Synaptic Plasticity in Pain

Marzia Malcangio
Editor

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Editor
Marzia Malcangio
Wolfson Centre for Age Related Diseases
King's College London
London SE1 1UL
United Kingdom
marzia.malcangio@kcl.ac.uk

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Contributors

Mark L. Baccei MED-Anaesthesiology, University of Cincinnati, OH, USA, bacceiml@ucmail.uc.edu

Rita Bardoni Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy, bardoni@unimo.it

Lucy Bee Neuroscience, Physiology and Pharmacology, University College London, London, UK, l.bee@ucl.ac.uk

Simon Beggs Program in Neurosciences and Mental Health, Hospital for Sick Children, University of Toronto Centre for the Study of Pain, Toronto, Ontario, Canada, simon.beggs@utoronto.ca

Fernando Cervero Anaesthesia Research Unit, McGill University, Montreal, Quebec, Canada, fernando.cervero@mcgill.ca

Victoria Chapman School of Biomedical Sciences, University of Nottingham, Nottingham, UK, victoria.chapman@nottingham.ac.uk

Anna K. Clark Wolfson Centre for Age Related Diseases, King's College London, London, UK, anna.clark@kcl.ac.uk

Joyce A. DeLeo Department of Pharmacology and Toxicology, Dartmouth College, Hanover, NH, USA; Neuroscience Center at Dartmouth, Dartmouth College, Hanover, NH, USA; Department of Anesthesiology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA, joyce.a.deleo@dartmouth.edu

Anthony Dickenson Neuroscience, Physiology and Pharmacology, University College London, London, UK, anthony.dickenson@ucl.ac.uk

Ruth Drdla Department of Neurophysiology, Center for Brain Research, Medical University of Vienna, Vienna, Austria, ruth.drdla@meduniwien.ac.at

Andrea Ebersberger Department of Physiology, Friedrich-Schiller-University of Jena, Jena, Germany, andrea.ebersberger@mti.uni-jena.de

Maria Fitzgerald Department of Anatomy and Developmental Biology, University College London, London, UK, m.fitzgerald@ucl.ac.uk

Xiao Ying Hua Moores Cancer Center, University of California, San Diego, CA, USA, xyhua@ucsd.edu

Rachel A. Ingram Department of Anatomy and Developmental Biology, University College London, London, UK, rachel.ingram@ucl.ac.uk

Ru-Rong Ji Department of Anesthesiology, Brigham and Women's Hospital and Harvard Medical School, Pain Research Center, Boston, MA, USA, rrji@zeus.bwh.harvard.edu

Torbjörn Johansson Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland; Institute of Pharmaceutical Sciences, ETH Zurich, Switzerland, johansson@pharma.uzh.ch

David Kendall School of Biomedical Sciences, University of Nottingham, Nottingham, UK, david.kendall@nottingham.ac.uk

Josephine Lai Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ, USA, lai@email.arizona.edu

Jennifer M.A. Laird Department of Pharmacology and Therapeutics and Alan Edwards Centre for Research on Pain, McGill University, Montréal, Quebec, Canada; AstraZeneca R&D Montréal, Montréal, Quebec, Canada, jennifer.laird@astrazeneca.com

Melissa J. Mahoney Department of Chemical and Biological Engineering, University of Colorado at Boulder, Boulder, CO, USA, melissa.mahoney@ colorado.edu

Marzia Malcangio Wolfson Centre for Age Related Diseases, King's College London, London, UK, marzia.malcangio@kcl.ac.uk

Juan Carlos Marvizon Center for Neurobiology of Stress, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, marvizon@ucla.edu

Adalberto Merighi Department of Veterinary Morphophysiology, University of Turin, Turin, Italy; Istituto Nazionale di Neuroscienze, Turin, Italy

Erin D. Milligan Department of Neurosciences, University of New Mexico Health Sciences Center, Albuquerque, NM, USA, emilligan@salud.unm.edu

Volker Neugebauer Department of Neuroscience and Cell Biology, The University of Texas Medical Branch, Galveston, TX, USA, voneugeb@utmb.edu

Frank Porreca Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ, USA, frankp@email.arizona.edu

John V. Priestley Barts and The London School of Medicine and Dentistry, Institute of Cell and Molecular Science, Neuroscience Centre, Queen Mary University of London, London, UK, j.v.priestley@qmul.ac.uk

Mirjana Randić Department of Biomedical Sciences, Iowa State University, Ames, IA, USA, mrandic@iastate.edu

Devi Rani Sagar School of Biomedical Sciences, University of Nottingham, Nottingham, UK, devi.sagar@nottingham.ac.uk

Jürgen Sandkühler Department of Neurophysiology, Center for Brain Research, Medical University of Vienna, Vienna, Austria, juergen.sandkuehler@meduniwien.ac.at

Hans-Georg Schaible Department of Physiology, Friedrich-Schiller-University of Jena, Jena, Germany, hans-georg.schaible@mti.uni-jena.de

Ryan G. Soderquist Department of Chemical and Biological Engineering, University of Colorado at Boulder, Boulder, CO, USA, ryan.soderquist@colodado.edu

Camilla I. Svensson Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, camilla.svensson@ki.se

Vivianne L. Tawfik Department of Pharmacology and Toxicology, Dartmouth College, Hanover, NH, USA; Dartmouth College, Neuroscience Center at Dartmouth, Hanover, NH, USA, vivianne.tawfix@dartmouth.edu

