MECHANISMS OF NEUROKININ$_1$ RECEPTOR ACTION IN THE DORSAL HORN OF THE SPINAL CORD

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DECLARATION

I hereby declare that the composition of this thesis and the work presented in it is entirely my own with the exception of the electrophysiological experiments, which were done in collaboration with Dr. S.M. Fleetwood-Walker, and neuropathic tissue collection and behavioural testing which was carried out by Dr. S.W.N Thompson. Some of the studies have been published and appear as reprints in an appendix.

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CONTENTS

ABSTRACT

This study addressed the role of neurokinin$_1$ (NK_1) receptors in nociceptive transmission and their participation in a series of events involving glycine and NMDA receptor-mediated effects on spinal neurones. Using an *in vivo* electrophysiology protocol utilising ionophoresis and extracellular recording from laminae III-V dorsal horn neurones of anaesthetised rats, the mechanisms of these interactions were assessed.

The functions of the inflammatory cytokine leukaemia inhibitory factor (LIF) were also considered. Injury-induced alterations in the spinal expression pattern of this factor and the consequences of these changes to neuropeptide and excitatory amino acid expression were measured using *in situ* hybridisation.

1. The involvement of NK_1 receptors in spinal pain transmission may be dependent upon the duration and intensity of the nociceptive stimulus. Since activation of spinal NK_1 receptors leads to increases in the concentration of glycine in the dorsal horn, the role of the inhibitory glycine receptor as a regulator of NK_1 receptor function was investigated. Ionophoretic application of GR82334, a selective NK_1 receptor antagonist did not alter activity evoked by cutaneous applications of mustard oil. However in the presence of the glycine antagonists strychnine or phenylbenzene-ω-phosphono- α-amino acid (PMBA), GR82334 displayed inhibitory properties. Therefore inhibitory glycine receptors may mask the contribution made by NK_1 receptors to nociceptive processing. This is discussed with reference to the role of NK_1 receptors during brief nociceptive transmission.

2. NK_1 receptors can contribute to the processing of sustained nociceptive stimuli by modulating excitatory amino acid-mediated transmission, particularly through potentiation of NMDA receptor activity. Experiments were carried out to investigate the possible role of the glycine site of the NMDA receptor (Gly_{NMDA}) and of protein kinase C (PKC) activation to the NK_1/NMDA interaction.

Ionophoresis of the NMDA receptor agonist 1-aminocyclobutane-*cis*-1,3-dicarboxylic acid (ACBD) produced a sustained increase in the firing rate of dorsal horn neurones that was facilitated by the NK_1 agonist acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-SP_{6-11} (Sar^9-SP). Gly_{NMDA} site antagonists 2-carboxy-4,6-dichloro-(1H)-indole-3-propanoic acid (MDL 29951), 7-chloro-3-(cyclopropylcarbonyl)-4-hydroxy-2(1H)-quinoline (L701,252), 5,7-dinitroquinaxoline-2,3-dione (MNQX) and 7-chlorothiokynurenic acid (7-CTK) or PKC inhibitors, chelerythrine and GF109203X significantly reduced this facilitation whilst having no effect on activity driven by ACBD alone. Like the NK_1 receptor agonist, a selective Gly_{NMDA} site agonist 1-

aminocyclopropanecarboxylic acid (ACPC) also caused facilitation of ACBD-evoked activity and this was inhibited by the Gly_{NMDA} site antagonist L701,252 in a similar manner. These data suggest that NK_1 receptors facilitate NMDA receptor by potentiating the positive influence of the Gly_{NMDA} site. This may be bought about by the NK_1 receptor-induced glycine release and/or by a PKC mediated increase in the sensitivity of the Gly_{NMDA} site. This demonstrates that co-transmitters released from fine primary afferent fibres may interact postsynaptically to alter central hyperexcitability, particularly that associated with more prolonged noxious events.

3. LIF is a neuroactive cytokine that is associated with peripheral nerve injury. Using *in situ* hybridisation, the present study has examined the distribution of LIF mRNA in the spinal cord, normally or following peripheral inflammation or nerve injury and determined the consequences of intrathecally applied LIF on spinal expression of NK_1 receptor and the high affinity glutamate transporter, EAAT2.

In control animals, dorsal horn expression of LIF was high in superficial laminae I-II with only light expression in the deeper laminae III-V and in the ventral horn. Both peripheral inflammation and neuropathy significantly increased levels of LIF mRNA in the dorsal horn and this was most evident in the lateral parts of laminae I and II.

NK_1 and EAAT2 expression was normally associated with cells in laminae I-II, IV-V and ventral horn motorneurones. Intrathecal LIF administration significantly increased this expression for all three mRNAs and resulted in dense hybridisation throughout the dorsal horn.

These results show that LIF is normally expressed in the spinal cord in a specific laminar pattern that increases dramatically following peripheral inflammation or nerve damage. Furthermore, LIF upregulates the expression of NK_1 receptors and EAAT2 transporters indicating that it may be a critical regulator of the central changes that occur following peripheral injury.

Interactions within the spinal cord may underlie the plasticity of the dorsal horn in sensory processing. This has been discussed with reference to the regulation of short-term co-operation between NK_1 and NMDA receptors by glycine and to long-term modifications of peptide and excitatory amino acid neurotransmission by altered LIF gene expression.

ABBREVIATIONS

^{35}S-dATP	Deoxyadenosine [α-^{35}S]-triphosphate
7-CTK	7-chlorothiokynurenic acid
ACBD	1-aminocyclobutane-*cis*-1,3-dicarboxylic acid
ACPC	1-aminocyclopropanecarboxylic acid
ACPD	1-aminocyclopentane-1,3-dicarboxylate
ALS	Amyotrophic lateral sclerosis
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
AP7	2-amino-7-phosphonoheptanoic acid
APV	2-amino-5-phosphonovalerate
BDNF-LI	Brain derived neurotrophic factor-like immunoreactivity
CaM-KII	Calcium/calmodulin-dependent protein kinase II
CDF	Cholinergic nerve differentiation factor
CFA	Complete Freund's adjuvant
CGRP	Calcitonin gene related peptide
CNQX	6-cyano-2,3-dihydroxy7-nitroquinoxaline
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CNTFR	CNTF receptor
CPP	4-[3-phophonopropyl]-2-piperazine-carboxylic acid
CRE	Cytokine response element
DAGO	D-Ala2,NmePhe4,gly-ol5]enkephalin
DEPC	Diethyl pyrocarbonate
DH	Dorsal horn
DNIC	Diffuse noxious inhibitory control
DRG	Dorsal root ganglion
EAA	Excitatory amino acid
EAAT	Excitatory amino acid transporter
FA	Fast adapting
GABA	γ-aminobutyric acid
GFAP	Glial fibrillary acidic protein
Gly_{NMDA}	Glycine site of the NMDA receptor
GLYR	Strychnine-sensitive glycine receptor
GLYT	Glycine transporter
HRP	Horse radish peroxidase

IL-6	Interleukin-6
IL-6R	IL-6 receptor
Jak	Janus kinase
LCN	Lateral cervical nucleus
LI	Like immunoreactivity
LIF	Leukaemia inhibitory factor
LIF-IF	LIF-induction factor
LIF-R	LIF receptor
LTD	Long term depression
LTP	Long term potentiation
MAPK	Mitogen activated protein kinase
MDL 29951	2-carboxy-4,6-dichloro-(1H)-indole-3-propanoic acid
mGluR	Metabotropic glutamate receptor
MNQX	5,7-dinitroquinaxoline-2,3-dione
NGF	Nerve growth factor
NK_1	$Neurokinin_1$ receptor
NKA	Neurokinin A
NKB	Neurokinin B
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
OSM-R	Oncostatin M receptor
PBS	Phosphate buffered saline
PHA-L	*Phaseolus vulgaris* leuko-agglutinin
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PMBA	phenylbenzene-ω-phosphono- α-amino acid
PPT	Preprotachykinin
PSDC	Postsynaptic dorsal column
PTX	Pertussis toxin
rhLIF	Recombinant human LIF
RSA	Rat serum albumin
RT-PCR	Reverse transciption-polymerase chain reaction
SA	Slowly adapting
Sar^9-SP	Acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-SP_{6-11}
SCT	Spinocervical tract
SMT	Spinomesencephalic tract

SP	Substance P
SPOMe	SP methyl ester
SRT	Spinal reticular tract
SSC	Standard sodium citrate
STAT3	Signal transduction and activators of transcription 3
STT	Spinothalamic tract
TDT	Terminal deoxynucleotidyl transferase
TH-IR	Tyrosine hydroxylase-immunoreactive
Tm	Melting temperature
TNF	Tumour necroses factor
Trk A	Tyrosine kinase A
TTX	Tetrodotoxin
VH	Ventral horn
VIP	Vasoactive intestinal peptide
WDR	Wide-dynamic range

CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Understanding the neural mechanisms that contribute to the perception of sensation has been a principal task of sensory physiology. The category of cutaneous sensation has been central to this issue and since the early 19th century significant advances in the knowledge of brain systems have been made from this field.

