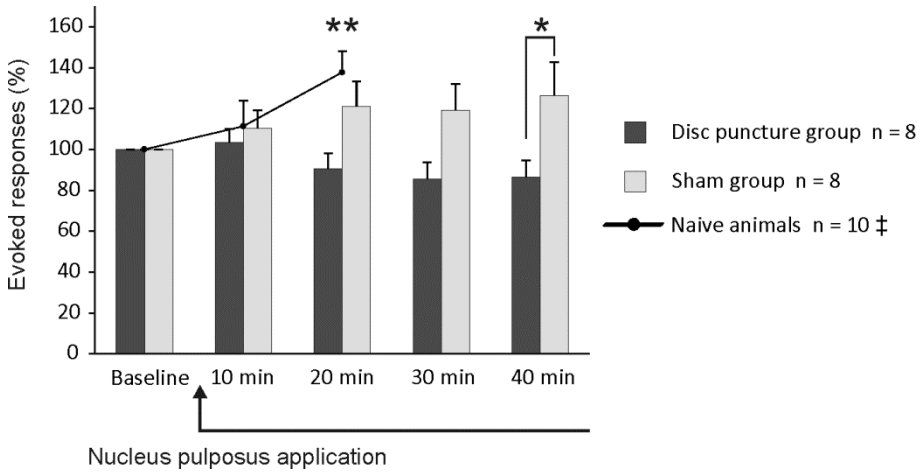

*Figure 6. Effects of nucleus pulposus application. Mean number of evoked responses in the contralateral VPL, expressed as percentage of baseline records. The data presented were from previously disc-punctured or sham-operated animals. ‡Previously published results from application of nucleus pulposus in naïve animals are also presented for comparison (Brisby and Hammar 2007). Mean ± SEM. *p < 0.05, **p < 0.01.*

Application of 25,000 notochordal cells to the DRG resulted in a statistically significant decrease in evoked thalamic activity within 10 minutes (Figure 7), which lasted throughout the 40 minutes of recording. In contrast, application of 25,000 chondrocyte-like cells did not evoke any statistically significant changes in thalamic activity during the 40 minutes of recording. However,

when comparing the mean number of evoked thalamic responses after 40 minutes, a statistically significant difference between notochordal and chondrocyte-like cells was apparent. However, none of the individually tested populations induced an increase in evoked responses similar to those previously observed (Brisby and Hammar 2007).

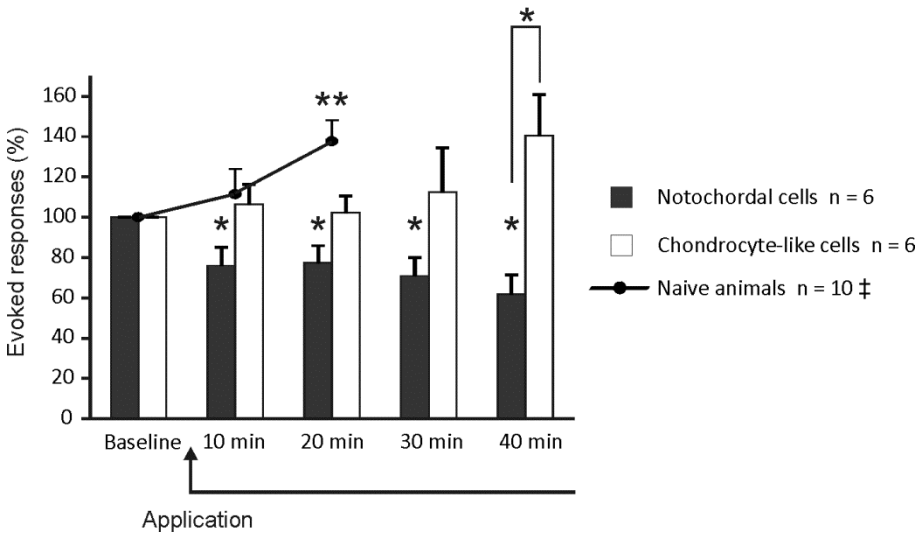

*Figure 7. Effects of 25,000 notochordal and chondrocyte-like cells individually applied to the DRG. The mean number of evoked responses is expressed as percentage of baseline records. ‡Previously published results from application of nucleus pulposus in naïve animals are also presented for comparison (Brisby and Hammar 2007). Mean ± SEM. *p < 0.05, **p < 0.01.*

Application of the higher amount of 150,000 chondrocyte-like cells did not show a demonstrable responsiveness similar to the lower amount of such cells on the DRG. Application of 30,000 cells in a combination of both notochordal and chondrocyte-like cells to the DRG (1,500 notochordal cells and 28,500 chondrocyte-like cells in the ratio 1/20) did not induce any changes in evoked thalamic activity during 40 minutes of recording. A summary of effects on evoked thalamic responses observed after the different interventions on the DRG is given in Table 1. Cell suspension medium alone was not shown to induce any changes in evoked thalamic activity (Figure 1 of paper III).

*Table 1. Responses in evoked thalamic neuronal activity at the end of recordings. ‡ Results previously published by Brisby and Hammar (Brisby and Hammar 2007). *The mean evoked responses recorded in sham-operated animals were significantly different from the disc-punctured group at 40 minutes after application of nucleus pulposus (NP) but there was no significant difference from baseline during this time (see Discussion). N/A, not applicable, i.e. not tested.*

Intervention on L4 DRG	Animal intervention		
	Naïve	24 hours NP	24 hours sham
Light mechanical compression	↓	↓	↓
Nucleus pulposus	↑‡	–	(↓)*
Nucleus pulposus application while mechanical compression still in situ	↑	↑	↑
25,000 Notochordal cells	↓	N/A	N/A
25,000 Chondrocyte-like cells	–	N/A	N/A
150,000 Chondrocyte-like cells	–	N/A	N/A
The combination (1,500 notochordal + 28,500 chondrocyte-like cells)	–	N/A	N/A

5.2.2 Von Frey withdrawal threshold

No statistically significant changes in between disc-punctured and sham-operated animals or between the left and right hind paws were found. Also, no significant changes in withdrawal threshold were observed 24 hours after surgery compared to the preoperative tests, but mechanical thresholds varied between test occasions and the mean standard errors were considerable during the preoperative tests (Figure 8).

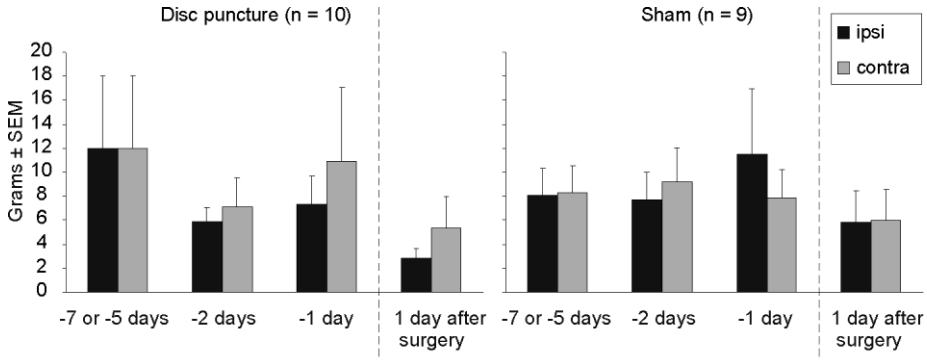

Figure 8. Mechanical thresholds tested with von Frey filaments. The disc-punctured and sham operated groups were tested three times before surgery and on the day after surgical intervention. The data are mean ± SEM.

6 DISCUSSION

Spinal structures such as the intervertebral disc may activate nociceptive fibres, thereby contributing to the experience of low back pain (LBP) (Deyo and Weinstein 2001). Such activation may also attenuate the activity in the low back muscles, i.e. by changes in motor control of these muscles (Kaigle, Wessberg et al. 1998; Zedka, Prochazka et al. 1999; Hodges, Galea et al. 2009). The aim of this thesis was to study the parts of the neuronal networks involved in low back pain, and the investigations were made by evaluating experimentally induced effects in neuronal networks of both the ascending and the descending pathways that may be involved in development and maintenance of low back pain.

6.1 Paper I

6.1.1 The contribution to control of erector spinae motoneurones by pyramidal and reticulospinal tracts neurones

In paper I, we investigated synaptic actions mediated by pyramidal tract and reticulospinal neurones to motoneurones of paraspinal muscles. There are several possible ways in which these spinal muscle motoneurones can be activated, as indicated in Figure 9. However, by comparing latencies, thresholds, and amplitudes of postsynaptic potentials (PSPs) evoked from the ipsilateral and contralateral pyramidal tract and the medial longitudinal fascicle (MLF), one can sort out their contribution in the network.