Stephen W.N. Thompson Biomedical Science, University of Plymouth, Plymouth, PL4 8AA, UK, stephen.thompson@plymouth.ac.uk

Andrew J. Todd Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK, a.todd@bio.gla.ac.uk

Ruizhong Wang Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ, USA, wangrz@email.arizona.edu

Robert Witschi Institute of Pharmacology and Toxicology, University of Zurich, **Zurich**, Switzerland; Institute of Pharmaceutical Sciences, ETH Zurich, Switzerland, witschi@pharma.unizh.ch

Tony L. Yaksh Molecular Biology, University of California, San Diego, CA, USA, tyaksh@ucsd.edu

Hanns Ulrich Zeilhofer Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland; Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland, zeilhofer@pharma.uzh.ch

Introduction

The chapters in this book have evolved around the concept that the first sensory synapse between the central terminals of primary sensory neurons and dorsal horn neurons in the spinal cord is plastic and modifiable. Thus, the book title reflects the effort of the several authors to address this idea of plasticity in pain from their own perspective. I am grateful to colleagues who have contributed with enthusiasm and competence to this task and particularly Dr. Sandkuhler for his advice and suggestions.

As extensively stressed throughout the book chapters, the detection and perception of pain have multi-dimensional nature and pain is defined by the International Association for the Study of Pain (IASP) as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'.

Primary sensory neurons respond to peripheral stimulation and project to the spinal cord. Specifically, the population of neurons which respond to damaging stimuli terminate in the superficial layers of the dorsal horn. The passage of sensory inputs from the dorsal horn of the spinal cord to higher centres in the brain is modulated by both descending facilitatory and inhibitory neurones. Therefore, the dorsal horns constitute the first relay site for nociceptive fibre terminals which make synaptic contacts with second order neurons. It has recently become clear that the strength of this first sensory synapse is plastic and modifiable by several modulators, including neuronal and non-neuronal regulators. Undoubtedly, the studies on the fundamental processes regulating the plasticity of the first pain synapse have resulted in the identification of new targets for the treatment of chronic pain.

This book includes six sections which start from the delineation of some anatomical circuits for pain in the dorsal horn.

The next two sections are focussed on the main players of the fast and slow transmissions at the pain synapse including GABA and the opioids as well as substance P, calcitonin gene-related peptide and brain derived neurotrophic factor.

The fourth section is concerned with synaptic plasticity and the application of sensory information in the dorsal horn of the spinal cord.

The final section consists of several chapters on mechanisms and targets for chronic pain in the dorsal horn, including the arthritic pain, visceral pain and neuropathic pain. Specifically, a number of contributors have expressed their views on the role played in the modulation of pain mechanisms by non neuronal cells, astrocytes and microglia which have recently become the focus of intensive research.

This book will be of interest to a wide readership in the pain field including PhD students, post-doc scientists and academics. Drug discovery teams in the private sector will find in this book some solid scientific support to their research.

Furthermore this book will arouse scientists interested in synaptic plasticity associated with other CNS functions such as hippocampal plasticity in learning processes.

Finally, I wish to thank Ann Avouris from Springer who has ideated this project and has supported this initiative with optimism.

Part I
Anatomical Plasticity of Dorsal Horn Circuits

Chapter 1
Changes in NK1 and Glutamate Receptors in Pain

Andrew J. Todd

Abstract The amino acid glutamate and the neuropeptide substance P are contained in many nociceptive primary afferents that terminate mainly in the superficial part of the dorsal horn. Both glutamate and substance P are released from the central terminals of nociceptive afferents following noxious stimulation. Glutamate acts on a variety of ionotropic and metabotropic receptors, while substance P acts on the neurokinin 1 receptor (NK1r), and both transmitters contribute to the processing of nociceptive information at the spinal level. Noxious stimulation of the hindpaw causes rapid (within minutes) internalisation of the NK1r, phosphorylation of the GluR1 subunit of the AMPA-type glutamate receptor and phosphorylation of the NR1 subunit of NMDA-type glutamate receptors. These plastic changes of SP and glutamate receptors that occur in acute and chronic pain states presumably contribute to sensitisation of dorsal horn neurons (central sensitization).

Abbreviations

AMPA	α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanic acid
CGRP	calcitonin gene related peptide
CFA	complete Freund's adjuvant
CVLM	caudal ventrolateral medulla
DRG	dorsal root ganglion
LPb	lateral parabrachial area
LTP	long term potentiation
NK1r	neurokinin 1 receptor
NMDA	N-methyl-D-aspartate
PAG	periaqueductal grey

A.J. Todd (✉)
Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK
e-mail: a.todd@bio.gla.ac.uk

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1.1 Introduction

Substance P, and the neurokinin 1 receptor (NK1r) on which it acts, have long been thought to play an important role in pain mechanisms. Substance P is contained in many nociceptive primary afferents, released following noxious stimulation and activates NK1rs on certain dorsal horn neurons. Glutamate is also released by central terminals of nociceptive afferents and acts on a variety of ionotropic and metabotropic receptors in the dorsal horn. The receptors that form the subject of this chapter are therefore activated following noxious stimulation and contribute to the processing of nociceptive information at the spinal level. The chapter will focus on aspects of dorsal horn anatomy that are relevant to substance P and the NK1r and to glutamatergic transmission, and will discuss evidence for plasticity involving these receptors. Only a brief description of other aspects of dorsal horn anatomy will be given here, and for more detailed descriptions the reader is referred to other recent reviews (Todd and Koerber, 2005; Ribeiro-da-Silva and De Koninck, 2008). This account is based on findings in the rat (unless stated otherwise), since most anatomical data have been obtained in this species.