Historically, two major theories on mechanisms of cutaneous sensation have been proposed, the specificity theory (Muller, 1842) and the pattern theory (Nafe, 1997). The specificity theory stated that separate specialised nerve endings accounted for each variety of the touch sensation on the basis that the skin could be mapped into distinct and fixed sensory spots of differing sensation (Goldscheider, 1987). Early histological evidence of several types of cutaneous sense organ were then used by the protagonists of this theory as conclusive proof. However, matches between sensory receptors and sensory spots proposed by Von Frey (1906) were found to be incorrect and the finding that most tactile stimuli would activate a large number of sensory units led to the development of the pattern theory (Weddell and Miller, 1962). This theory states that sensory stimuli are encoded by trains of nerve impulses and a patterned sequence of nerve activity leads to a learned sensation (Sinclair, 1955), but the theory is flawed by histological evidence on the existence of specialised sensory receptors. A further modification, the gate control theory (Melzack and Wall, 1965) tried to account for this discrepancy by describing central summation of peripheral inputs. It was suggested that slowly conducting fibres signalling pain were under the segmental control of fast fibres that gated the information as a continuous process of filtering throughout the central nervous system (CNS). However, the neuronal circuit proposed by Melzack and Wall has been challenged and direct experimental evidence for the theory is contentious (Cervero and Iggo, 1980).

Currently the predominant understanding of cutaneous sensation relies on sensory channels where each modality of sensation depends upon information transmitted along these channels (Willis and Coggeshall, 1991). However, the idea of a hard-wired, modality-specific system of nerve fibres running from the periphery to cortex has attracted much controversy (Wall, 1996). For example, psychophysical studies have shown that the perceptual nature of a stimulus can be profoundly altered by the environmental situation (McGrath, 1994) and in clinical studies it is known that surgical lesions of pain pathways rarely produce lasting analgesic effects (Gybels and Sweet, 1989). This contextual nature of

sensory perception emphasises the plasticity of the somatosensory system and the complexity of its underlying mechanisms.

1.2 PERIPHERAL SENSORY NERVES AND RECEPTORS

Peripheral sensory nerves pass information on the intensity, modality and location of a stimulus to the spinal cord. These nerves have been classified according to their conduction velocity and hence the diameter and myelination properties of their axons. In cutaneous nerves, the largest sensory axons belong to the Aβ class conducting at 30-100m/s, the small myelinated fibres are called Aδ fibres (4-30m/s) and unmyelinated nerves are designated as C fibres (less than 2.5m/s) (Willis and Coggeshall, 1991).

A different terminology is used for nerves innervating muscle and joint where myelinated axons are divided into groups I. II and III and unmyelinated axons, group IV (Willis and Coggeshall, 1991).

The threshold for electrical excitation of sensory axons is inversely proportional to their diameter. It is therefore possible to experimentally activate Aβ fibres alone, all classes of A fibre, or all A and C fibres simulating different intensities and modalities of natural activation. However a further classification of sensory nerves based on receptor properties gives a more accurate definition of the modality of the fibre.

Cutaneous receptors can be classified into mechanoreceptors, nociceptors and thermoreceptors. Here only mechanoreceptors and nociceptors will be considered.

1.2.1 Mechanoreceptors

Mechanoreceptors are activated by mechanical and occasionally thermal stimuli. They can be organised as slowly adapting receptors that discharge continuously during stimulation and signal stimulation pressure, and rapidly adapting receptors discharging only when a stimulus is applied and signalling the rate of stimulation or transition (Burgess and Perl, 1973).

There are 2 types of slowly adapting (SA) mechanoreceptors associated with different receptor structures. SA1 receptors are linked to dome-like Merkel cell complexes (Iggo, 1963) and SA2 receptors have been identified with Ruffini endings located in the dermis (Chambers *et al.* 1972). Both of these signal skin deformation.

Rapidly adapting (FA) mechanoreceptors can be further divided upon their location in either hairy or glabrous skin. There are at least 5 types of cell in hairy skin (tylotrich hairs, down hairs, field receptors and C mechanoreceptors) signalling velocity (the rate at which skin is displaced) and transition (the acceleration of the stimulus; guard hairs) and 2 types in glabrous skin: Meissner's corpuscles (Kruase's end bulb in the cat) for stimulus velocity

(FA1) and Pacinian corpuscles (FA2) for transients in skin indentation (Willis and Coggeshall, 1991).

1.2.2 Nociceptors

Nociceptors are free nerve endings that are activated by stimuli that threaten or actually damage tissue. There are 2 major types of nociceptor, Aδ mechanical nociceptors that respond to mechanical stimuli that damage the skin (Burgess and Perl, 1967) and C polymodal nociceptors that are activated by noxious mechanical, thermal and chemical stimuli (Bessou and Perl, 1969). There are also other types of less common nociceptor including the C mechanical nociceptor (Bessou and Perl, 1969) and the Aδ mechanoheat nociceptor (Iggo and Ogawa, 1971). In addition to their transduction of noxious stimuli many of these receptors may also signal in the innocuous range at lower intensities (Willis and Coggeshall, 1991).

The response properties of sensory neurones are therefore dependent upon a number of factors including their degree of myelination, their axonal diameter and the type of sensory receptor they innervate. However, these responses do not necessarily reflect the perception of a given stimulus and it is only when the stimulus can be shown to activate a certain fibre and activity in the fibre correlates with the perceptive quality of the stimulus that the specificity of the system can be proven (Sinclair, 1981). Recently this assumption was tested using intraneural microneurography in human subjects (Torebjörk and Ochoa, 1980). A single peripheral nerve was stimulated with a microelectrode and its psychophysical properties investigated. The electrode was then switched to recording mode and the receptive field, response properties and conduction velocity of the sensory neurone analysed. Invariably, the referred receptive field matched with the recorded field and the evoked sensation correlated with the type of stimulation needed to activate that class of neurone. For example, repetitive stimulation of a SA1 fibre evoked the feeling of sustained pressure whereas FA2 fibres (Pacinian corpuscles) evoked a vibration sensation. Importantly, it was also found that pain could not be generated from mechanoreceptors and required the activation of either Aδ mechanical nociceptors (a sharp pain) or C polymodal nociceptors (a dull burning pain). These experiments provided substantial evidence that primary afferents are specific to both stimulus and evoked sensation.

From the periphery, sensory neurones extend to their cell bodies at the dorsal root ganglion (DRG). DRG cells are usually divided into two categories, large clear cells and small dark cells and these categories correlate with axon diameter and function (Ramon y Cajal, 1909). This has led to the characterisation of peptide and enzyme morphological markers for these

cells on the assumption that differential labelling of small or large cells may help elucidate a functional role for the markers.

Extending from the DRG to the CNS, the processes of primary afferents are referred to as dorsal root axons and these branch into a series of rootlets as they approach the cord. Here, primary afferents terminate in a strict topographical and organisational pattern in the dorsal horn of the spinal cord.