The fact that several stimuli to the pyramidal tracts were required to evoke a response in the motoneurones and that the amplitude of the response grew with increasing number of stimulations is an indication that two or more synapses are present between the point of stimulation and the evoked response. The majority of pyramidal tract fibres cross the midline and affect the motoneurones on the contralateral side (Brodal 1981; Lemon 2008), and this was also observed in the low back paraspinal muscle motoneurones. Hemisection on the contralateral side of the spinal cord below the level of decussation of most corticospinal fibres induced only minor effects on longissimus motoneurones while a hemisection on the ipsilateral side induced more severe effects. The ipsilateral hemisection—cutting of both the crossed fibres from the contralateral pyramidal tract and the fewer ipsilaterally descending fibres from the ipsilateral pyramidal tract—caused that several more stimuli from the contralateral pyramidal tract neurones were required to

evoke the same response. This illustrates the importance of the crossing pyramidal tract neurones for motor control in erector spinae motoneurones.

Regarding MLF stimulation, the short latencies of less than one ms for the evoked earliest responses together with responses of similar amplitude evoked irrespective of the number of stimulations applied, is an indication of monosynaptic coupling from the reticular formation to back motoneurones.

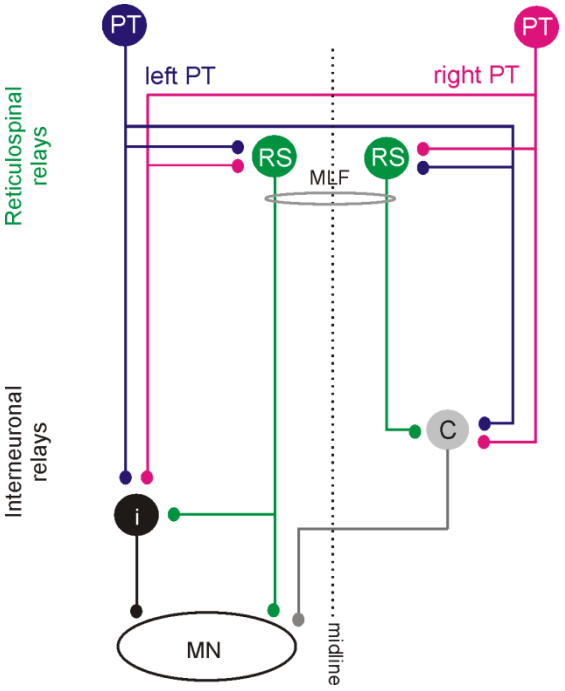

Figure 9. Possible neuronal pathways for innervation of pyramidal tract and reticulospinal neurones to erector spinae motoneurones (MN). Modified from Galea et al. (Galea, Hammar et al. 2010). Blue and red circles and lines represent left- and right-side pyramidal tract (PT) neurones and their projections. Green circles represent reticulospinal (RS) neurones with axons with descending projections within the medial longitudinal fascicle (MLF). Grey and black circles represent spinal target neurones of the pyramidal tract and reticulospinal neurones, with black neurones projecting ipsilaterally (i), and grey neurones contralaterally (c).

6.1.2 Corticospinal excitatory actions on erector spinae motoneurones are relayed by reticulospinal neurones

Reticulospinal neurones can be co-activated by pyramidal tract neurones from both hemispheres (He and Wu 1985; Matsuyama and Drew 1997; Kably and Drew 1998). If activity in pyramidal tract neurones activated from one hemisphere is relayed by reticulospinal neurones, they could mediate excitation of motoneurones on both the left and the right sides of the spinal cord (Jankowska, Hammar et al. 2003; Davidson and Buford 2004; Schepens and Drew 2006). In paper I, excitatory postsynaptic potentials (EPSPs) evoked by pyramidal tract stimulation disappeared following a lesion to the MLF, indicating an unreserved importance of reticulospinal neurones for pyramidal tract excitatory actions on back motoneurones. Inhibitory pyramidal tract actions, on the other hand, appeared to depend on reticulospinal neurones to a lesser extent, as they were still present after MLF lesion. A contribution of spinal interneurones activated more directly by pyramidal tract fibres would therefore be a more likely explanation for the inhibitory effect on the back motoneurones. Thus, excitatory actions on erector spinae motoneurones are mediated by reticulospinal neurones, while a limited proportion may also be mediated by interneurones activated by pyramidal tract neurones and the inhibitory actions of pyramidal tract neurones are not as dependent on the reticulospinal neurones. The fact that a large degree of the pyramidal tract activity was found to activate motoneurones primarily via reticulospinal neurones indicates a high degree of bilaterality of the motor control on the low back muscles.

6.1.3 Possible neuronal network involved in low back pain

While involuntary contraction of muscles surrounding a painful body part in order to immobilize the area can be valuable by itself, a muscle spasm can be a source of pain when the duration is sustained. Although a paraspinal muscle spasm—defined as prolonged involuntary contraction of back muscles—is not a consistent finding in low back pain patients, it has been observed nevertheless (Nouwen and Bush 1984). In such cases, a direct connection has been shown between the back pain experienced and the degree of muscle tension (Nouwen and Solinger 1979).

The cause of paraspinal muscle hyperactivity has been proposed to be a reflex in response to the pain, and experimental evidence for a reflex-spasm theory was published by Pedersen et al. (Pedersen, Blunck et al. 1956) who showed that mechanical stimulation of deep structures of the lower back of

the cat—whether joint, fascia, ligament, or periost—not only causes nociceptive responses but also elicits spontaneous motor activity in the back muscles. Since then, other investigators have shown similar responses to painful stimuli (Indahl, Kaigle et al. 1995; Arendt-Nielsen, Graven-Nielsen et al. 1996; Indahl, Kaigle et al. 1997; Zedka, Prochazka et al. 1999).

In animal studies, EMG responses have been demonstrated in the multifidus muscle in response to electrical stimulation of the annulus fibrosus of the intervertebral disc (Indahl, Kaigle et al. 1995). Disc lesion has also been shown to induce rapid changes in motor cortex excitability in the area representing the paraspinal muscles at the injured segment (Hodges, Galea et al. 2009). Changes in motor cortex activity appear to be a common feature in patients suffering from low back pain, apparent as altered motor co-ordination of trunk muscles (van Dieen, Cholewicki et al. 2003; van Dieen, Selen et al. 2003; Nelson-Wong, Alex et al. 2012) and reduced (Danneels, Coorevits et al. 2002) and delayed activity (Hodges and Richardson 1996; Leinonen, Kankaanpaa et al. 2001; MacDonald, Moseley et al. 2009) in deep back muscles. A physiological link between the ascending tracts and corticospinal activity was not investigated. Whether the neuronal networks involved in such a motor response are entirely spinal or whether they include supraspinal components remains unknown.

One possible pathway for how the paraspinal muscles can be affected by nociceptive stimuli to structures in the back is through activation of the reticular formation. The reticular formation in rats has been shown to receive collateral projections from spinothalamic neurones (Rossi and Brodal 1957; Kevetter and Willis 1983) and spinoreticulo-thalamic pathway (Peschanski and Besson 1984). The spinoreticulo-thalamic pathway has also been associated with behavioural reactions from painful stimuli (Casey 1971; Casey 1971; Peschanski and Besson 1984), indicating that there might be a more direct coupling between ascending nociceptive input and motor output.

The investigation in paper I was performed in cats because electrophysiology done *in vivo* is one possible way to be able to target specific motoneurones in a network and to evaluate their responses to stimulation of different tract neurones. The data in paper I indicating a bilateral motor output from corticospinal activation through reticulospinal neurones are compatible with the bilateral EMG responses following electrical stimulation of the annulus fibrosus of the intervertebral disc in pigs (Indahl, Kaigle et al. 1995). This bilateral innervation has also been suggested in human axial muscles (Marsden, Farmer et al. 1999). In low back pain patients, multifidus muscle atrophy has been found to be bilateral although the pain is unilateral (Beneck and Kulig), and experimentally induced unilateral back pain evokes a bilateral reduction of activity in the erector spinae muscles (Boudreau, Farina et al.). Taken together, the bilateral

motor responses observed elsewhere, might be a result of corticospinal activity mediated via reticulospinal neurones.

6.2 Papers II and III

In papers II and III, thalamic effects evoked by DRG exposure to components that have been suggested to be involved in disc hernia-related pain (Smyth and Wright 1958; Rydevik, Brown et al. 1984; McCarron, Wimpee et al. 1987) were investigated. The thalamus is a relay structure for ascending sensory information from the spinal cord towards the cortex (Brodal 1981) and might therefore be considered to be relevant for the study of nociceptive transmission that could eventually lead to the experience of, for example, low back pain, but before the actual involvement of cortical processing. In papers II and III, the thalamus was therefore chosen to study possible changes in nociceptive transmission caused by an experimental disc hernia. The ventral posterior lateral (VPL) thalamic nucleus of the rat is one of the nuclei in which nociceptive stimulation, i.e. applied to the hind paw (the plantar surface where the nociceptive information is mediated to the spinal cord via the sciatic nerve), induces an increase in total responses measured as the total number of firing neurones (Zhang, Wang et al. 2011). Since the VPL relays nociceptive information from several tracts (Peschanski and Besson 1984; Giesler, Bjorkeland et al. 1988), the changes demonstrated in papers II and III, may be—without any possibility of differentiating them—considered to be the net sum of responses in a number of different pathways.