1.2 Anatomical Components of the Dorsal Horn

The dorsal horn receives its major input from primary afferent axons, which arborise in a modality-specific pattern. It contains a diverse collection of neurons that can be divided into two main classes: (1) those with axons that remain in the spinal cord (interneurons), and (2) projection neurons, with axons that ascend through the white matter and terminate in the brain, forming a major output from the dorsal horn. The interneurons include both excitatory (glutamatergic) and inhibitory (mainly GABAergic) cells, while most projection neurons are glutamatergic (Broman, 1994; Todd and Koerber, 2005). The dorsal horn also receives inputs from descending axons that originate in several brain regions and modulate the transmission of sensory information (see Chapter 19).

Rexed (1954) divided the dorsal horn of the cat spinal cord into 6 laminae and this scheme, which has been applied to other species, is widely used for descriptive purposes. Laminae I and II are often referred to as the superficial dorsal horn, and form the main termination zone for nociceptive primary afferents. Laminae III–VI (the deep dorsal horn) receive their major primary afferent input from low-threshold mechanoreceptive afferents. However, this region is also important in pain mechanisms, since it contains projection neurons that convey nociceptive information, and because the low-threshold afferents that terminate within it can give rise to tactile allodynia (touch-evoked pain) in certain pain states.

1.3 Substance P and the NK1r

1.3.1 Sources of Substance P in the Dorsal Horn

Lamina I and the outer part of lamina II (IIo) contain a dense plexus of substance P-containing axons, most of which are of primary afferent origin. In addition, scattered substance P axons (including primary afferents) terminate in deeper laminae. Information about the functions of substance P-containing primary afferents has come from studies in which immunostaining has been carried out after electrophysiological characterisation of individual afferents recorded in the guinea pig dorsal root ganglion (DRG) (Lawson et al., 1997). Substance P was found most commonly in cells that gave rise to unmyelinated (C) fibres, but also in some with small (Aδ) or large (Aβ) myelinated axons. All substance P-containing afferents were nociceptors, but not all nociceptive afferents contained the peptide. Substance P was particularly associated with nociceptive afferents that had deep cutaneous receptive fields, although it was also seen in some polymodal C nociceptors that innervated glabrous skin. In the rat, all peptidergic primary afferents contain calcitonin gene-related peptide (CGRP) (Ju et al., 1987), which is only found in primary afferent axons in the dorsal horn. Therefore the presence of CGRP can be used in double-labelling immunocytochemical studies to distinguish between substance P-containing axons that are primary afferents and those that are not (Sakamoto et al., 1999). Cell bodies that contain substance P or the mRNA for its precursor protein (preprotachykinin 1) are present in the dorsal horn (Hökfelt et al., 1977; Warden and Young, 1988) and give rise to the non-primary substance P-containing axons.

1.3.2 Anatomical Distribution of NK1r

Several immunocytochemical studies have described the distribution of the NK1r in the spinal dorsal horn (Bleazard et al., 1994; Liu et al., 1994; Nakaya et al., 1994; Brown et al., 1995; Littlewood et al., 1995; Mantyh et al., 1995; Todd et al., 1998). NK1r-immunoreactivity is present on the cell bodies and dendrites of certain dorsal horn neurons, but not on axons in the spinal cord. Immunostaining for the receptor is particularly dense in lamina I and is scattered throughout the deeper laminae (III–VI), but is present on very few neurons in lamina II. Within the dorsal horn, we have estimated that ~45% of neurons in lamina I and 10–30% of those in laminae III–VI are NK1r-immunoreactive. Most of the dendrites of the NK1r-immunoreactive lamina I cells are restricted to this lamina, where they make up a dense plexus. Cheunsuang and Morris (2000) have demonstrated that there is a bimodal size distribution of NK1r-positive neurons in lamina I, with a population of small weakly stained cells, and a group of large cells that generally show strong immunoreactivity. Among the NK1r-expressing neurons in deeper laminae,

there is a population of large neurons with dendrites that travel dorsally to enter lamina I (Liu et al., 1994; Brown et al., 1995; Littlewood et al., 1995; Mantyh et al., 1995; Naim et al., 1997). Although these cells are very distinctive, there are only ~20–25 of them on either side in each mid-lumbar segment and slightly fewer per segment in the cervical enlargement (Todd et al., 2000; Al-Khater et al., 2008).

1.3.3 Projection Neurons and the NK1r

Cell bodies of projection neurons can be identified by injection of retrograde tracers into brain regions where their axons terminate. Studies of this type have shown that in rat lumbar enlargement projection neurons are concentrated in lamina I and scattered throughout laminae III–VI and the lateral spinal nucleus. Many of these cells have axons that cross the midline and ascend in the contralateral white matter to terminate in various regions of the brainstem and thalamus (Todd, 2002). Brainstem regions that receive inputs from lamina I projection neurons include the caudal ventrolateral medulla (CVLM), lateral parabrachial area (LPb) and periaqueductal grey matter (PAG).

It has been estimated that lamina I contains approximately 400 projection neurons on each side in the L4 segment in the rat (Todd et al., 2000; Spike et al., 2003). Most of these (~85%) project to contralateral LPb, with around 30% sending collaterals to the PAG. Only ~15 lamina I neurons/segment project to the thalamus from the midlumbar cord, although the number of lamina I spinothalamic neurons is much higher (~90 cells/segment) in the cervical enlargement (Al-Khater et al., 2008). Most lamina I projection neurons send their axons only to contralateral brain targets, but some have bilateral projections (Spike et al., 2003).