1.3 THE DORSAL HORN

The dorsal horn is the first synapse for incoming sensory information (Figure 1.1). The area is rich in interneurones and descending inputs exerting both positive and negative effects and initial processing and modulation of sensory signals may occur here. There are 3 major functional types of neurone present in the dorsal horn; low threshold (class I) which respond to only innocuous information, wide-dynamic range (WDR or class II) which respond to both innocuous and noxious stimulation and high threshold (class III) neurones which are activated by only noxious inputs (Mendell, 1966; Handwerker *et al.* 1975; Cervero *et al.* 1976).

The dorsal horn has a laminar structure that was initially described by Rexed (1952) in the cat (Figure 1.2). This anatomical classification has since been correlated with physiological and pharmacological observations and it appears that these laminae are divided on both structural and functional properties. This study will concentrate on the organisation of lumbar spinal cord and hence on laminae I-VI which extend from the dorsal surface of the grey matter towards the central canal.

1.3.1 Lamina I

The neurones of lamina I can be divided into small-unclassified cells and larger marginal cells that have wide ranging dendrites often elongated in the rostrocaudal direction (Light *et al.* 1979). These neurones respond chiefly to noxious stimuli, although some WDR neurones are also present , and in agreement with this, electrical activation of Aδ and C fibres brings about activity in lamina I (Christensen and Perl, 1970; Handwerker *et al.* 1975; Cervero *et al.* 1976; Menetrey *et al.* 1977; Rethelyi *et al.* 1983). Anatomical tracing studies using retrograde transport of horseradish peroxidase (HRP) or more recently a plant lectin, phaseolus vulgaris leuko-agglutinin (PHA-L), have also shown that primary afferent innervation of lamina I consists mainly of small myelinated and unmyelinated fibres (Light and Perl, 1979; Gobel *et al.* 1981; Sugiura *et al.* 1986).

1.3.2 Lamina II

Lamina II, or the "substantia gelatinosa" is characterised by many small cells giving it a gelatinous appearance (Cervero and Iggo, 1980). These cells have been divided into the outer most stalked cells and the more central islet cells (Gobel, 1975; Gobel, 1978). At the synaptic level, neurones of this and deeper laminae often consist of glomeruli – morphological structures consisting of a central primary afferent terminal surrounded by dendrites and other axonal terminals (Ralston, 1968; Ralston, 1971). These structures allow both presynaptic and postsynaptic modification of primary afferent input and may contribute to modulation of information transfer in the dorsal horn.

Histological studies indicate that unmyelinated fibres form most of the primary afferent input into lamina II (LaMotte, 1977; Rethelyi, 1977; Ralston and Ralston, 1979; Sugiura *et al.* 1989) and accordingly electrical stimulation of C-fibres activates lamina II neurones (Kumazawa and Perl, 1976; Kumazawa and Perl, 1978). However many groups report Aδ and even Aβ activation of lamina II, although longer latencies indicate that this may be due to polysynaptic activation (Bennett *et al.* 1979; Wall *et al.* 1979; Fitzgerald, 1981). Natural stimulation of lamina II cells supports the above evidence but some responses may be subject to more complex form of regulation. For example habituation to repeated stimulation, strong inhibition and fluctuating receptive field sizes are sometimes seen (Wall *et al.* 1979).

1.3.3 Lamina III-VI

Neurones in laminae III-VI increase in size towards the deeper dorsal horn. Lamina III contains many small cells whilst lamina IV has a more heterogeneous population, laminae V and VI contain fewer but larger neurones (Ramon y Cajal, 1909; Rexed, 1952). A characteristic of many of these cells is their dorsally directed projections to laminae I and II and these interlaminar neurones allow direct input from superficial to deeper laminae (Willis and Coggeshall, 1991).

Primary afferent input into these areas is predominantly from large myelinated fibres. Lamina III and IV receive both Aβ and Aδ fibres and consequently most neurones are class 2 WDR (Kumazawa and Perl, 1977; Menetrey *et al.* 1977). Many of these myelinated fibres have specific patterns of innervation; for example inputs from large hair follicles follow a longitudinal plane whereas inputs from SAII mechanoreceptors are transversely orientated (Brown, 1981). Laminae V and VI are also innervated by Aβ and Aδ fibres and both high threshold (class 3) and WDR neurones are found in this area (Kumazawa and Perl, 1977; Menetrey *et al.* 1977; Rethelyi *et al.* 1982). Afferents from visceral, joint and muscle also terminate in the deeper dorsal horn (Willis and Coggeshall, 1991).

1.4 ASCENDING SOMATOSENSORY PATHWAYS

Sensory information passes from the dorsal horn to the brain through ascending sensory pathways in the white matter. Historically these pathways have attracted both experimental and clinical interest and consequently a major strategy for pain relief has been to lesion specific parts of the ascending tracts.

If the spinal cord is divided into three parts through the cross-section a loosely defined anatomical and physiological classification of the pathways can be made. In the dorsal most division, the dorsal funiculus (or posterior column in humans) is generally thought to transmit discrimination, vibration and positional sense and surgical lesions of this area do not usually relieve pain (Nathan *et al.* 1986). Further laterally, the dorsal lateral funiculus (posterior lateral funiculus in humans) contains pathways that convey information similar to that transmitted in the dorsal funiculus, although in humans, lesions in this area produce little alteration in perception of sensations. However, in animals, combined interruptions of both the dorsal lateral funiculus and the dorsal funiculus produce more profound changes indicating that these areas act together to contribute to sensory discrimination (Nathan *et al.* 1986). The most ventral section, the ventral quadrant (the anterolateral quadrant in humans) contributes to pain and temperature sensation. This area has attracted the most interest from clinical research as lesions here may reduce chronic pain without affecting innocuous tactile sensation. However cordotomy often only produces temporary pain respite emphasising the general plasticity of these pathways and the ability of the spinal cord to redirect inputs after interruption (White and Sweet, 1969).

Specific pathways in each of these areas will be considered.

1.4.1 Sensory Pathways in the Dorsal Funiculus

The dorsal funiculus consists of branches of primary afferent fibres and second order neurones which belong to the postsynaptic dorsal column pathway (PSDC). The primary afferents enter the cord through the cervical and lumbar dorsal roots, turn rostrally and ascend to the dorsal column nuclei, and the PSDC system receives input from the spinal cord grey matter (Ramon y Cajal, 1909).

1.4.1.1 Postsynaptic Dorsal Column Pathway

Neurones in the PSDC project from laminae III-V of the dorsal horn to the nucleus gracilis and nucleus cuneatus (Giesler *et al.* 1984). These neurones have convergent inputs from low and high threshold cutaneous afferents and are best activated by innocuous stimuli and strong mechanical stimuli, however very few are activated by noxious heat in the rat indicating that this pathway is probably not involved in nociception in this animal (Giesler and Cliffer, 1985). In the cat, PSDC neurones may be low threshold, WDR or high threshold

cells responding to innocuous and noxious mechanical or thermal stimuli and these cells have receptive fields on both glabrous and hairy skin (Angaut-Petit, 1975; Brown *et al.* 1983).

1.4.2 Sensory Pathways in the Dorsal Lateral Funiculus

The dorsal lateral funiculus consists of two major pathways, the spinocervical and spinomedullary tract. Since the spinomedullary tract is mainly concerned with type I muscle afferents only the spinocervical tract will be considered here.

1.4.2.1 Spinocervicothalamic Tract

The spinocervical tract (SCT) originates in the dorsal horn and terminates in the lateral cervical nucleus (LCN). Neurones from this area then project to the contralateral thalamus, the midbrain or both (Morin, 1955). Using retrograde labelling of HRP injected into the LCN, it has been shown that SCT cells initially synapse in laminae III-IV of the dorsal horn and then extend into the dorsal lateral funiculus (Brown *et al.* 1980).