6.2.1 Mechanical compression of the DRG

In paper II, acute mechanical compression was induced by gentle dislocation of the left L4 DRG. The compression was estimated to be light based on visual appearance, the remaining nerve function while the nerve was compressed, and the fast recovery when the compression was removed (Figure 3, paper II). Mechanical compression was applied to the DRG in three groups of rats: naïve, previously sham-operated, and previously disc-punctured. The compression was shown to induce a decrease in the evoked responses in all of the groups tested.

The time course differed slightly between the groups before the decrease in evoked neuronal response was shown to be significant (Figure 5). The disc-punctured group and the sham-operated group showed a more slowly developing decrease than the naïve animal group. It is possible that this was a result of the initial surgical intervention affecting the general status of the rats during the second electrophysiological experiments. Additionally, the larger number of animals in the naïve group (n = 11), especially compared to the

previously sham-operated group (n = 8), may have resulted in the observed statistically significant differences found at an earlier time point in the naïve group compared to the two intervention groups.

Light mechanical compression applied to a DRG in a naïve animal could, based on previous knowledge, be expected to induce a decrease in the total amount of neurones firing towards the spinal cord (Bentley and Schlapp 1943). Whether similar depressive effects on neuronal activity following compression of a single DRG would be represented also in more supraspinal structures such as for example, the VPL had not been investigated previously. However, the fact that in all groups the response to mechanical compression of the DRG was a reduced activity within 40 minutes or less indicates that neuronal activity in the VPL could be regarded as fairly directly representing the afferent input from the nerve root.

6.2.2 Application of nucleus pulposus to the DRG

Nucleus pulposus applied to the DRG after 40 minutes of mechanical compression and with the compression still remaining in situ resulted in an increase—or at least a reversal of the decrease—in evoked responses so that the decrease caused by mechanical compression was no longer significant. The result was similar in all groups, however, indicating that no additional effects occurred in the group in which nucleus pulposus was re-applied to the DRG (the disc-punctured group), and none of the groups showed the rapid increase observed in a previous study by Brisby and Hammar (Brisby and Hammar 2007). Thus, the effect of mechanical compression on evoked neuronal activity in the VPL was counteracted by application of nucleus pulposus in all the groups tested.

In paper II, an additional series was performed in which nucleus pulposus was applied to the DRG without any mechanical compression being involved. Similarly to the first series, the evoked neuronal effect was investigated in disc-punctured and sham-operated rats. In contrast to application of nucleus pulposus while mechanical compression still remained, the evoked neuronal responses solely after re-application of nucleus pulposus to the DRG (the disc-punctured group) differed from those after first-time application of nucleus pulposus (sham-operated animals), after 40 minutes of recording (Figure 6). This lack of responsiveness of the DRG may have been due to the previous exposure to nucleus pulposus. Since the re-application of nucleus pulposus in the first series could reverse the reduced neuronal activity evoked by mechanical compression, it might indicate that it was not merely the nucleus pulposus tissue applied the day before that was left on the DRG inhibiting any response on the second day experiment. None of the groups, however, had significant different values from baseline at any

time and the rapid increase previously noted from application of nucleus pulposus in naïve animals (Brisby and Hammar 2007) could not be repeated. If anything, application of nucleus pulposus to the DRG in the sham-operated group showed a tendency to increase similar to the result published by Brisby and Hammar (Brisby and Hammar 2007).

6.2.3 DRG sensitivity after application of nucleus pulposus

One of the aims in paper II was to evaluate whether or not the DRG could be sensitized by previous application of nucleus pulposus before later application of mechanical compression and/or nucleus pulposus. However, there were no clear indications of such sensitization. Mechanical compression and subsequent application of nucleus pulposus showed similar results in the disc-punctured group as in both the naïve and sham-operated animals. As previously mentioned, the effect was significantly different between the previously sham-operated and disc-punctured rats after 40 minutes, when nucleus pulposus alone was applied. This could be an indication of a still unmeasured increase in thalamic activity after the first DRG exposure to nucleus pulposus. It could, however, also be regarded as being a result of reduced sensitivity of the DRG because of the previous exposure to nucleus pulposus. In the human situation, we do not know which one of the two factors (mechanical compression or leakage of nucleus pulposus) is the most critical in giving disc hernia-related pain involving the DRG directly. We also do not know how the pain corresponds with the order in which the two appear. To evaluate at least the nociceptive transmission, it would be most interesting to carry out a more long-term study with, if possible, several applications of nucleus pulposus to study altered sensitivity of the DRG over time.

6.2.4 Effects on evoked thalamic neuronal activity after application of notochordal- and chondrocyte-like cells

Two different cell populations of nucleus pulposus, the notochordal cells and the chondrocyte-like cells, have previously been shown to affect nervous tissue *in vitro* (Larsson, Brisby et al. 2011; Larsson, Runesson et al. 2011). In paper III, the aim was therefore to investigate effects on evoked neuronal activity in the VPL after application of these cell populations to the DRG. After 40 minutes of recording, the evoked neuronal responses following application of 25,000 chondrocyte-like cells to the DRG differed from those measured after application of the same number of notochordal cells (Figure

7). The difference appears to be a result of the effects after application of notochordal cells to the DRG, which induced a decrease in the evoked neuronal activity as early as 10 minutes after application; this lasted for the rest of the experiment. Chondrocyte-like cells, on the other hand, were not found to give a significant different effect from that at baseline—not even after application of a substantially larger amount of cells (150,000), more than two times the amount of cells in one rat intervertebral disc (Larsson, Runesson et al. 2011). The combination of chondrocyte-like cells and notochordal cells applied to the DRG did not induce any changes in evoked neuronal responses during the time of the experiment, and neither of the individual cell populations nor the combination of the two induced an effect similar to the one previously reported after application of whole nucleus pulposus (Brisby and Hammar 2007).

Studies investigating neuronal activity following application of either cell population derived from nucleus pulposus are—to my knowledge—rare. Overall, there have been few studies on the histological effects of application of individual cell populations from nucleus pulposus. In two previous studies, the notochordal population of nucleus pulposus was shown to affect nervous tissue negatively by inhibiting neurite outgrowth (Larsson, Runesson et al. 2011) and reducing neurite diameter (Larsson, Brisby et al. 2011) in DRGs cultured *in vitro*. In this *in vitro* model, the inhibitory effect of chondrocyte-like cells was not observed following application of 20,000 cells to a culture well but it could be shown after application of 40,000 cells (Larsson, Runesson et al. 2011). The results in paper III indicate that neither of the cell populations was the sole factor responsible for the increased neuronal thalamic activity reported after application of whole nucleus pulposus (Brisby and Hammar 2007). Thus, separately, they are most likely unable to induce the increased excitability reported in the DRG (Takebayashi, Cavanaugh et al. 2001; Kallakuri, Takebayashi et al. 2005) and spinal cord (Anzai, Hamba et al. 2002; Cuellar, Montesano et al. 2004) after application of nucleus pulposus to the nerve root.

Since 99% of the intervertebral disc is thought to be extracellular matrix (Roberts, Evans et al. 2006), one could question the importance of the cells in nucleus pulposus for the aforementioned effects on neuronal excitability. It is also possible that soluble factors produced by the nucleus pulposus cells are relevant for a potential effect. The matrix contribution is, however, more difficult to investigate. In a previous study by Olmarker et al. (Olmarker, Brisby et al. 1997), nucleus pulposus was frozen prior to application on the porcine cauda equina before investigating changes in conduction velocity. It was assumed that the freezing procedure would preserve various molecules of the nucleus pulposus, but lyse the cells. The nerve conduction velocity was unaffected by the frozen nucleus pulposus and reduced by the non-frozen

nucleus pulposus used as control. The investigators draw the conclusion that the nucleus pulposus-induced effects were therefore related to its cell population.

One of the limitations of study III was the difficulty in choosing a reasonable number of cells to apply to the DRG. Twenty-five thousand cells were chosen because it was a similar amount to that tested in two previously published studies by Larsson et al. (Larsson, Brisby et al. 2011; Larsson, Runesson et al. 2011). These studies were, however, carried out *in vitro* in a cell culture well where, even if the cell number was similar, the cell concentration in each well was most likely less than the concentration applied to the DRG *in vivo* in paper III. Moreover, the time period in which the cell populations were able to exert an effect differed in the *in vitro* and *in vivo* studies. *In vitro*, the cells were left for 24 hours before neurite evaluation while the cells were applied only for 40 minutes for recording of evoked thalamic activity in the *in vivo* study.

To conclude, the study in paper III, might be looked upon as a first attempt to investigate the effects of individual nucleus pulposus-derived cell populations regarding neuronal activity. The results, however, reveal the more complicated nature of how the nucleus pulposus tissue can affect neuronal activity, thereby contributing to the experience of disc herniа-related pain (McCarron, Wimpee et al. 1987; Byrod, Rydevik et al. 1998; Yabuki, Kikuchi et al. 1998; Murata, Rydevik et al. 2005).

6.2.5 Effects induced by nucleus pulposus

Although the cellular mechanisms underlying the results reported in papers II and III could not be investigated in this rat model, it is reasonable to assume that changes in evoked neuronal thalamic activity following stimulation of the sciatic nerve are a result of altered excitability—either in the soma or axons of the DRG neurones, or at the synapses in the dorsal horn of the spinal cord, or both.