Since the NK1r is present on many lamina I neurons, several studies have investigated the extent to which the receptor is expressed by projection neurons in this lamina (Ding et al., 1995; Marshall et al., 1996; Li et al., 1998; Todd et al., 2000; Spike et al., 2003; Al-Khater et al., 2008). We have estimated that ~80% of lamina I neurons that project to thalamus, LPb, PAG or the medulla are NK1r-immunoreactive (Marshall et al., 1996; Todd et al., 2000; Spike et al., 2003; Al-Khater et al., 2008), and these correspond to the large NK1r-positive cells identified by Cheunsuang and Morris (2000) (Polgár et al., 2002). All of the large NK1r-positive cells in laminae III and IV with long dorsal dendrites that enter the superficial dorsal horn are projection neurons, since virtually all of them can be labelled with tracer injected into the CVLM, while two-thirds project to LPb (Todd et al., 2000). We have recently shown that approximately 20% of these cells in the lumbar enlargement, and about 85% of those at cervical levels, belong to the spinothalamic tract (Al-Khater et al., 2008).

The NK1r-immunoreactive projection neurons in lamina I, as well as those located in laminae III and IV, receive a dense synaptic input from substance

P-containing primary afferents (Naim et al., 1997; Todd et al., 2002). These afferents not only innervate dendrites of these cells that lie within the dense plexus of substance P axons in laminae I–IIo, but also make numerous synapses on dendrites of the lamina III/IV cells that lie below the plexus. For the lamina III/IV cells, it has been shown that primary afferent inputs are organised in a selective manner, since these cells receive very few contacts from C fibres that do not contain substance P (Sakamoto et al., 1999).

1.3.4 Plasticity of NK1rs in the Dorsal Horn

Mantyh et al. (1995) demonstrated that acute noxious stimulation of the rat hindpaw caused internalisation of NK1rs on many lamina I neurons and on dorsal dendrites of the large lamina III/IV cells. Internalisation causes loss of NK1r-immunoreactivity from the plasma membrane and the appearance of immunoreactive endosomes (Fig. 1.1). There is also a structural alteration, with thin dendrites showing a marked beading. These changes develop rapidly

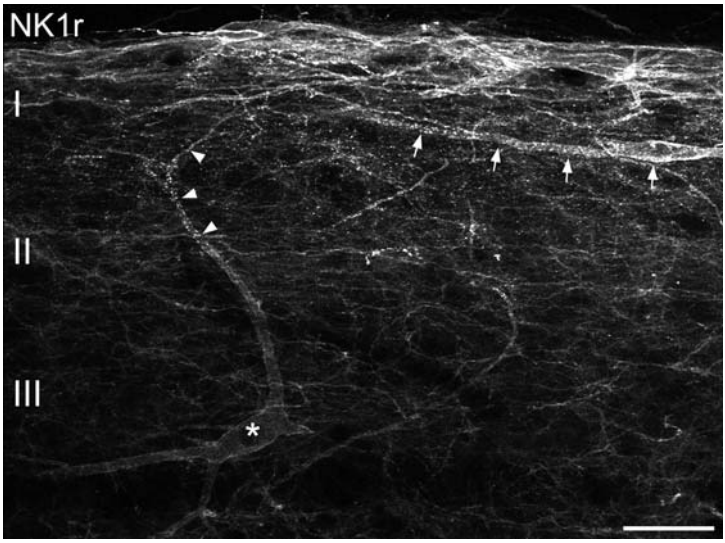

Fig. 1.1 Internalisation of NK1 receptor following acute noxious stimulation. A confocal image showing part of a parasagittal section through the dorsal horn of the L4 segment of a rat stained for the NK1r. The rat had received noxious mechanical stimulation of the ipsilateral hindpaw (pinching of the skin) under terminal general anaesthesia 5 minutes before perfusion fixation. Internalisation of the receptor is seen on a dendrite that belongs to a lamina I neuron (*arrows*) and on the distal part of a dorsal dendrite of a large lamina III cell (*arrowheads*). The soma (*asterisk*) and proximal dendrites of the lamina III cell show the normal distribution of NK1r on the surface. Scale bar = 50 μm. Modified from Polgár et al. (2007) with permission from BioMed Central

(within 1 minute) and last less than an hour. On lamina I cells, the entire somatodendritic membrane is affected, and since substantial parts of these cells are not in contact with substance P-containing axons (Todd et al., 2002), this is consistent with the view that substance P diffuses from its release sites and acts through volume transmission.

It was subsequently shown that following inflammation of the hindpaw with complete Freund's adjuvant (CFA), internalisation of the NK1r in the ipsilateral dorsal horn was substantially increased (Abbadie et al., 1997). This change was reflected in an increase in the number of lamina I neurons that showed internalisation after noxious stimulation, as well as significant internalisation in NK1r-positive cell bodies in deeper laminae, which was not seen in normal rats. In addition, previously innocuous mechanical stimuli could cause internalisation of the receptor. Allen et al. (1999) reported that during CFA-induced inflammation, NK1r internalisation was evoked by electrical stimulation of sciatic nerve only at Aδ or C fibre strength. This indicates that the internalisation seen during inflammation is caused by substance P released from Aδ and C fibres. Increased internalisation of the receptor may also play a role in visceral pain and hyperalgesia, since Honoré et al. (2002) reported that colonic inflammation led to increased internalisation in lamina I neurons following noxious colo-rectal distension, as well as internalisation following non-noxious visceral stimuli (see Chapter 13).