SCT neurones are activated by electrical stimulation of cutaneous Aβ, Aδ and C fibres (Mendell, 1966) and by noxious and innocuous natural stimuli especially hair movement (Brown and Franz, 1969). However this tract is under tonic inhibitory modulation via descending fibres such that the proportion of nociceptive responses transmitted by the SCT increases dramatically when the descending inhibition is reduced (Kenshalo, Jr. *et al.* 1982). The receptive fields of these neurones are confined to the hairy skin in the cat and tend to be small with an adjacent inhibitory field (Hongo *et al.* 1968). This may serve to enhance the contrast of inputs and contribute to the tactile discrimination of the very small hair movements.

1.4.3 Sensory Pathways in the Ventral Quadrant

There are three major pathways in the white matter of the ventral quadrant of the spinal cord. These are the spinothalamic, spinoreticular and spinomesencephalic tracts.

1.4.3.1 Spinothalamic Tract

The spinothalamic tract (STT) passes sensory information from the dorsal horn to the thalamus. Using HRP tracing methods, it has become apparent that there are 3 components of the STT at the lumbosacral level: A projection that originates in lamina I and extends to the thalamus via the contralateral dorsal lateral funiculus (Willis and Leonard, 1978), a projection from laminae IV- VI that travels via the contralateral ventral quadrant to the thalamus (Giesler *et al.* 1981) and a third component that arises from laminae VII-X and again ascends through the contralateral ventral quadrant (Giesler *et al.* 1981; Eccleston,

1995). Neurones from all 3 components are activated by Aβ, Aδ and C fibres but receptive field properties differ (Foreman *et al.* 1975; Chung *et al.* 1979). Lamina I STT cells have small cutaneous receptive fields and are mostly WDR types with a subpopulation being thermoreceptive (Willis *et al.* 1974; Applebaum *et al.* 1975), whereas Laminae IV-VI STT cells have larger receptive fields and are usually WDR or sometimes high threshold or low threshold cells (Chung *et al.* 1979; Giesler *et al.* 1981).

Due to their putative involvement in pain transmission, STT neurones have attracted additional attention as potential analgesic targets. Initial attempts to relieve chronic pain by surgically lesioning the anterolateral quadrant in humans have produced mixed results. The current consensus is that this form of analgesia is usually only temporary and is often replaced by neurogenic pain of a different and sometimes worse quality (White and Sweet, 1969; Tasker and Dostrovsky, 1989). However STT cells are still potential pharmacological targets and demonstrations that these cells can become sensitised to repetitive input and contribute to behavioural hyperalgesia emphasises the likely importance of this tract in nociceptive transmission (Kenshalo *et al.* 1979).

1.4.3.2 Spinoreticular Tract

The spinal reticular tract (SRT) projects from the spinal cord to the brain stem reticular formation, which in turn relays information to the thalamus. Most SRT cells are contralateral to their reticular formation targets and are located in Laminae V, VII, VIII and X with a few in laminae I and VI (Kevetter and Willis, 1983). SRT cells receiving cutaneous input have restricted receptive fields and may be low threshold, high threshold or WDR neurones. Many SRT cells have visceral, muscle or joint inputs (Fields *et al.* 1977).

1.4.3.3 Spinomesencephalic Tract

Spinomesencephalic tract (SMT) cells project from the dorsal horn to the mesencephalic reticular formation and the periaquaductal grey matter with some collaterals reaching the thalamus. There are 2 populations of SMT cells, one that extends from laminae I and the lateral spinal nucleus through the contralateral dorsal lateral funiculus and another that originates in laminae V, VII and X and travels through the contralateral ventral quadrant (Menetrey *et al.* 1982; Yezierski and Mendez, 1991). SMT cells may be low-threshold, high threshold or WDR neurones and many of these also respond to noxious heat (Menetrey *et al.* 1980; Yezierski and Broton, 1991).

1.5 Descending Inputs to the Dorsal Horn

In addition to primary afferents, intrinsic interneurones and ascending fibres, the dorsal horn has a significant population of neurones descending from the brain. These exert important modulatory actions and allow higher levels of the CNS to influence sensory input. Some of these neurones may have excitatory actions but greater attention has been paid to descending inhibitory control, particularly tonic inhibition of dorsal horn nociceptive interneurones. Using reversible cold block, properties of dorsal horn neurones can be examined before and after the block to ascertain the degree of inhibition (Brown, 1971). It has been found that nociceptive responses of neurones in laminae I and IV-VI (Wall, 1967; Besson *et al.* 1975; Handwerker *et al.* 1975; Cervero *et al.* 1976; Duggan *et al.* 1981) but not those in lamina II are subject to inhibitory control (Cervero *et al.* 1979). Lesion studies have identified an important source of this inhibition as the nucleus paragigantocellularis lateralis and microinjection of a GABA agonist in this area reduces the inhibition (Foong and Duggan, 1986).

Inhibition can also be evoked in a phasic manner by stimulation of many sites in the brain including the periaquaductul grey, the raphe nuclei and the locus coeruleus. These supraspinal sites exert their influence via specific pathways and are often analgesic (Reynolds, 1969; Olivéras *et al.* 1974; Segal and Sandberg, 1977).

An important supraspinal loop that brings about a powerful and selective inhibition known as Diffuse Noxious Inhibitory Control (DNIC) has been observed by Le Bars *et al.* (1979a and 1979b). They reported that inhibition of WDR neurones occurs after noxious stimuli are applied to any part of the body or face. It is specific to WDR neurones, is graded according to stimulus intensity and depends upon ascending pathways in the ventrolateral quadrant (Villanueva *et al.* 1986b) and descending pathways in the dorsal lateral funiculus (Villanueva *et al.* 1986a). This form of descending control may enhance the contrast of noxious signals reaching the brain, as DNIC will suppress the activity of WDR neurones with inactive receptive fields.

The pharmacology of descending inhibition depends upon several inhibitory neurotransmitters. These include the opioid peptides, serotonin, noradrenaline, dopamine and the inhibitory amino acids GABA and glycine. Descending pathways probably exert much of their influence indirectly through excitation of interneurones emphasising the complexity of the chemical anatomy of these projections (Besson and Chaouch, 1987).

FIGURE 1.1

Schematic organisation of the neuronal organisation of the superficial dorsal horn and its afferent input

A hypothetical transverse section of the dorsal horn is represented, showing the types of neurones and afferent fibres that terminate there. The receptors, which are associated with each fibre type, are listed on the left of the diagram. Indicated on the right are the laminar divisions of Rexed (1952), along with the corresponding anatomical nomenclature. The neurones schematised are typical of those found in each lamina: (*from top to bottom*) a marginal cell, and SG limiting cell, two SG central cells and two neurones of the nucleus proprius, the most superficial of which has dendrites penetrating lamina II (Cervero and Iggo, 1980).