Nucleus pulposus produces several cytokines (Takahashi, Suguro et al. 1996; Yoshida, Nakamura et al. 2005) and the one that has received the most interest is tumour necrosis factor α (TNF-α) (Olmarker and Larsson 1998; Igarashi, Kikuchi et al. 2000). TNF-α has been shown to elicit spontaneous action potentials and to increase discharge rates in primary nociceptive neurons when applied locally (Sorkin, Xiao et al. 1997; Liu, Li et al. 2002), supposedly by altering Na^+ currents (Jin and Gereau 2006; He, Zang et al. 2010; Chen, Pang et al. 2011), thereby increasing neuronal excitability.

Additional mechanisms have been suggested. Ohtori et al. (Ohtori, Inoue et al. 2006) proposed that application of nucleus pulposus affects neuronal excitability through upregulation of the sodium selective acid-sensing ion

channel 3 (ASIC3) in the DRG. Otoshi et al. (Otoshi, Kikuchi et al. 2009) demonstrated the capacity of nucleus pulposus to activate satellite glial cells, which are believed to be of importance for the development of pathological pain (Watkins, Milligan et al. 2001). Nucleus pulposus has also been shown to induce morphological changes such as Schwann cell damage (Byrod, Rydevik et al. 1998), changes in elastic fibres in the capsule surrounding the DRG (Murata, Rydevik et al. 2005), and reduced blood flow in the DRG (Yabuki, Kikuchi et al. 1998) within hours of exposure.

6.2.6 Comparison with a previous study in the rat model

None of the groups tested in papers II and III, perhaps with the exception of the sham-operated group in which nucleus pulposus was applied solely to the DRG (as previously discussed), showed a facilitatory effect like the one published by Brisby and Hammar (Brisby and Hammar 2007). The rapid increase in evoked neuronal activity demonstrated by Brisby and Hammar was, however, found to appear after application of nucleus pulposus to DRGs in a group of naïve rats in which nucleus pulposus was used not only as a primary intervention but also after application of fat tissue. Application of fat tissue to the DRG has been used previously as a control group when evaluating neuronal electrical activity in the adjacent nervous tissue or spinal cord dorsal horn, but with varied result. Some investigators have found similar effects of application of nucleus pulposus as with application of fat tissue (Anzai, Hamba et al. 2002; Kallakuri, Takebayashi et al. 2005) while others have been able to demonstrate a difference between the two (Takebayashi, Cavanaugh et al. 2001; Cuellar, Montesano et al. 2004). None of them showed an inhibitory effect on neuronal activity as seen in the VPL by Brisby and Hammar (Brisby and Hammar 2007). The effects that can be induced by fat tissue fall outside the scope of this thesis. However, the possible effect of first applying fat and then nucleus pulposus was not evaluated in the study by Brisby and Hammar (Brisby and Hammar 2007), leaving the possibility that the potent increase in evoked neuronal activity reported was a combinatory effect and might therefore not be fully compatible with the lack of effect of application of nucleus pulposus or cell populations alone, or in a combination, as described in papers II and III.

6.2.7 Von Frey test

In order to link our data to the published literature on experimental disc hernia in rats, animals in paper II were subjected to the same surgical procedures and tested for changes in paw withdrawal thresholds with von Frey filaments. Previous behavioural studies using a combination of

application of nucleus pulposus and subsequent DRG compression (Omarker and Myers 1998) concluded that the increased mechanical sensitivity determined by von Frey test was however more pronounced when the DRG was simultaneously subjected to chronic mechanical compression. The lack of behavioural changes measured with von Frey filaments 24 hours after disc puncture or sham surgery in the present study is compatible with data reported by Olmarker and Myers (Omarker and Myers 1998), indicating that our findings are comparable with the results using the same model for experimental disc hernia in rodents reported elsewhere.

6.2.8 Limitations

One limitation of the papers presented in this thesis was that all electrophysiological investigations were undertaken under anaesthesia. There are electrophysiological investigations published elsewhere in which recordings from neurones were made in awake animals after the recording electrodes had been placed in the nervous system during anaesthesia. These are, however, limited to studies on neuronal activity, i.e. during behavioural tasks (Laubach, Wessberg et al. 2000) or in models for learning processes (Mostofi, Holtzman et al. 2010). It was simply not possible to perform the larger surgical procedures preceding the experiments in papers I–III in awake animals. In the ethical point of view it was also not possible to perform studies on nociceptive transmission without adequate anaesthesia.

In papers II and III, a rather new rat model was used for investigation of neuronal activity in the VPL of the thalamus with subsequent experimental disc hernia affecting a DRG. This model has previously been used to demonstrate a rapid and potent increase in thalamic activity after exposure to the DRG to nucleus pulposus (Brisby and Hammar 2007). The results of the two papers included in this thesis have, however, not shown such clear responses. Since the responses evoked in the VPL are the net sum of those from several ascending tracts with one or more synapses, the effects induced by mechanical compression or application of nucleus pulposus to the DRG in an animal that might already be in pain from previous surgery might therefore induce responses in the ascending tracts that are too weak to induce a measurable effect.

It is possible that a methodological error was introduced, as the baseline was set to 100% at the beginning of each electrophysiological experiment. The baseline was therefore assumed to be the same for all groups regardless of whether or not the animals were previously operated on. It cannot be discounted that a surgical procedure performed one day earlier might alter the neuronal activity in the neurones investigated. In addition to this, the leakage of nucleus pulposus onto the DRG on the day before might already have

altered the excitability. Takebayashi et al. (Takebayashi, Cavanaugh et al. 2001) demonstrated that the spontaneous discharge rate in the DRG gradually increases in the hours following application of nucleus pulposus to the DRG.

One way to improve the experimental setup would be to expand the facetectomy to expose both the L4 and L5 DRGs. Since both L4 and L5 spinal nerves together receive most of the sciatic sensory fibres (Swett, Torigoe et al. 1991) one could transect them separately and place them on two separate stimulating electrodes. Most likely, activation of these two would induce activity in the same or closely related areas in the contralateral VPL. The evoked activity by stimulating the L5 spinal nerve could be used as a control if an intervention is made on the L4 DRG. Theoretically, the activity evoked from L5 should not change after application of mechanical compression or nucleus pulposus on the L4 DRG.

6.2.9 Future perspectives

In order to investigate a possible mechanism of how ascending nociceptive transmission from structures in the spine, for example the intervertebral disc, can affect low back motoneurones, one could investigate longissimus motoneurone responses electrophysiologically in the cat to a subsequent stimulation of afferents from structures in the lower back. The stimulation could be injury to the intervertebral disc as investigated in pigs by Indahl et al. (Indahl, Kaigle et al. 1995), or hypertonic saline injected into the human erector spinae muscle as reported by Zedka et al. (Zedka, Prochazka et al. 1999). Responses evoked in the back motoneurones might perhaps correlate with the previously reported muscle activity and altered patterns of voluntary movements. If it does so, one could continue the investigation by lesioning the MLF to investigate whether the evoked responses in the motoneurones disappear, which would suggest that the previously published effects (Indahl, Kaigle et al. 1995; Zedka, Prochazka et al. 1999) are mediated via reticulospinal neurones. Moreover, the latencies for the evoked responses might be an indication of whether the response is mediated via collaterals from ascending nociceptive tracts to the reticular formation (Rossi and Brodal 1957; Kevetter and Willis 1983; Pechura and Liu 1986) or whether higher centres are involved.

Furthermore, in the clinical situation it is not fully known whether the mechanical compression or the leakage of nucleus pulposus—if both are required—is the first to appear, to induce disc hernia-related pain. It would therefore be interesting to investigate the effect of prior mechanical compression instead of application of nucleus pulposus to the DRG in the rat model of evoked activity in the VPL. There is, however, a risk that the reported reduction in conduction velocity due to mechanical compression of a

nerve would develop into a complete block (Bentley and Schlapp 1943) making the second-day electrophysiological investigation less interesting. The compression caused by gently dislocating the DRG, as in paper II, has been reported before—both alone and together with subsequent application of nucleus pulposus. It has been shown to induce behavioural changes (Olmarker, Iwabuchi et al. 1998; Omarker and Myers 1998; Olmarker, Storkson et al. 2002) and sole mechanical compression, if anything, has been related to an increase rather than a decrease in sensitivity in the affected paw.

Another important feature in the clinical situation is an even longer time of exposure, which was not investigated in this thesis. Even though longer time, i.e. one week, of application of nucleus pulposus to the DRG has been shown to induce histological changes (Olmarker, Rydevik et al. 1993; Olmarker, Nordborg et al. 1996), it is unclear how long the nucleus pulposus will remain and affect the DRG. Whether or not the mechanical compression of the DRG is still present can, on the other hand, be easily seen at the end of the experiment and perhaps a future investigation could include a few weeks of mechanical compression before application of nucleus pulposus during the electrophysiological investigation.

To continue the investigation of how neuronal excitability is affected by individual cell populations from nucleus pulposus, it would be of interest to study individual neurones in the DRG or spinal cord. This would lead to a more direct response to the presence of a cell population compared to the rat model presented in papers II and III, in which there is a net sum of effects in networks of neurones for the evoked response.