There is also evidence for increased NK1r expression in the ipsilateral dorsal horn following inflammation or nerve injury. There is a higher level of NK1r mRNA after injection of CFA (Schäfer et al., 1993; McCarson and Krause, 1994), while NK1r-immunoreactivity increases after inflammatory stimuli or various types of nerve injury (Abbadie et al., 1996, 1997; Goff et al., 1998; Honoré et al., 1999) (Fig. 1.2). Up-regulation of NK1r was seen in both lamina I

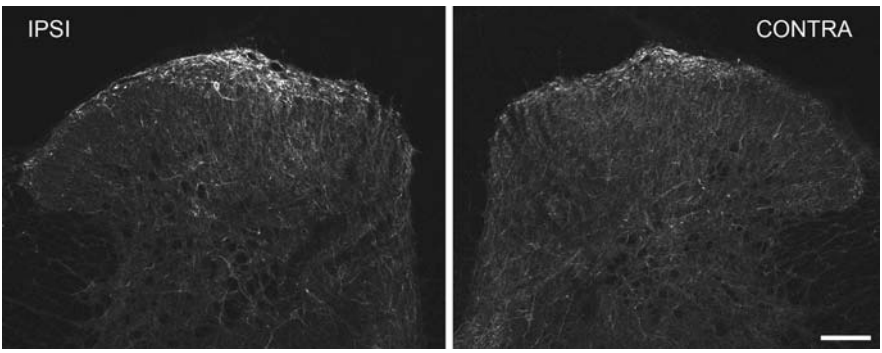

Fig. 1.2 Upregulation of NK1r in the L4 dorsal horn of a rat two weeks after chronic constriction injury of the left sciatic nerve. NK1r-immunoreactivity is densest in lamina I on both sides, but the staining is considerably stronger on the ipsilateral side (ipsi), compared to that on the contralateral side (contra). Scale bar = 100 μm. Reproduced from Todd and Ribeiro-da-Silva (2007) with permission

and laminae III–IV, and extended into regions outside those innervated by the affected nerves or skin territories. Abbadie et al. (1997) reported that the number of NK1r-positive lamina I neurons was not altered following inflammation with CFA, although their staining intensity increased. However, palecek et al. (2003) reported that inflammation of the colon resulted in *de novo* expression of NK1r by a small number of lamina III/IV projection neurons belonging to the post-synaptic dorsal column pathway, which are not normally NK1r-immunoreactive (Polgár et al., 1999).

1.4 Sources of Glutamatergic Input to the Dorsal Horn

All primary afferents use glutamate as a neurotransmitter (De Biasi and Rustioni, 1988; Broman et al., 1993), and their central terminations are arranged in a highly ordered way that depends on fibre diameter and sensory modality (Ribeiro-da-Silva and De Koninck, 2008). Unmyelinated afferents, most of which function as nociceptors or thermoreceptors, can be divided into two main groups, those with neuropeptides and those without. Most peptidergic afferents project to laminae I–IIo, with some arborising further ventrally. In contrast, the majority of non-peptidergic C fibres terminate in a narrow band that occupies the central part of lamina II. Aδ afferents project to two different regions in the dorsal horn: Aδ nociceptors terminate mainly in lamina I (with additional branches to laminae V and X), while those that innervate down hairs (D-hair afferents) arborise in inner lamina II (IIi) and lamina III. Low-threshold mechanoreceptive Aβ afferents end in a region extending ventrally from lamina IIi.

Excitatory interneurons and projection cells in the dorsal horn provide another major source of glutamatergic axons. Between 25 and 40% of neurons in laminae I–III are GABA-immunoreactive (Polgár et al., 2003) and the remainder are thought to be excitatory, glutamatergic cells. Until the discovery of the vesicular glutamate transporters it was difficult to identify the axons of these cells. However, it is now known that most (if not all) glutamatergic neurons in the dorsal horn express VGLUT2, and these cells are likely to give rise to the great majority of VGLUT2-immunoreactive boutons that are present in large numbers throughout the dorsal horn (Oliveira et al., 2003; Todd et al., 2003; Alvarez et al., 2004).

There are also descending glutamatergic axons (e.g. corticospinal tract), although little is known about the synaptic arrangements that these form.

1.5 Glutamate Receptors

1.5.1 Ionotropic Receptors at Glutamatergic Synapses

In situ hybridization studies have shown that all 3 types of ionotropic glutamate receptor (NMDA, AMPA and kainate) are present in the dorsal horn

(Furuyama et al., 1993; Tölle et al., 1993; Watanabe et al., 1994). However, although immunocytochemistry can be used to reveal the subunits of these receptors at non-synaptic sites such as the perikaryal cytoplasm (Tachibana et al., 1994; Jakowec et al., 1995; Popratiloff et al., 1996), it is difficult to detect receptors at synapses because of the extensive cross-linking of synaptic proteins that results from fixation. We have therefore used an antigen retrieval method based on pepsin treatment to reveal the synaptic distribution of AMPA (Nagy et al., 2004a; Polgar et al., 2008) and NMDA (Nagy et al., 2004b) receptor subunits in the rat spinal cord.