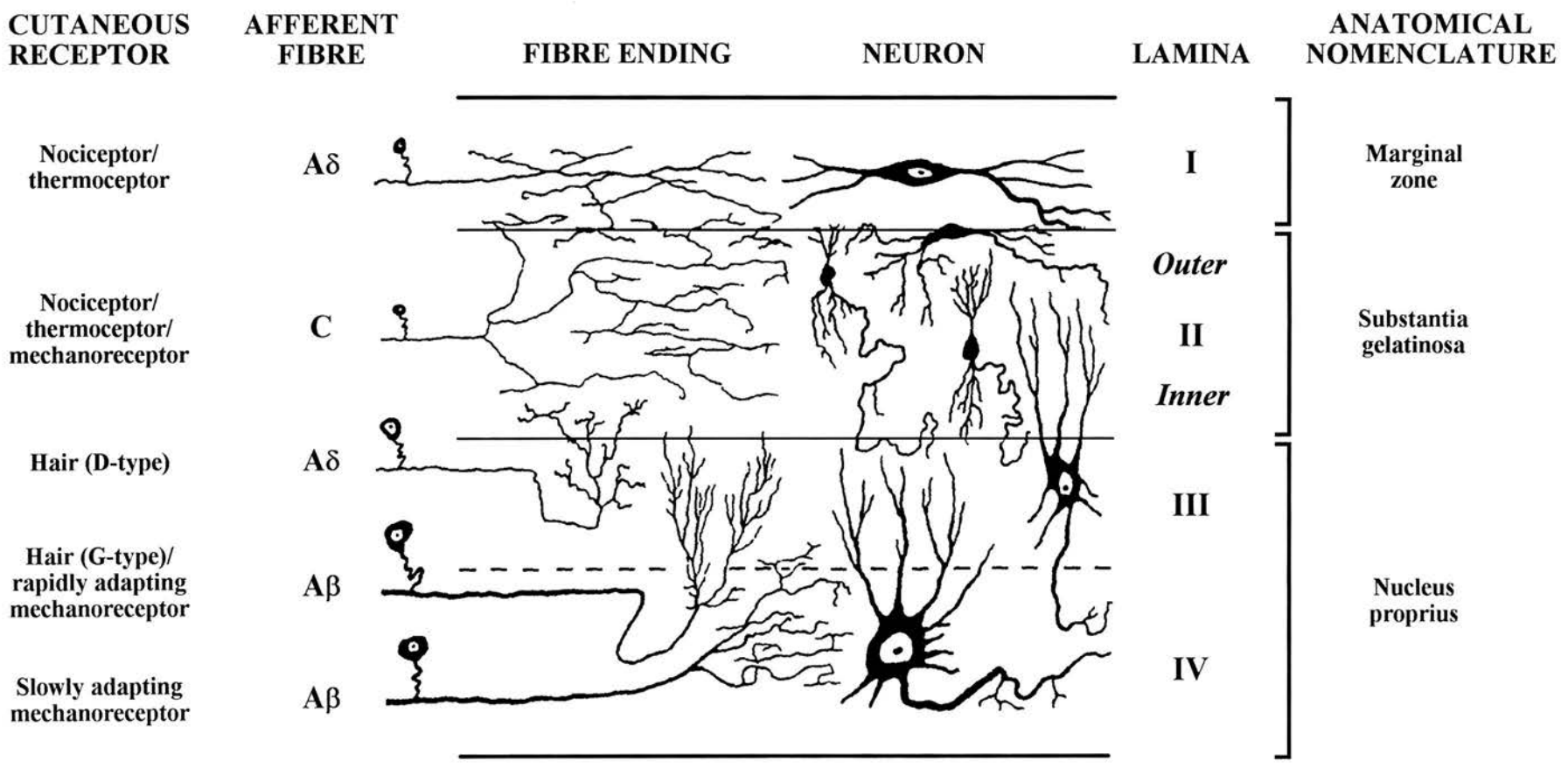
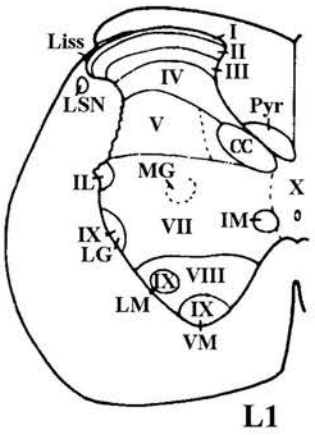

CUTANEOUS RECEPTOR
AFFERENT FIBRE
FIBRE ENDING
NEURON
LAMINA
ANATOMICAL NOMENCLATURE
Nociceptor/ thermoceptor
Aδ
I
Marginal zone
Nociceptor/ thermoceptor/ mechanoreceptor
C
Outer
II
Inner
Substantia gelatinosa
Hair (D-type)
Aδ
III
Hair (G-type)/ rapidly adapting mechanoreceptor
Aβ
Nucleus proprius
Slowly adapting mechanoreceptor
Aβ
IV

FIGURE 1.2

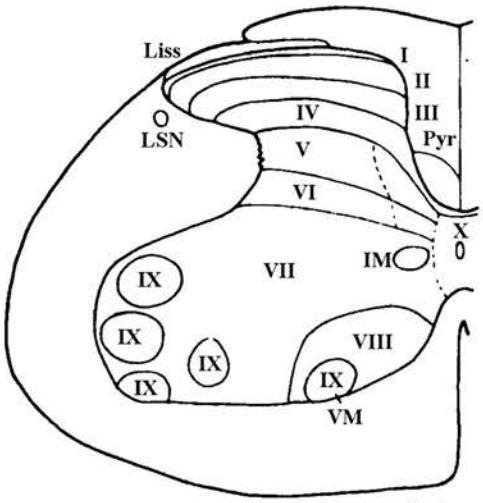
Schematic drawings of the cytoarchitectonic subdivisions of the segments L1-L6 of the rat spinal cord

The laminar divisions described by Rexed (1952) in the cat spinal cord can also be seen in the rat (Molander *et al.* 1984). The dorsal horn consists of laminae I-VI.

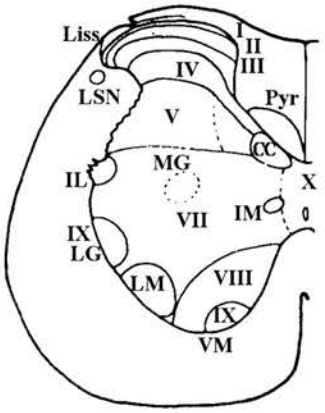
CC, column of Clarke; IL, intermedio-lateral nucleus; IM, intermedio-medial nucleus; LSN-lateral spinal nucleus; Liss, Lissauer's tract; LG-lateral group of large cells in the dorso-lateral part of the ventral horn; LM, latero-medial nucleus; MG, medial group of large neurones in the intermediate zone.



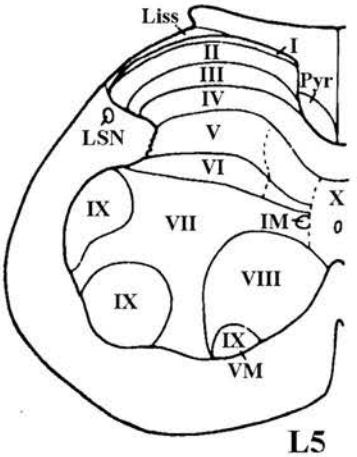
L1



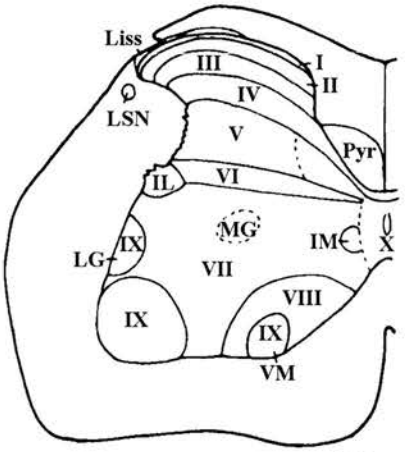
L4



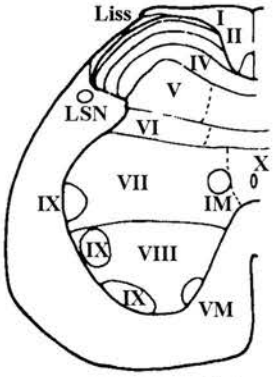
L2



L5



L3



L6

1.6 HYPERALGESIA AND SENSITISATION

The nociceptive system is unique amongst sensory systems in that is has the capacity to sensitise to repetitive stimulation. Nervous system responses are enhanced by continued activity and this leads to behavioural and perceptual changes with a corresponding increase in the intensity of the perceived pain. This behavioural response is called hyperalgesia and its physiological correlate is termed sensitisation.

Hyperalgesia is defined as a lowered pain threshold and an increased response to suprathreshold stimuli, similarly sensitisation is characterised by a lowered neuronal response threshold and enhanced responses in the suprathreshold range (Treede *et al.* 1992). Hyperalgesia can be further divided into primary and secondary types (Lewis, 1942; Hardy *et al.* 1950) where primary hyperalgesia refers to an enhancement at the site of injury and secondary hyperalgesia refers to changes in the surrounding undamaged skin. These types have different qualitative aspects with regard to the modality of the perceived pain such that primary hyperalgesia is characterised by enhanced responsiveness to mechanical and heat stimuli and secondary hyperalgesia is an enhancement to only mechanical stimuli. Consequently they may also utilise different neuronal mechanisms.