7 CONCLUSION

The conclusions from this thesis are:

- Motor control through pyramidal tract neurones to the erector spinae muscle is mainly mediated via reticulospinal neurones, and is to a lesser extent mediated via interneurones activated by the pyramidal tract neurones.

- The study of evoked neuronal changes in the thalamus presented here following mechanical compression, application of whole nucleus pulposus tissue, and application of the cell components thereof to the DRG indicates the complex nature of nociceptive transmission induced acutely in disc hernia.

- Previous exposure of the DRG to nucleus pulposus does not appear to sensitize the DRG to a second exposure to nucleus pulposus or mechanical compression.

- The study of neuronal networks involved in low back pain including acute nociceptive transmission is complex. In this thesis an animal model for such investigations is presented and the strengths and limitations of this model are discussed

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REFERENCES

Airaksinen, O., J. I. Brox, et al. (2006). "Chapter 4. European guidelines for the management of chronic nonspecific low back pain." Eur Spine J 15 Suppl 2: S192-300.

Alstermark, B., M. J. Pinter, et al. (1985). "Pyramidal effects in dorsal neck motoneurones of the cat." J Physiol 363: 287-302.

Andersson, G. B. (1998). "Epidemiology of low back pain." Acta Orthop Scand Suppl 281: 28-31.

Anzai, H., M. Hamba, et al. (2002). "Epidural application of nucleus pulposus enhances nociresponses of rat dorsal horn neurons." Spine (Phila Pa 1976) 27(3): E50-5.

Arendt-Nielsen, L., T. Graven-Nielsen, et al. (1996). "The influence of low back pain on muscle activity and coordination during gait: a clinical and experimental study." Pain 64(2): 231-40.

Armand, J. and H. G. Kuypers (1980). "Cells of origin of crossed and uncrossed corticospinal fibers in the cat: a quantitative horseradish peroxidase study." Exp Brain Res 40(1): 23-34.

Asato, F., M. Butler, et al. (2000). "Variation in rat sciatic nerve anatomy: implications for a rat model of neuropathic pain." J Peripher Nerv Syst 5(1): 19-21.

Barrot, M. (2012). "Tests and models of nociception and pain in rodents." Neuroscience 211: 39-50.

Beckstein, J. C., S. Sen, et al. (2008). "Comparison of animal discs used in disc research to human lumbar disc: axial compression mechanics and glycosaminoglycan content." Spine (Phila Pa 1976) 33(6): E166-73.

Beneck, G. J. and K. Kulig "Multifidus atrophy is localized and bilateral in active persons with chronic unilateral low back pain." Arch Phys Med Rehabil 93(2): 300-6.

Bentley, F. H. and W. Schlapp (1943). "The effects of pressure on conduction in peripheral nerve." J Physiol 102(1): 72-82.

Bessou, P. and E. R. Perl (1969). "Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli." J Neurophysiol 32(6): 1025-43.

Bester, H., S. Beggs, et al. (2000). "Changes in tactile stimuli-induced behavior and c-Fos expression in the superficial dorsal horn and in parabrachial nuclei after sciatic nerve crush." J Comp Neurol 428(1): 45-61.

Boden, S. D., D. O. Davis, et al. (1990). "Abnormal magnetic-resonance scans of the lumbar spine in asymptomatic subjects. A prospective investigation." J Bone Joint Surg Am 72(3): 403-8.

Bogduk, N. (1983). "The innervation of the lumbar spine." Spine (Phila Pa 1976) 8(3): 286-93.

Bogduk, N., W. Tynan, et al. (1981). "The nerve supply to the human lumbar intervertebral discs." J Anat 132(Pt 1): 39-56.
Boos, N., S. Weissbach, et al. (2002). "Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science." Spine (Phila Pa 1976) 27(23): 2631-44.
Boudreau, S., D. Farina, et al. "The relative timing of trunk muscle activation is retained in response to unanticipated postural-perturbations during acute low back pain." Exp Brain Res 210(2): 259-67.
Brisby, H. (2006). "Pathology and possible mechanisms of nervous system response to disc degeneration." J Bone Joint Surg Am 88 Suppl 2: 68-71.
Brisby, H. and I. Hammar (2007). "Thalamic activation in a disc herniation model." Spine (Phila Pa 1976) 32(25): 2846-52.
Brodal, A., Ed. (1981). Neurological Anatomy. Oxford, Oxford University Press.
Brosamle, C. and M. E. Schwab (1997). "Cells of origin, course, and termination patterns of the ventral, uncrossed component of the mature rat corticospinal tract." J Comp Neurol 386(2): 293-303.
Buckwalter, J. A. (1995). "Aging and degeneration of the human intervertebral disc." Spine (Phila Pa 1976) 20(11): 1307-14.
Byrod, G., B. Rydevik, et al. (1998). "Early effects of nucleus pulposus application on spinal nerve root morphology and function." Eur Spine J 7(6): 445-9.
Cao, L., F. Guilak, et al. (2007). "Three-dimensional morphology of the pericellular matrix of intervertebral disc cells in the rat." J Anat 211(4): 444-52.
Carr, J. H., R. B. Shepherd, et al. (1985). "Investigation of a new motor assessment scale for stroke patients." Phys Ther 65(2): 175-80.
Carstens, E. and D. L. Trevino (1978). "Laminar origins of spinothalamic projections in the cat as determined by the retrograde transport of horseradish peroxidase." J Comp Neurol 182(1): 161-65.
Casey, K. L. (1971). "Escape elicited by bulboreticular stimulation in the cat." Int J Neurosci 2(1): 29-34.
Casey, K. L. (1971). "Responses of bulboreticular units to somatic stimuli eliciting escape behavior in the cat." Int J Neurosci 2(1): 15-28.
Cassidy, J. D., P. Cote, et al. (2005). "Incidence and course of low back pain episodes in the general population." Spine (Phila Pa 1976) 30(24): 2817-23.
Chelberg, M. K., G. M. Banks, et al. (1995). "Identification of heterogeneous cell populations in normal human intervertebral disc." J Anat 186 (Pt 1): 43-53.
Chen, C., J. M. Cavanaugh, et al. (2004). "Effects of nucleus pulposus on nerve root neural activity, mechanosensitivity, axonal morphology, and sodium channel expression." Spine (Phila Pa 1976) 29(1): 17-25.

Chen, J., W. Yan, et al. (2006). "Molecular phenotypes of notochordal cells purified from immature nucleus pulposus." Eur Spine J 15 Suppl 3: S303-11.

Chen, X., R. P. Pang, et al. (2011). "TNF-alpha enhances the currents of voltage gated sodium channels in uninjured dorsal root ganglion neurons following motor nerve injury." Exp Neurol 227(2): 279-86.

Choi, K. S., M. J. Cohn, et al. (2008). "Identification of nucleus pulposus precursor cells and notochordal remnants in the mouse: implications for disk degeneration and chordoma formation." Dev Dyn 237(12): 3953-8.

Collins, J. G., M. Kawahara, et al. (1983). "Alpha-chloralose suppression of neuronal activity." Life Sci 32(26): 2995-9.

Craig, A. D., M. C. Bushnell, et al. (1994). "A thalamic nucleus specific for pain and temperature sensation." Nature 372(6508): 770-3.

Craig, A. D., Jr. and H. Burton (1979). "The lateral cervical nucleus in the cat: anatomic organization of cervicothalamic neurons." J Comp Neurol 185(2): 329-46.

Cuellar, J. M., P. X. Montesano, et al. (2004). "Role of TNF-alpha in sensitization of nociceptive dorsal horn neurons induced by application of nucleus pulposus to L5 dorsal root ganglion in rats." Pain 110(3): 578-87.

Dangaria, T. R. and O. Naesh (1998). "Changes in cross-sectional area of psoas major muscle in unilateral sciatica caused by disc herniation." Spine (Phila Pa 1976) 23(8): 928-31.

Danneels, L. A., P. L. Coorevits, et al. (2002). "Differences in electromyographic activity in the multifidus muscle and the iliocostalis lumborum between healthy subjects and patients with sub-acute and chronic low back pain." Eur Spine J 11(1): 13-9.

Davidson, A. G. and J. A. Buford (2004). "Motor outputs from the primate reticular formation to shoulder muscles as revealed by stimulus-triggered averaging." J Neurophysiol 92(1): 83-95.

Davidson, N. (1965). "The projection of afferent pathways on the thalamus of the rat." J Comp Neurol 124(3): 377-90.

Dekaban, A. (1953). "Human thalamus; an anatomical, developmental and pathological study. I. Division of the human adult thalamus into nuclei by use of the cyto-myelo-architectonic method." J Comp Neurol 99(3): 639-83.

Derby, R., B. J. Kim, et al. (2005). "The relation between annular disruption on computed tomography scan and pressure-controlled diskography." Arch Phys Med Rehabil 86(8): 1534-8.

Deyo, R. A. and J. N. Weinstein (2001). "Low back pain." N Engl J Med 344(5): 363-70.