AMPA receptors (AMPArs) are tetramers made up from 4 subunits (GluR1-4, or GluRA-D), and both heteromeric and homomeric arrangements can form functional receptors. Most AMPArs contain the GluR2 subunit, which renders them impermeable to Ca^{2+}. Following antigen retrieval, AMPArs can be detected at most if not all, glutamatergic synapses throughout the spinal grey matter (Nagy et al., 2004a; Polgar et al., 2008). Virtually all of these synapses contain GluR2, while the other 3 subunits have distinct laminar distributions. In laminae I–II, GluR1 and GluR3 are each present in ~60–65% of AMPAr-containing synaptic puncta, with only 10% of puncta lacking both of these subunits, whereas GluR4 is present in ~25% of puncta in lamina I and <10% of those in lamina II. Further ventrally, expression of GluR4 increases, while that of GluR1 decreases, and in lamina IV–IX most glutamatergic synapses contain GluR2, GluR3 and GluR4. Ca^{2+}-permeable (GluR2-lacking) AMPArs are present in the dorsal horn (Engelman et al., 1999) and since virtually all glutamatergic synapses appear to contain GluR2, this suggests that there is heterogeneity of subunit composition, with Ca^{2+}-permeable and - impermeable receptors being intermingled at synapses (Tong and MacDermott, 2006).

The NMDA receptor (NMDAr) is also a tetramer, but unlike the AMPAr only heteromeric arrangements form functional receptors. These normally contain two NR1 and two NR2 subunits. There are four different NR2 subunits (A–D) and 8 splice variants of the NR1 subunit, which result from inclusion or exclusion of two cassettes (N1 and C1) and a further modification that can lead to replacement of another cassette (C2 or C2'). In situ hybridisation data has suggested that all spinal neurons express the NR1 subunit (Tölle et al., 1993), and we have found with immunocytochemistry after antigen retrieval that synaptic staining for this subunit is widespread throughout the spinal grey matter (Nagy et al., 2004b). In contrast, NR2A puncta were most numerous in the deep dorsal horn (particularly laminae III–IV) and ventral horn, while NR2B puncta were concentrated in laminae I–II. As expected, both of these NR2 subunits showed substantial co-localisation with NR1. The laminar distribution of synaptic NR1, NR2A and NR2B subunits that we observed with immunocytochemistry closely matches the distributions of their mRNAs reported by Watanabe et al. (1994).

At present, little is known about the synaptic distribution of kainate receptors in the spinal cord.

1.5.2 Metabotropic Glutamate Receptors

Eight different metabotropic glutamate receptors (mGluR1-8) have been identified, and several of these are present in the spinal cord (Vidnyánszky et al., 1994; Ohishi et al., 1995; Boxall et al., 1998; Jia et al., 1999; Alvarez et al., 2000; Azkue et al., 2001; Walker et al., 2001). Staining for mGluR5 is very dense in the superficial laminae, where it is thought to be present in both local neurons and primary afferent terminals. mGluR1 is expressed by neurons in deeper laminae of the dorsal horn and in the ventral horn. Immunostaining with an mGluR2/3 antibody (probably representing mGluR3) is concentrated in laminae IIi and III, and is located in both intrinsic neurons and primary afferent terminals. mGluR4 and mGluR7 are both concentrated in the superficial dorsal horn, with much of the receptor being associated with primary afferent terminals and some with local neurons.

1.5.3 Plasticity Involving Glutamate Receptors

1.5.3.1 AMPA Receptors

Central sensitisation of dorsal horn neurons, which contributes to both inflammatory and neuropathic pain states, shows certain similarities to long-term potentiation (LTP) (see Chapter 9). LTP in the hippocampus is thought to involve insertion of AMPArs into the affected synapses, leading to an increase in synaptic strength. Phosphorylation of the GluR1 subunit seems to be required for this form of activity-dependent insertion during hippocampal LTP, and this also results in increased current flow through the receptor. Several studies have therefore examined whether there is upregulation or phosphorylation of AMPAr subunits following noxious stimulation, induction of inflammation, or nerve injury, since such changes might be at least partially responsible for central sensitisation in the dorsal horn.

There is evidence for an increase in the mRNA or protein level of all four AMPAr subunits in the ipsilateral dorsal horn after various types of nerve injury (Harris et al., 1996; Popratiloff et al., 1998; Garry et al., 2003; Yang et al., 2004). Popratiloff et al. (1998) carried out post-embedding immunogold labelling with an antibody that recognises both GluR2 and GluR3 subunits on spinal cords following sciatic nerve transection and reported that the density of receptors was increased at synapses formed by Aδ D-hair afferents. However, the functional significance of this observation is hard to interpret, as these afferents will have lost their peripheral receptive fields due to axotomy.