1.6.1 Primary Hyperalgesia

Primary hyperalgesia to heat can be accounted for by the sensitisation of peripheral nociceptors (Reeh *et al.* 1986). In hairy skin, this occurs predominantly in C-fibres (LaMotte *et al.* 1982) whereas in glabrous skin, Aδ mechanical nociceptors become sensitised and expand their modality range to include heat (Campbell *et al.* 1979). This sensitisation is believed to occur through a chemical cascade involving many inflammatory mediators including histamine, serotonin, bradykinin, substance P and prostaglandins and have been collectively referred to as an "inflammatory soup" (Handwerker and Reeh, 1991).

In contrast, there is less evidence to suggest that nociceptor sensitisation can fully account for primary hyperalgesia to mechanical stimulation. Whilst some investigators report that mechanical sensitisation can be induced by tissue acidosis or injection of inflammatory mediators (for review see Meyer *et al.* 1994) the majority of studies suggest that quantitatively, nociceptor sensitisation cannot readily account for the large changes in mechanical threshold observed in primary hyperalgesia (see Treede and Magerl, 1995). Several alternative mechanisms have been suggested, such as expansions of the receptive fields of Aδ fibres to include initially unresponsive branches (Reeh *et al.* 1987), recruitment of previously silent afferents that become sensitised and then respond to mechanical stimulation (Davis *et al.* 1990), and changes in the response properties of neurones in the CNS (Laird and Cervero, 1989).

1.6.2 Secondary Hyperalgesia

The mechanism of secondary hyperalgesia has historically been the subject of controversy. Arguments have been based upon the location of the mechanism and whether it occurs in the peripheral nervous system or centrally. The major protagonist for a peripheral site was Lewis (1942) and using evidence from a series of nerve block experiments he argued for the existence of a network of "nocifensor" nerves that release substances and sensitise nociceptors adjacent to the injury site. This theory accords well with the phenomenon of neurogenic inflammation where cutaneous vasodilatation and inflammation occurs after antidromic stimulation (Chapman *et al.* 1961). However, secondary hyperalgesia reflects an increase in sensitivity to mechanical stimulation and most evidence implies that nociceptors cannot be sensitised in this way. In agreement with this, Reeh *et al.* (1986) have demonstrated that mechanical sensitisation does not occur after antidromic nerve stimulation. Furthermore, it has recently become apparent that there is no anatomical evidence for a nocifensor network of nerves and very little support for the idea that secondary hyperalgesia results from sensitised primary afferents. Instead, current opinion supports the ideas of Hardy *et al.* (1950) who proposed that secondary hyperalgesia is mediated via enhanced sensitivity of central neurones.

Indirect evidence for central sensitisation was first demonstrated by Woolf who reported changes in the excitability of the hamstring flexor withdrawal reflex after conditioning stimuli were applied to C-fibres from different origins (Woolf and Wall, 1986). This work was followed up with studies recording directly from dorsal horn neurones where it was found that sustained activation of C-fibres modified both the receptive field size and response properties of central neurones for a prolonged period of time (Cook *et al.* 1987).

The perceptual counterpart of central sensitisation is an enhanced sensitivity to mechanical stimulation where low intensity input becomes noxious. For this to occur in the absence of nociceptor sensitisation there must be either an increased summation of nociceptor input by central neurones or an enhanced or altered connectivity of low threshold mechanoreceptor connections in the dorsal horn. Evidence from a study by Torebjork *et al.* (1992) supports the latter. Using intraneural microstimulation in humans they demonstrated that weak electrical stimulation of low threshold mechanoreceptors elicited a tactile sensation in the projected receptive field. When this field became part of a zone of secondary hyperalgesia, the tactile sensation was accompanied by pain. This indicated that low threshold mechanoreceptors were transmitting painful information and mechanoreceptors might have access to high threshold and/or WDR central neurones during secondary hyperalgesia.

The principal mechanisms of secondary hyperalgesia therefore appear to lie in the plasticity of dorsal horn neurones. These neurones must be able to increase their receptive field size to accommodate the extended hyperalgesic zone and enhance their excitability to transmit

previously innocuous information as painful. It has been demonstrated that STT neurones maintain these properties and following peripheral injury high threshold neurones develop sensitivity to low-intensity mechanical input and WDR neurones increase their discharge (Simone *et al.* 1991). An essential component of this process may be the complicated pharmacology of the dorsal horn. Here, interactions between excitatory systems and disinhibition of negative inputs define the plasticity of these neurones and contribute to the behavioural changes.

Ma and Woolf (1996) recently described an extension of the demonstrations of Torebjork *et al.* on the enhanced connectivity of low threshold mechanoreceptors. They reported that following peripheral inflammation, light touch or electrical stimulation of rat Aβ fibres at 5 minute intervals brought about an incremental increase in the excitability of the spinal cord that was not seen in normal animals. They called this process progressive tactile hypersensitivity and stressed that it was quite different from central sensitisation in that it had a slow build up, relied on activity in Aβ fibres and was dependent on the presence of inflammation. Progressive tactile hypersensitivity was further characterised by this group and shown to be due to a nerve growth factor dependent phenotypic switch in myelinated sensory neurones (Ma and Woolf, 1997) such that they increased their expression and release of substance P in the same way as pain fibres (Neumann *et al.* 1996). This process may therefore contribute to the enhanced mechanical sensitivity seen in primary and secondary hyperalgesia.

Central sensitisation is similar to many other kinds of neuronal plasticity in the CNS such as hippocampal long-term potentiation (LTP) and ocular dominance, however there are some fundamental differences (Treede and Magerl, 1995). Most importantly, central sensitisation is a heterosynaptic event i.e. activity in nociceptors alters synaptic efficacy for inactive nonnociceptive contacts. In contrast LTP is homosynaptic and any heterosynaptic events must occur simultaneously and will result in long term depression (LTD). Similarly the type of stimulation required to induce LTP is vastly different from that required to induce central sensitisation; LTP requires repetitive trains of synchronous input at frequencies of about 100Hz whereas central sensitisation needs only C-fibre input at 1 Hz. Finally the time course of each type of potentiation is different. LTP may last for weeks after stimulation of only a few seconds, but central sensitisation is dependent on continued nociceptive input and decays within hours of cutaneous nerve stimulation. The mechanisms of hyperalgesia are therefore relatively short and unstable and this is reflected in the physiological role of this phenomenon, i.e. to promote wound healing by protecting the injured site from further damage for only the duration of the injury. However there are certain pathological situations such as neuropathic pain where the pain outlasts the injury and these may also to be due to altered sensory processing in the periphery and CNS.

Hyperalgesia is a lowered threshold and enhanced response to painful stimulation and is caused by both peripheral and central mechanisms. In the CNS, the plasticity of dorsal horn neurones contributes to the process and this is mediated via pharmacological processes such as interactions between fast and slow excitatory transmission and disinhibition of negative inputs. These regulatory aspects will now be discussed with reference to excitatory amino acids, tachykinin transmitters and the inhibitory amino acid glycine.

1.7 GLUTAMATE AS A NEUROTRANSMITTER

There is now considerable evidence that glutamate and other excitatory amino acids (EAAs) such as aspartate are major neurotransmitters eliciting fast excitatory responses in the CNS (Watkins and Evans, 1981; Mayer and Westbrook, 1987). The first demonstration that glutamate could depolarise neurones was made in the spinal cord of the cat by Curtis *et al.* (1960), however technical difficulties associated with the extensive involvement of glutamate in cellular metabolism initially hindered its establishment as an excitatory neurotransmitter. Despite this, histochemical techniques including glutamine uptake assays (Duce and Keen, 1983) and immunohistochemical localisation of glutaminase (Cangro *et al.* 1985), glutamine or glutamate itself (Wanaka *et al.* 1987a; Battaglia and Rustioni, 1988a) initially demonstrated higher levels of glutamate throughout primary afferents fibres and dorsal horn interneurones.