Dudley, R. E., S. R. Nelson, et al. (1982). "Influence of chloralose on brain regional glucose utilization." Brain Res 233(1): 173-80.

Edgley, S. A., E. Jankowska, et al. (2004). "Ipsilateral actions of feline corticospinal tract neurons on limb motoneurons." J Neurosci 24(36): 7804-13.
Errington, R. J., K. Puustjarvi, et al. (1998). "Characterisation of cytoplasm-filled processes in cells of the intervertebral disc." J Anat 192 (Pt 3): 369-78.
Flor, H., C. Braun, et al. (1997). "Extensive reorganization of primary somatosensory cortex in chronic back pain patients." Neurosci Lett 224(1): 5-8.
Freemont, A. J., T. E. Peacock, et al. (1997). "Nerve ingrowth into diseased intervertebral disc in chronic back pain." Lancet 350(9072): 178-81.
Galea, M. P., I. Hammar, et al. (2010). "Bilateral postsynaptic actions of pyramidal tract and reticulospinal neurons on feline erector spinae motoneurons." J Neurosci 30(3): 858-69.
Gan, J. C., P. Ducheyne, et al. (2003). "Intervertebral disc tissue engineering II: cultures of nucleus pulposus cells." Clin Orthop Relat Res(411): 315-24.
Garrett, K. M. and J. Gan (1998). "Enhancement of gamma-aminobutyric acidA receptor activity by alpha-chloralose." J Pharmacol Exp Ther 285(2): 680-6.
Gauriau, C. and J. F. Bernard (2002). "Pain pathways and parabrachial circuits in the rat." Exp Physiol 87(2): 251-8.
Giesler, G. J., Jr., M. Bjorkeland, et al. (1988). "Organization of the spinocervicothalamic pathway in the rat." J Comp Neurol 268(2): 223-33.
Gilson, A., M. Dreger, et al. (2010). "Differential expression level of cytokeratin 8 in cells of the bovine nucleus pulposus complicates the search for specific intervertebral disc cell markers." Arthritis Res Ther 12(1): R24.
Greene (1959). Anatomy of the rat. Philadelphia, Hafner Publishing Co.
Grillner, S., T. Hongo, et al. (1968). "Reciprocal effects between two descending bulbospinal systems with monosynaptic connections to spinal motoneurones." Brain Res 10(3): 477-80.
Gronblad, M., J. N. Weinstein, et al. (1991). "Immunohistochemical observations on spinal tissue innervation. A review of hypothetical mechanisms of back pain." Acta Orthop Scand 62(6): 614-22.
Guehring, T., J. P. Urban, et al. (2008). "Noninvasive 3D vital imaging and characterization of notochordal cells of the intervertebral disc by femtosecond near-infrared two-photon laser scanning microscopy and spatial-volume rendering." Microsc Res Tech 71(4): 298-304.
Guilak, F., H. P. Ting-Beall, et al. (1999). "Viscoelastic properties of intervertebral disc cells. Identification of two biomechanically distinct cell populations." Spine (Phila Pa 1976) 24(23): 2475-83.
Hartell, N. A. and P. M. Headley (1990). "Spinal effects of four injectable anaesthetics on nociceptive reflexes in rats: a comparison of

electrophysiological and behavioural measurements." Br J Pharmacol 101(3): 563-8.
He, X. H., Y. Zang, et al. (2010). "TNF-alpha contributes to up-regulation of Nav1.3 and Nav1.8 in DRG neurons following motor fiber injury." Pain 151(2): 266-79.
He, X. W. and C. P. Wu (1985). "Connections between pericruciate cortex and the medullary reticulospinal neurons in cat: an electrophysiological study." Exp Brain Res 61(1): 109-16.
Hides, J. A., M. J. Stokes, et al. (1994). "Evidence of lumbar multifidus muscle wasting ipsilateral to symptoms in patients with acute/subacute low back pain." Spine (Phila Pa 1976) 19(2): 165-72.
Hodges, P., A. K. Holm, et al. (2006). "Rapid atrophy of the lumbar multifidus follows experimental disc or nerve root injury." Spine (Phila Pa 1976) 31(25): 2926-33.
Hodges, P. W., M. P. Galea, et al. (2009). "Corticomotor excitability of back muscles is affected by intervertebral disc lesion in pigs." Eur J Neurosci 29(7): 1490-500.
Hodges, P. W., G. L. Moseley, et al. (2003). "Experimental muscle pain changes feedforward postural responses of the trunk muscles." Exp Brain Res 151(2): 262-71.
Hodges, P. W. and C. A. Richardson (1996). "Inefficient muscular stabilization of the lumbar spine associated with low back pain. A motor control evaluation of transversus abdominis." Spine (Phila Pa 1976) 21(22): 2640-50.
Holstege, G. and H. G. Kuypers (1982). "The anatomy of brain stem pathways to the spinal cord in cat. A labeled amino acid tracing study." Prog Brain Res 57: 145-75.
Howe, J. F., J. D. Loeser, et al. (1977). "Mechanosensitivity of dorsal root ganglia and chronically injured axons: a physiological basis for the radicular pain of nerve root compression." Pain 3(1): 25-41.
Hu, S. J. and J. L. Xing (1998). "An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat." Pain 77(1): 15-23.
Hunter, C. J., J. R. Matyas, et al. (2003). "The three-dimensional architecture of the notochordal nucleus pulposus: novel observations on cell structures in the canine intervertebral disc." J Anat 202(Pt 3): 279-91.
Hunter, C. J., J. R. Matyas, et al. (2004). "Cytomorphology of notochordal and chondrocytic cells from the nucleus pulposus: a species comparison." J Anat 205(5): 357-62.
Ichimura, K., H. Tsuji, et al. (1991). "Cell culture of the intervertebral disc of rats: factors influencing culture, proteoglycan, collagen, and deoxyribonucleic acid synthesis." J Spinal Disord 4(4): 428-36.
Igarashi, T., S. Kikuchi, et al. (2000). "2000 Volvo Award winner in basic science studies: Exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Molecular, histologic, and

behavioral comparisons in rats." Spine (Phila Pa 1976) 25(23): 2975-80.
Indahl, A., A. Kaigle, et al. (1995). "Electromyographic response of the porcine multifidus musculature after nerve stimulation." Spine (Phila Pa 1976) 20(24): 2652-8.
Indahl, A., A. M. Kaigle, et al. (1997). "Interaction between the porcine lumbar intervertebral disc, zygapophysial joints, and paraspinal muscles." Spine (Phila Pa 1976) 22(24): 2834-40.
Inoue, G., S. Ohtori, et al. (2006). "Exposure of the nucleus pulposus to the outside of the anulus fibrosus induces nerve injury and regeneration of the afferent fibers innervating the lumbar intervertebral discs in rats." Spine (Phila Pa 1976) 31(13): 1433-8.
Jack, J. J. B. (1978). Studies in Neurophysiology. Some methods for selective activation of muscle afferent fibers. Cambridge, University Press.
Jankowska, E., I. Hammar, et al. (2003). "Neuronal basis of crossed actions from the reticular formation on feline hindlimb motoneurons." J Neurosci 23(5): 1867-78.
Jankowska, E. and K. Stecina (2007). "Uncrossed actions of feline corticospinal tract neurones on lumbar interneurones evoked via ipsilaterally descending pathways." J Physiol 580(Pt 1): 133-47.
Jin, X. and R. W. t. Gereau (2006). "Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha." J Neurosci 26(1): 246-55.
Jonsson, B. and B. Stromqvist (1996). "Clinical appearance of contained and noncontained lumbar disc herniation." J Spinal Disord 9(1): 32-8.
Kably, B. and T. Drew (1998). "Corticoreticular pathways in the cat. I. Projection patterns and collaterization." J Neurophysiol 80(1): 389-405.
Kaigle, A. M., S. H. Holm, et al. (1997). "1997 Volvo Award winner in biomechanical studies. Kinematic behavior of the porcine lumbar spine: a chronic lesion model." Spine (Phila Pa 1976) 22(24): 2796-806.
Kaigle, A. M., P. Wessberg, et al. (1998). "Muscular and kinematic behavior of the lumbar spine during flexion-extension." J Spinal Disord 11(2): 163-74.
Kallakuri, S., T. Takebayashi, et al. (2005). "The effects of epidural application of allografted nucleus pulposus in rats on cytokine expression, limb withdrawal and nerve root discharge." Eur Spine J 14(10): 956-64.
Kevetter, G. A. and W. D. Willis (1983). "Collaterals of spinothalamic cells in the rat." J Comp Neurol 215(4): 453-64.
Kim, J. H., B. M. Deasy, et al. (2009). "Differentiation of intervertebral notochordal cells through live automated cell imaging system in vitro." Spine (Phila Pa 1976) 34(23): 2486-93.

Kim, S. H. and J. M. Chung (1992). "An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat." Pain 50(3): 355-63.

Konttinen, Y. T., M. Gronblad, et al. (1990). "Neuroimmunohistochemical analysis of peridiscal nociceptive neural elements." Spine (Phila Pa 1976) 15(5): 383-6.