Plasticity involving AMPArs has also been investigated following acute noxious stimuli or during inflammation. Zhou et al. (2001) examined AMPAr subunit mRNA levels after injection of CFA into one hindpaw, and reported that there were changes in the ipsilateral dorsal horn. mRNA for GluR1 was upregulated within the first few hours, while mRNAs for GluR2 and GluR3

showed a slower increase (5–24 hours after CFA injection). Fang et al. (2003) performed Western blots on tissue from both dorsal horns in rats that had received injections of capsaicin into the hindpaw. They observed an increase in the level of phosphorylation of the GluR1 subunit at two serine residues (S831 and S845) on the ipsilateral side, which developed within 5 minutes and lasted for 1 hour after the stimulus. They also reported an increase in staining in the ipsilateral dorsal horn with antibodies that recognised the two phosphorylated forms of GluR1. However, it is unlikely that this staining represents synaptic receptors, since these are generally masked by tissue fixation (see above). We have used antigen retrieval to investigate phosphorylation of GluR1 at synapses in the dorsal horn (Nagy et al., 2004a). We found that the basal level of S845 phosphorylation was very low, but that 10 minutes after injection of capsaicin into the hindpaw there was a significant increase in the level of GluR1-S845 at synapses in laminae I and II in the somatotopically appropriate part of the ipsilateral dorsal horn (Fig. 1.3a, b). In contrast, there was a significant basal

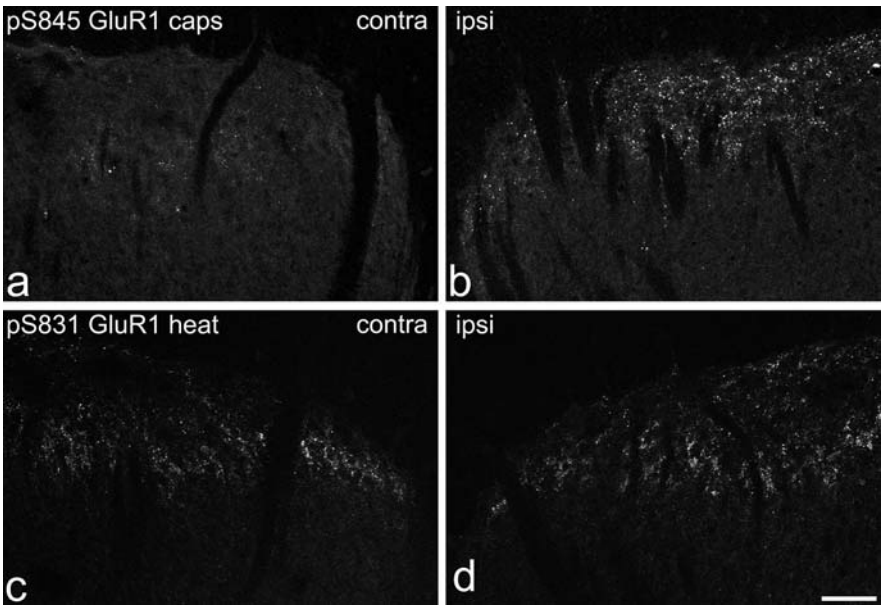

Fig. 1.3 Immunostaining for GluR1 phosphorylated at S845 **a**,**b** or S831 **c**,**d** residues following antigen retrieval. The confocal images show transverse sections from the L4 segment of rats that had received a capsaicin injection into one hindpaw **a**,**b** or had one hindpaw immersed in water at 52°C for 45 seconds **c**,**d**. In each case the stimulus was applied under terminal general anaesthesia 10 minutes prior to perfusion fixation, and the sections were reacted by an antigen-retrieval method that reveals synaptic receptors. **a**,**b**: Note that there is little phospho-S845-GluR1 on the contralateral (contra) side, but a relatively high level in laminae I and II on the ipsilateral (ipsi) side. **c**,**d**: there is significant basal level of phospho-S831-GluR1 (as seen on the contralateral side), and this is not altered following the stimulus. Scale bar = 50 μm. Reproduced from Todd (2008) with permission

level of GluR1 phosphorylated at S831, and this did not change after noxious stimulation (Nagy and Todd, unpublished observations) (Fig. 1.3c, d). We have also found that phosphorylation of GluR1 on S845 at synapses in laminae I–II can be induced by noxious thermal stimulation and that it begins within 5 minutes of the stimulus and lasts for at least 2 hours (E. Polgár and A.J. Todd, unpublished observations). This provides direct anatomical evidence for a type of plasticity involving AMPArs at glutamatergic synapses in the dorsal horn. Galan et al. (2004) observed a rapid increase in the level of GluR1 (but not of GluR2/3) in the plasma membrane fraction of dorsal horns from rats in a visceral pain model, and a similar finding was reported by Pezet et al. (2008) after formalin injection into the hindpaw. These results suggest that insertion of GluR1 subunits into dorsal horn synapses contributes to hyperalgesia.

1.5.3.2 NMDA Receptors

NMDA receptor subunits have numerous serine and tyrosine phosphorylation sites in their C terminal regions, and several studies have investigated phosphorylation of these in inflammatory or neuropathic pain states. NR1 can be phosphorylated at 3 sites, S890, S896 and S897, which appear in the C1 cassette (see above). Phosphorylation of these sites may result in increased or decreased clustering of the receptor at synapses (Chen and Roche, 2007). NR1 subunits in the Golgi apparatus and endoplasmic reticulum are highly phosphorylated, but they appear to be rapidly dephosphorylated following exit from the endoplasmic reticulum, suggesting a role for these phosporylation sites in intracellular trafficking (Scott et al., 2003). Zou et al. (2000) found that the number of spinothalamic tract neurons showing cytoplasmic staining with an antibody that recognises NR1 phosphorylated at the S897 site was increased in the ipsilateral dorsal horn following injection of capsaicin into one hindpaw, while Brenner et al. (2004) reported an increase in the number of neurons staining with antibody against NR1 phosphorylated at S896 after a noxious heat stimulus. There is also evidence that NR1 phosphorylation is increased following nerve injury, since Gao et al. (2005) showed that spinal nerve ligation led to an increase in the number of phospho-S897-NR1-immunoreactive neurons in the ipsilateral dorsal horn that lasted from 3 days to 4 weeks after the operation. These results provide evidence that trafficking of the NR1 subunit is altered in both acute and neuropathic pain states, although it is not yet clear whether this is reflected in an alteration in the number of NMDA receptors at synapses, or in their functional properties. An additional issue that needs to be considered is that of splice variants. Prybylowski et al. (2001) reported that only ~5% of the NR1 protein in the spinal cord contained the C1 cassette, which includes these phosphorylation sites. Although there may be significant regional differences within the grey matter, this suggests that the changes in phophorylation reported above may affect only a minority of NMDA receptors in the dorsal horn.