In DRG cells, glutamate has been localised immunologically in both small cells and large cells (Wanaka *et al.* 1987a; Battaglia and Rustioni, 1988a). More general evidence such as higher concentrations of glutamate-like immunoreactivity (-LI) in unmyelinated dorsal root axons (De Biasi and Rustioni, 1988; Westlund *et al.* 1989), confinement of glutaminase immunoreactivity to smaller cells (Cangro *et al.* 1985) and the specific accumulation of radioactive glutamine by this subpopulation (Duce and Keen, 1983) indicated a more prominent role for glutamate in small cells. Similarly, in the dorsal horn, glutamate terminals, axons and cells were found in laminae I-II (Greenamyre *et al.* 1984; Magnusson *et al.* 1986; De Biasi and Rustioni, 1988; Broman *et al.* 1993) the area of termination for fine primary afferents. Using double-labelling immunocytochemistry, Battaglia and Rustioni (1988b) showed that staining for the metabolic marker cytochrome oxidase did not correlate with glutamate staining and, glutamate-LI was seen in synaptic vesicles in the central terminals of glomeruli (Maxwell *et al.* 1990). Overall this evidence supports a neurotransmitter function for glutamate beyond its metabolic roles.

In agreement with the histochemical data, electrophysiological experiments also implicated a role for glutamate as a spinal neurotransmitter. Since Curtis *et al.* first demonstrated that ionophoretic application of glutamate produced an excitation of dorsal horn neurones, many

groups have reported similar excitation both *in vivo* and *in vitro* (Zieglgänsberger and Herz, 1971; Davies and Watkins, 1983; Peet *et al.* 1983; Jahr and Jessell, 1985; Schneider and Perl, 1985; King *et al.* 1988; Yoshimura and Jessell, 1990). Interestingly, these responses were often most prominent in neurones concentrated in laminae I and II and Schneider and Perl (1985a and 1985b) demonstrated that these same neurones could be activated by C fibre volleys. However several of these groups reported that almost all spinal cord interneurones were responsive to glutamate. This discrepancy may be resolved by the possible influence of uptake systems on exogenously applied amino acid. Cells and synapses that are not affected by glutamate might be surrounded by uptake sites that control the concentration of exogenous agent and reduce the level of excitation. These sites could also exert their effects under more physiological conditions and participate in the control of glutamate neurotransmission (Headley and Grillner, 1990).

In addition to their fast effects, there is evidence that EAAs are also involved in long-term functional changes such as central sensitisation. Spinal application of glutamate produced an incremental increase in the activity of dorsal horn neurones that altered receptive field sizes (Zieglgänsberger and Herz, 1971) and enhanced neuronal responses to innocuous and noxious stimulation (Aanonsen *et al.* 1990b; Dougherty and Willis, 1991b). Similarly, dorsal horn neurones sensitised by peripheral inflammation showed increased responsiveness to ionophoretic application of EAAs (Dougherty and Willis, 1992) and intrathecal application of these compounds produced an increase in the excitability of flexor efferents (Woolf and Wiesenfeld-Hallin, 1985).

Taken together, the histochemical and electrophysiological data suggest that glutamate has a more pronounced role in the transmission of nociceptive responses and this is supported by experiments examining the release of EAAs and from behavioural evidence. For example, noxious simulation and peripheral inflammation raised levels of extracellular glutamate in the spinal cord (Skilling *et al.* 1988; Sorkin *et al.* 1992), and intrathecally applied EAAs also produced nociceptive behaviours, (Aanonsen and Wilcox, 1987) secondary hyperalgesia (Coderre and Melzack, 1991a) and enhanced formalin-induced behaviour (Coderre and Melzack, 1992). However evidence from some studies supports a role for glutamate in mediating low threshold inputs. This will be considered below with reference to the receptor subgroups involved.

1.7.1 Glutamate Transporters

A fundamental requirement for fast and accurate information processing and hence glutamatergic neurotransmission is the existence of a system to rapidly terminate glutamate action. Unlike many other neurotransmitters, there is no extracellular enzyme to do this and

instead a family of uptake carriers or transporters terminates the synaptic action of glutamate through removal from the synaptic cleft.

Currently, four glutamate transporters have been cloned to form a new gene family with significant differences from other transporter systems. Excitatory amino acid transporter 1 (EAAT1, also known as GLAST) and EAAT2 (or GLT-1) are located predominantly in glial cells in the CNS and disruption of these systems leads to a general rise in extracellular glutamate often resulting in excitotoxicity and cell death. EAAT3 (or EAAC1) is expressed in neurones throughout the brain and deletion of this molecule can cause epileptiform seizures. EAAT4, the most recently cloned transporter is located in the cerebellum but its cellular distribution is not yet known (Rothstein *et al.* 1994; Fairman *et al.* 1995; Rothstein *et al.* 1996).

All four glutamate transporters share similar mechanisms of glutamate uptake. Briefly, this is coupled to cotransport of two Na^+ into the cell with the counter transport of one K^+ and either the cotransport of one H^+ or the countertransport of one OH^- (Takahashi *et al.* 1997). Glutamate transfer may also activate a chloride conductance through the transporter channel that if located presynaptically, would presumably clamp the terminal at a relatively negative potential and further reduce glutamate release (Wadiche *et al.* 1995b; Wadiche *et al.* 1995b). During hypoxia or ischemia the fall in brain ATP levels leads to a slowing of the pump that may eventually result in a reversal and release of glutamate back into the extraceullar space (Attwell *et al.* 1993). In transient situations this process is slowed by the accompanying acidic pH (Billups and Attwell, 1996), but sustained ischemia can lead to excessive glutamate release and neuronal cell death. Similarly, neurodegeneration associated with amyotrophic lateral sclerosis (ALS) might also be due to glutamate excitotoxicity. Chronic excess glutamate has been shown to contribute to selective motorneurone degeneration as a result of impaired functioning of the EAAT2 type of transporter highlighting the importance of glutamate regulation and transmitter uptake (Smith and Dewey, 1992).

1.8 GLUTAMATE RECEPTORS

The actions of glutamate are mediated through several receptor subtypes. These are divided into the ionotropic ligand gated ion channels and the metabotropic G-protein linked receptors. Further classification based upon pharmacological analysis subdivides these into the ionotropic NMDA, AMPA and kainate receptors named after their selective agonists, and the metabotropic groups I-III, organised by signal transduction mechanisms. The molecular structure of all of these receptors has now been ascertained and this substantiates the pharmacological data.

1.8.1 Metabotropic Glutamate Receptors

Glutamate was initially thought to activate only ligand-gated ion channels. However, in 1985, it became apparent that glutamate was able to stimulate phospholipase C (PLC) presumably through a receptor coupled to a GTP-binding protein (Sladeczek *et al.* 1985). This was confirmed in 1987 using the *Xenopus* oocyte system (Sugiyama *et al.* 1987) and in 1991 the first metabotropic glutamate receptor (mGluR) was cloned with a functional expression screening procedure (Masu *et al.* 1991). Since then, the number of receptors has grown to include a family of eight, numbered sequentially as mGluR1 – mGluR8. MGluRs are similar to all other G-protein linked receptors in that they have 7 transmembrane domains. However, they are much larger and do not share any close sequence homology with that gene superfamily and therefore represent a new class of G-protein-coupled receptor (for review see Hollmann and Heinemann, 1994; Pin and Duvoisin, 1995).