Kortelainen, P., J. Puranen, et al. (1985). "Symptoms and signs of sciatica and their relation to the localization of the lumbar disc herniation." Spine (Phila Pa 1976) 10(1): 88-92.

Lacroix, S., L. A. Havton, et al. (2004). "Bilateral corticospinal projections arise from each motor cortex in the macaque monkey: a quantitative study." J Comp Neurol 473(2): 147-61.

Larsson, K., H. Brisby, et al. (2011). "Electron Microscopy Analysis of Neurites Extending from Dorsal Root Ganglia in vitro following Exposure to Intervertebral Disc Cells." Cells Tissues Organs.

Larsson, K., E. Runesson, et al. (2011). "Effects of intervertebral disc cells on neurite outgrowth from dorsal root ganglion explants in culture." Spine (Phila Pa 1976) 36(8): 600-6.

Laubach, M., J. Wessberg, et al. (2000). "Cortical ensemble activity increasingly predicts behaviour outcomes during learning of a motor task." Nature 405(6786): 567-71.

Le Bars, D., M. Gozariu, et al. (2001). "Animal models of nociception." Pharmacol Rev 53(4): 597-652.

Leinonen, V., M. Kankaanpaa, et al. (2001). "Disc herniation-related back pain impairs feed-forward control of paraspinal muscles." Spine (Phila Pa 1976) 26(16): E367-72.

Lemon, R. N. (2008). "Descending pathways in motor control." Annu Rev Neurosci 31: 195-218.

Liu, B., H. Li, et al. (2002). "Increased sensitivity of sensory neurons to tumor necrosis factor alpha in rats with chronic compression of the lumbar ganglia." J Neurophysiol 88(3): 1393-9.

Lynn, B. (1977). "Cutaneous hyperalgesia." Br Med Bull 33(2): 103-8.

MacDonald, D., G. L. Moseley, et al. (2009). "Why do some patients keep hurting their back? Evidence of ongoing back muscle dysfunction during remission from recurrent back pain." Pain 142(3): 183-8.

Maldonado, B. A. and T. R. Oegema, Jr. (1992). "Initial characterization of the metabolism of intervertebral disc cells encapsulated in microspheres." J Orthop Res 10(5): 677-90.

Malinsky, J. (1959). "The ontogenetic development of nerve terminations in the intervertebral discs of man. (Histology of intervertebral discs, 11th communication)." Acta Anat (Basel) 38: 96-113.

Mannion, A. F. (1999). "Fibre type characteristics and function of the human paraspinal muscles: normal values and changes in association with low back pain." J Electromyogr Kinesiol 9(6): 363-77.

Mannion, A. F., L. Kaser, et al. (2000). "Influence of age and duration of symptoms on fibre type distribution and size of the back muscles in chronic low back pain patients." Eur Spine J 9(4): 273-81.
Marsden, J. F., S. F. Farmer, et al. (1999). "The unilateral and bilateral control of motor unit pairs in the first dorsal interosseous and paraspinal muscles in man." J Physiol 521 Pt 2: 553-64.
Martin, M. D., C. M. Boxell, et al. (2002). "Pathophysiology of lumbar disc degeneration: a review of the literature." Neurosurg Focus 13(2): E1.
Martini (2003). Human Anatomy. New Jersey, Pearson Education, Inc.
Massion, J. (1992). "Movement, posture and equilibrium: interaction and coordination." Prog Neurobiol 38(1): 35-56.
Massion, J. (1994). "Postural control system." Curr Opin Neurobiol 4(6): 877-87.
Matsuyama, K. and T. Drew (1997). "Organization of the projections from the pericruciate cortex to the pontomedullary brainstem of the cat: a study using the anterograde tracer Phaseolus vulgaris-leucoagglutinin." J Comp Neurol 389(4): 617-41.
Matsuyama, K., F. Mori, et al. (1999). "Morphology of single pontine reticulospinal axons in the lumbar enlargement of the cat: a study using the anterograde tracer PHA-L." J Comp Neurol 410(3): 413-30.
McCarron, R. F., M. W. Wimpee, et al. (1987). "The inflammatory effect of nucleus pulposus. A possible element in the pathogenesis of low-back pain." Spine (Phila Pa 1976) 12(8): 760-4.
Mengiardi, B., M. R. Schmid, et al. (2006). "Fat content of lumbar paraspinal muscles in patients with chronic low back pain and in asymptomatic volunteers: quantification with MR spectroscopy." Radiology 240(3): 786-92.
Mientjes, M. I. and J. S. Frank (1999). "Balance in chronic low back pain patients compared to healthy people under various conditions in upright standing." Clin Biomech (Bristol, Avon) 14(10): 710-6.
Mitani, A., K. Ito, et al. (1988). "Descending projections from the gigantocellular tegmental field in the cat: cells of origin and their brainstem and spinal cord trajectories." J Comp Neurol 268(4): 546-66.
Mixter and Barr (1934). "Rupture of the intervertebral disc with involvement of the spinal canal." N Engl J Med 211: 210-215.
Miyagi, M., T. Ishikawa, et al. (2011). "Assessment of gait in a rat model of myofascial inflammation using the CatWalk system." Spine (Phila Pa 1976) 36(21): 1760-4.
Mostofi, A., T. Holtzman, et al. (2010). "Electrophysiological localization of eyeblink-related microzones in rabbit cerebellar cortex." J Neurosci 30(26): 8920-34.
Murata, Y., B. Rydevik, et al. (2005). "Incision of the intervertebral disc induces disintegration and increases permeability of the dorsal root ganglion capsule." Spine (Phila Pa 1976) 30(15): 1712-6.

Nelson-Wong, E., B. Alex, et al. (2012). "Altered muscle recruitment during extension from trunk flexion in low back pain developers." Clin Biomech (Bristol, Avon).

Nouwen, A. and C. Bush (1984). "The relationship between paraspinal EMG and chronic low back pain." Pain 20(2): 109-23.

Nouwen, A. and J. W. Solinger (1979). "The effectiveness of EMG biofeedback training in low back pain." Biofeedback Self Regul 4(2): 103-11.

Nyberg-Hansen, R. and A. Brodal (1963). "Sites of termination of corticospinal fibers in the cat. An experimental study with silver impregnation methods." J Comp Neurol 120: 369-91.

Ohtori, S., G. Inoue, et al. (2006). "Up-regulation of acid-sensing ion channel 3 in dorsal root ganglion neurons following application of nucleus pulposus on nerve root in rats." Spine (Phila Pa 1976) 31(18): 2048-52.

Olmarker, K., H. Brisby, et al. (1997). "The effects of normal, frozen, and hyaluronidase-digested nucleus pulposus on nerve root structure and function." Spine (Phila Pa 1976) 22(5): 471-5; discussion 476.

Olmarker, K., M. Iwabuchi, et al. (1998). "Walking analysis of rats subjected to experimental disc herniation." Eur Spine J 7(5): 394-9.

Olmarker, K. and K. Larsson (1998). "Tumor necrosis factor alpha and nucleus-pulposus-induced nerve root injury." Spine (Phila Pa 1976) 23(23): 2538-44.

Olmarker, K., C. Nordborg, et al. (1996). "Ultrastructural changes in spinal nerve roots induced by autologous nucleus pulposus." Spine (Phila Pa 1976) 21(4): 411-4.

Olmarker, K., B. Rydevik, et al. (1993). "Autologous nucleus pulposus induces neurophysiologic and histologic changes in porcine cauda equina nerve roots." Spine (Phila Pa 1976) 18(11): 1425-32.

Olmarker, K., R. Storkson, et al. (2002). "Pathogenesis of sciatic pain: a study of spontaneous behavior in rats exposed to experimental disc herniation." Spine (Phila Pa 1976) 27(12): 1312-7.

Omarker, K. and R. R. Myers (1998). "Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion." Pain 78(2): 99-105.

Otoshi, K., S. Kikuchi, et al. (2009). "The reactions of glial cells and endoneurial macrophages in the dorsal root ganglion and their contribution to pain-related behavior after application of nucleus pulposus onto the nerve root in rats." Spine (Phila Pa 1976) 35(3): 264-71.

Pazzaglia, U. E., J. R. Salisbury, et al. (1989). "Development and involution of the notochord in the human spine." J R Soc Med 82(7): 413-5.