There is also evidence that NR2B subunits in the spinal cord are phosphorylated during inflammatory and neuropathic pain states (Guo et al., 2002; Abe

et al., 2005). NR2B has several potential phosphorylation sites and specific antibodies have been used to demonstrate phosphorylation of serine 1303 and tyrosine 1472 (Abe et al., 2005; Zhang et al., 2005; Pezet et al., 2008). Phosphorylation at these sites may result in retention of the receptors at the synapse and increased current flow. Abe et al. (2005) reported that after transection of the L5 spinal nerve, there was a substantial increase in the level of NR2B phosphorylated at tyrosine 1472 in the superficial dorsal horn, and showed with electron microscopy that some of this was located at synapses. Caudle et al. (2005) reported a progressive down-regulation of NR2B protein from the spinal cord during the five days following injection of carrageenan into the hindpaw, although Guo et al. (2002) found no change in NR2B protein level following inflammation induced with CFA.

1.5.3.3 Metabotropic Glutamate Receptors

There have been relatively few studies of plasticity involving mGluRs. Boxall et al. (1998) reported that ultraviolet-irradiation of one hindpaw sufficient to cause hyperalgesia was associated with a significant bilateral increase in the level of mRNA for mGluR3 in the dorsal horn, while Dolan et al. (2003, 2004) demonstrated an increase in mRNA and protein for both mGluR3 and mGluR5 in the dorsal horn of the sheep in cases of persistent inflammatory pain due to foot infection, and in a post-surgical pain model. Evidence for plasticity of mGluR expression following nerve injury has been provided by Hudson et al. (2002) who reported an increase in mGluR5 protein in A fibre somata in L4 and L5 DRG, the proximal sciatic nerve stump and the ipsilateral superficial dorsal horn after sciatic nerve transection. Following L5 spinal nerve ligation, they observed upregulation of mGluR5 not only in the affected ganglion, but also in the undamaged L4 DRG, and these increases occurred mainly in ganglion cells that gave rise to myelinated fibres. A recent study by Pitcher et al. (2007) has investigated possible redistribution of mGluR1 and mGluR5 in the ipsilateral dorsal horn following injection of CFA into one hindpaw. They found evidence for up-regulation of mGluR5 and a significant increase in the amount associated with the plasma membrane. Although there was no difference in the amount of mGluR1 labelling associated with the membrane, immunoparticles coding for mGluR1 were located significantly closer to synapses. These results therefore suggest that there is trafficking of the group I mGluRs towards the plasma membrane and towards synaptic active zones, presumably resulting in increased efficiency of synaptic transmission involving these receptors.

1.6 Concluding Remarks

The ionotropic and metabotropic receptors for glutamate and the NK1r for SP undergo plastic changes in the dorsal horn of the spinal cord in models of acute as well as chronic pain. Given the important roles of both glutamate and

substance P in nociceptive signalling in the dorsal horn, these changes are likely to contribute to the central sensitisation of dorsal horn neurons that underlies hyperalgesia.

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Chapter 2
Trophic Factors and Their Receptors in Pain Pathways

John V. Priestley

Abstract Trophic factors play a key role in the plasticity of pain pathways. They shape the circuitry and neurochemistry of acute pain pathways and also contribute to changes that occur in chronic inflammatory and neuropathic pain. Adult dorsal root ganglion (DRG) neurons express neurotrophin receptors and localization studies have shown that different DRG subtypes express different receptors. Thus large diameter neurons (low threshold mechanoreceptors) express either trkB (the receptor for BDNF) or trkC (the receptor for neurotrophin-3, NT-3) and small diameter neurons express trkA (the receptor for nerve growth factor, NGF). However the trkA expression is confined to the population of nociceptors that constitutively express neuropeptides (peptidergic nociceptors). Another population of nociceptors exists (non-peptidergic nociceptors) which normally do not express neuropeptides, which can be identified using the lectin *Griffonia simplifolia* IB4, and which express receptor components for GDNF. After nerve injury or inflammation major changes take place that contribute to the development of chronic pain and many of these changes appear to be driven by changes in the availability of growth factors. The role of NGF in such changes has been well documented but less is known about the role of GDNF. However there is growing evidence that endogenous GDNF contributes to inflammatory pain, and that exogenous GDNF can be used to treat neuropathic pain. In each case the GDNF effects are mediated primarily by the non-peptidergic (IB4) population of nociceptors. There is also evidence that neuropoetic cytokines act on non-peptidergic nociceptors.

J.V. Priestley (✉)
Barts and The London School of Medicine and Dentistry, Institute of Cell and Molecular Science, Neuroscience Centre, Queen Mary University of London, 4 Newark Street, Whitechapel, London E1 2AT, UK
e-mail: j.v.priestley@qmul.ac.uk

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