Pechura, C. M. and R. P. Liu (1986). "Spinal neurons which project to the periaqueductal gray and the medullary reticular formation via axon

collaterals: a double-label fluorescence study in the rat." Brain Res 374(2): 357-61.
Pedersen, H. E., C. F. Blunck, et al. (1956). "The anatomy of lumbosacral posterior rami and meningeal branches of spinal nerve (sinu-vertebral nerves); with an experimental study of their functions." J Bone Joint Surg Am 38-A(2): 377-91.
Peng, B., W. Wu, et al. (2005). "The pathogenesis of discogenic low back pain." J Bone Joint Surg Br 87(1): 62-7.
Pengel, L. H., R. D. Herbert, et al. (2003). "Acute low back pain: systematic review of its prognosis." BMJ 327(7410): 323.
Peschanski, M. and J. M. Besson (1984). "A spino-reticulo-thalamic pathway in the rat: an anatomical study with reference to pain transmission." Neuroscience 12(1): 165-78.
Peschanski, M., P. W. Mantyh, et al. (1983). "Spinal afferents to the ventrobasal thalamic complex in the rat: an anatomical study using wheat-germ agglutinin conjugated to horseradish peroxidase." Brain Res 278(1-2): 240-4.
Peterson, B. W., N. G. Pitts, et al. (1979). "Reticulospinal connections with limb and axial motoneurons." Exp Brain Res 36(1): 1-20.
Poiraudeau, S., I. Monteiro, et al. (1999). "Phenotypic characteristics of rabbit intervertebral disc cells. Comparison with cartilage cells from the same animals." Spine (Phila Pa 1976) 24(9): 837-44.
Radebold, A., J. Cholewicki, et al. (2001). "Impaired postural control of the lumbar spine is associated with delayed muscle response times in patients with chronic idiopathic low back pain." Spine (Phila Pa 1976) 26(7): 724-30.
Raj, P. P. (2008). "Intervertebral disc: anatomy-physiology-pathophysiology-treatment." Pain Pract 8(1): 18-44.
Reighard, J. (1949). Anatomy of the Cat. New York, Henry Holt and Company.
Risbud, M. V., T. P. Schaer, et al. (2010). "Toward an understanding of the role of notochordal cells in the adult intervertebral disc: from discord to accord." Dev Dyn 239(8): 2141-8.
Robbins, A., D. W. Pfaff, et al. (1992). "Reticulospinal and reticuloreticular pathways for activating the lumbar back muscles in the rat." Exp Brain Res 92(1): 46-58.
Roberts, S., S. M. Eisenstein, et al. (1995). "Mechanoreceptors in intervertebral discs. Morphology, distribution, and neuropeptides." Spine (Phila Pa 1976) 20(24): 2645-51.
Roberts, S., H. Evans, et al. (2006). "Histology and pathology of the human intervertebral disc." J Bone Joint Surg Am 88 Suppl 2: 10-4.
Rossi, G. F. and A. Brodal (1957). "Terminal distribution of spinoreticular fibers in the cat." AMA Arch Neurol Psychiatry 78(5): 439-53.

Rydevik, B., M. D. Brown, et al. (1984). "Pathoanatomy and pathophysiology of nerve root compression." Spine (Phila Pa 1976) 9(1): 7-15.

Rydevik, B. and C. Nordborg (1980). "Changes in nerve function and nerve fibre structure induced by acute, graded compression." J Neurol Neurosurg Psychiatry 43(12): 1070-82.

Sasaki, N., M. Sekiguchi, et al. (2011). "A comparison of pain-related behavior following local application of nucleus pulposus and/or mechanical compression on the dorsal root ganglion." Fukushima J Med Sci 57(2): 46-53.

Schepens, B. and T. Drew (2006). "Descending signals from the pontomedullary reticular formation are bilateral, asymmetric, and gated during reaching movements in the cat." J Neurophysiol 96(5): 2229-52.

Shimamura, M., T. Yamauchi, et al. (1968). "Effects of chloralose anesthesia on spinal reflexes." Jpn J Physiol 18(6): 788-97.

Shvartzman, L., E. Weingarten, et al. (1992). "Cost-effectiveness analysis of extended conservative therapy versus surgical intervention in the management of herniated lumbar intervertebral disc." Spine (Phila Pa 1976) 17(2): 176-82.

Silfies, S. P., D. Squillante, et al. (2005). "Trunk muscle recruitment patterns in specific chronic low back pain populations." Clin Biomech (Bristol, Avon) 20(5): 465-73.

Sinclair, D. C., W. H. Feindel, et al. (1948). "The intervertebral ligaments as a source of segmental pain." J Bone Joint Surg Br 30B(3): 515-21.

Smyth, M. J. and V. Wright (1958). "Sciatica and the intervertebral disc; an experimental study." J Bone Joint Surg Am 40-A(6): 1401-18.

Sorkin, L. S., W. H. Xiao, et al. (1997). "Tumour necrosis factor-alpha induces ectopic activity in nociceptive primary afferent fibres." Neuroscience 81(1): 255-62.

Stecina, K. and E. Jankowska (2007). "Uncrossed actions of feline corticospinal tract neurones on hindlimb motoneurones evoked via ipsilaterally descending pathways." J Physiol 580(Pt 1): 119-32.

Steffens, H., P. Dibaj, et al. (2012). "In vivo measurement of conduction velocities in afferent and efferent nerve fibre groups in mice." Physiol Res 61(2): 203-14.

Strutton, P. H., S. Theodorou, et al. (2005). "Corticospinal excitability in patients with chronic low back pain." J Spinal Disord Tech 18(5): 420-4.

Study, R. E. and J. L. Barker (1981). "Diazepam and (--)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons." Proc Natl Acad Sci U S A 78(11): 7180-4.

Suzuki, M., G. Inoue, et al. (2009). "Nuclear factor-kappa B decoy suppresses nerve injury and improves mechanical allodynia and

thermal hyperalgesia in a rat lumbar disc herniation model." Eur Spine J 18(7): 1001-7.
Swett, J. E., Y. Torigoe, et al. (1991). "Sensory neurons of the rat sciatic nerve." Exp Neurol 114(1): 82-103.
Takahashi, H., T. Suguro, et al. (1996). "Inflammatory cytokines in the herniated disc of the lumbar spine." Spine (Phila Pa 1976) 21(2): 218-24.
Takebayashi, T., J. M. Cavanaugh, et al. (2001). "Effect of nucleus pulposus on the neural activity of dorsal root ganglion." Spine (Phila Pa 1976) 26(8): 940-5.
Torvik, A. and A. Brodal (1957). "The origin of reticulospinal fibers in the cat; an experimental study." Anat Rec 128(1): 113-37.
Trout, J. J., J. A. Buckwalter, et al. (1982). "Ultrastructure of the human intervertebral disc: II. Cells of the nucleus pulposus." Anat Rec 204(4): 307-14.
Trout, J. J., J. A. Buckwalter, et al. (1982). "Ultrastructure of the human intervertebral disc. I. Changes in notochordal cells with age." Tissue Cell 14(2): 359-69.
Tsao, H., K. J. Tucker, et al. (2010). "Experimentally induced low back pain from hypertonic saline injections into lumbar interspinous ligament and erector spinae muscle." Pain 150(1): 167-72.
Wagner, J. G., D. J. Weidler, et al. (1977). "Pharmacokinetics of peniobarbital in the cat and comparisons with the rabbit and man." Res Commun Chem Pathol Pharmacol 16(2): 375-84.
van Dieen, J. H., J. Cholewicki, et al. (2003). "Trunk muscle recruitment patterns in patients with low back pain enhance the stability of the lumbar spine." Spine (Phila Pa 1976) 28(8): 834-41.
van Dieen, J. H., L. P. Selen, et al. (2003). "Trunk muscle activation in low-back pain patients, an analysis of the literature." J Electromyogr Kinesiol 13(4): 333-51.
Wasiak, R., J. Kim, et al. (2006). "Work disability and costs caused by recurrence of low back pain: longer and more costly than in first episodes." Spine (Phila Pa 1976) 31(2): 219-25.
Watkins, L. R., E. D. Milligan, et al. (2001). "Glial activation: a driving force for pathological pain." Trends Neurosci 24(8): 450-5.
Weiler, C., A. G. Nerlich, et al. (2010). "Immunohistochemical identification of notochordal markers in cells in the aging human lumbar intervertebral disc." Eur Spine J 19(10): 1761-70.
Willis, W. D., Jr., X. Zhang, et al. (2001). "Projections from the marginal zone and deep dorsal horn to the ventrobasal nuclei of the primate thalamus." Pain 92(1-2): 267-76.
Wilson, V. J. and M. Yoshida (1968). "Vestibulospinal and reticulospinal effects on hindlimb, forelimb, and neck alpha motoneurons of the cat." Proc Natl Acad Sci U S A 60(3): 836-40.
www.FASS.se (2012). FASS, Läkemedelsindustriföreningens Service AB.

Yabuki, S., S. Kikuchi, et al. (1998). "Acute effects of nucleus pulposus on blood flow and endoneurial fluid pressure in rat dorsal root ganglia." Spine (Phila Pa 1976) 23(23): 2517-23.

Yayama, T., S. Kobayashi, et al. (2010). "Effects of graded mechanical compression of rabbit sciatic nerve on nerve blood flow and electrophysiological properties." J Clin Neurosci 17(4): 501-5.

Yoshida, M., T. Nakamura, et al. (2005). "Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model." Spine (Phila Pa 1976) 30(1): 55-61.

Yoshihara, K., Y. Shirai, et al. (2001). "Histochemical changes in the multifidus muscle in patients with lumbar intervertebral disc herniation." Spine (Phila Pa 1976) 26(6): 622-6.

Zedka, M., A. Prochazka, et al. (1999). "Voluntary and reflex control of human back muscles during induced pain." J Physiol 520 Pt 2: 591-604.

Zhang, Y., N. Wang, et al. (2011). "Ensemble encoding of nociceptive stimulus intensity in the rat medial and lateral pain systems." Mol Pain 7: 64.