The amino acid sequence of mGluRs reveals that they can be divided into three groups based upon sequence homology. Group I consists of mGluR1 and mGluR5, group II, mGluR2 and mGluR3 and group III, all the others. This classification is also supported by their pharmacology (Houamed *et al.* 1991; Abe *et al.* 1992; Tanabe *et al.* 1992) and respective transduction mechanisms. Group I receptors stimulate PLC via a pertussis toxin (PTX) insensitive G-protein (Houamed *et al.* 1991; Masu *et al.* 1991) resulting in an increase in phosphoinositide turnover and Ca^{2+} release from internal stores (Tanabe *et al.* 1992). In addition, these receptors may have auxiliary linkages to adenylyl cyclase signal transduction and arachadonic acid release (Aramori and Nakanishi, 1992). Group II and III mGluRs activate a PTX-sensitive G-protein and are linked to the inhibition of adenylyl cyclase (Abe *et al.* 1992). The activation of these second messenger systems may result in regulation of ion channels with either inhibitory or excitatory pre- and postsynaptic effects. These include, NMDA, AMPA and GABA-gated channels as well as voltage-gated Ca^{2+} channels and K^{+} channels, indeed mGluR agonists probably exert slow depolarisations and neuronal excitation through these types (Pin and Duvoisin, 1995).

MGluRs are distributed throughout the CNS with a degree of regionally specific expression for each subtype. For example, group I receptors are highly expressed in the hippocampus (Masu *et al.* 1991) and mGluR2 and mGluR4 are found in the cerebellum and olfactory bulb as is mGluR8 (Houamed *et al.* 1991). Interestingly mGluR8 mRNA is expressed exclusively in the retina whilst mGluR7 has a widespread distribution (Pin and Duvoisin, 1995). MGluRs are also present in the spinal cord.

1.8.1.1 Metabotropic Glutamate Receptors and Sensory Processing

Distribution of mGluRs in the spinal cord implies a nociceptive function for these receptors. Immunocytochemical studies have located Group I receptors in the superficial laminae I-III

(Fotuhi *et al.* 1993; Romano *et al.* 1995) on dendritic profiles targeted by synaptic boutons with the morphological characteristics of C fibre terminals (Vidnyanszky *et al.* 1994) Furthermore mRNA for these receptors is present in these areas indicating that a major source of receptor is intrinsic interneurones (Shigemoto *et al.* 1992). Other mGluR subtypes have not been extensively studied in the spinal cord. However there is evidence that mGluR7-LI is localised in neurones double labelled with either substance P or the isolectin I-B4 (Li *et al.* 1997). The authors of this study suggest that mGluR7 is found on nociceptive primary afferent fibres.

Electrophysiological and behavioural evidence also implicates a role for mGluRs in nociceptive transmission. Ventral root potentials evoked by electrical or chemical stimulation (Boxall *et al.* 1996) and dorsal horn neuronal responses to joint (Neugebauer *et al.* 1994b) and cutaneous inflammation (Young *et al.* 1994b; Young *et al.* 1995b) were reduced by mGluR antagonists as was behaviour induced by peripheral injection of the algogen carrageenan (Young *et al.* 1995a). However, formalin induced nociceptive behaviour was only slightly reduced by a mGluR antagonist whilst it was significantly facilitated by an agonist (Fisher and Coderre, 1996). This action may reflect an interaction between metabotropic and ionotropic glutamate receptors. Co-operation between these types of receptors has been previously demonstrated in isolated dorsal horn neurones (Bleakman *et al.* 1992) and in *in vivo* preparations (Bond and Lodge, 1995), but the relative contributions of specific cellular mechanisms versus increases in general excitability may complicate the data (Jones and Headley, 1995). Interestingly, Mellar *et al.* have suggested that mechanical hyperalgesia requires an interaction between mGluRs and AMPA receptors whilst thermal hyperalgesia relies on NMDA receptor activity alone (Meller *et al.* 1993).

The role of mGluRs in nociceptive processing appears to be confined to situations of spinal cord sensitisation and they have only a minimal role in transmitting innocuous or brief noxious inputs. This is in direct contrast to the AMPA and kainate group of glutamate receptors.

1.8.2 AMPA and Kainate Receptors

The first glutamate receptors to be characterised were ligand-gated ion channels. Pharmacological identification of these receptors divided them into the NMDA, AMPA and kainate subtypes named after their respective agonists. However, no glutamate receptor antagonist clearly distinguished between AMPA and kainate receptors, hence they were often collectively referred to as non-NMDA receptors.

Recent advances in molecular biology have now determined the structural composition of non-NMDA receptors and using expression cloning, a total of 9 receptor subunits have been identified. These are divided into 3 groups of closely related receptor genes comprising of

AMPA (GluR1-GluR4 each with a flip and flop splice variant) (Hollmann *et al.* 1989; Bettler *et al.* 1990; Boulter *et al.* 1990), low-affinity kainate receptors (GluR5-GluR7) (Bettler *et al.* 1990; Egebjerg *et al.* 1991; Bettler *et al.* 1992) and high-affinity kainate receptors with subunits KA1 and KA2 (Werner *et al.* 1991; Herb *et al.* 1992). These subunits form functional homomeric and heteromeric receptor configurations with distinct functional properties. For example, the Ca^{2+} permeability of AMPA receptors is regulated via the GluR2 subunit and receptors consisting of this type are not permeable to Ca^{2+} (Hume *et al.* 1991) Likewise, co-expression of GluR6 with KA2 increases the response to AMPA, and heteromeric AMPA receptors are responsive to kainate (Herb *et al.* 1992). However, there is little data on the configuration of native receptors *in vivo* although there is evidence that the subunits GluR1-GluR4 can form heteromeric complexes with each other and GluR6 and GluR7 form native heteromeric complexes with KA2 but not with GluR1-GluR4 (Hollmann and Heinemann, 1994).

The structure of non-NMDA receptors was believed to be similar to the nicotinic acetylcholine receptor, however there is no sequence homology between these receptors or with any other ligand-gated ion channel, including GABA, $5\text{-}HT_3$ or glycine receptors and a revised model proposed some significant differences. The topology of non-NMDA receptors is currently believed to contain the usual 4 transmembrane domains, however domain 2 does not cross the membrane and is located close to the cytoplasmic side (Wo and Oswald, 1994). As with other ligand gated ion channels, non-NMDA receptors form pentameric structures (for review see Bettler and Mulle, 1995).

1.8.2.1 Non-NMDA Receptors and Sensory Processing

Due to the functional organisation of DRG cells and of dorsal horn laminae, the distribution patterns of non-NMDA receptors can give important information on their prospective sensory processing roles. Whilst there is plenty of data on the location of AMPA receptors, that for kainate receptors is relatively sparse.

Ligand-binding studies showed that non-NMDA receptors were present in the superficial dorsal horn extending from laminae I-III (Wrathall *et al.* 1992; Henley *et al.* 1993). Immunohistochemical experiments supported this evidence and gave further information on subunit distribution. The immunolabelling of the spinal cord revealed that GluR1, GluR2 and GluR3 subunits were seen predominantly in laminae I,II and III indicating that they may well receive input from Aδ and C fibre nociceptors (Furuyama *et al.* 1993; Tachibana *et al.* 1994; Popratiloff *et al.* 1996). Furthermore, these receptors were upregulated following peripheral nerve injury and could therefore contribute to hyperalgesia (Harris *et al.* 1996). However, other groups have reported the existence of AMPA subunits in laminae III, in which case they could mediate Aβ fibre input (Kiyama *et al.* 1993; Martin *et al.* 1993). At the electron

microscope level, it has been suggested that GluR1 receptors face synapses from unmyelinated primary afferents and GluR2/3 receptors synapse with fine myelinated fibres (Popratiloff *et al.* 1996). This would imply that different subtypes might mediate different types of sensation.

In situ hybridisation studies broadly agreed with the immunohistochemical data. Generally, GluR1/2 mRNAs predominated in the dorsal horn and GluR3-4 in the ventral horn (Furuyama *et al.* 1993; Henley *et al.* 1993; Tolle *et al.* 1993). The cells that expressed GluR1 and GluR2 were mainly in laminae I, II and the upper part of lamina III. GluR2 had a much higher intensity of expression compared to GluR1 and interestingly GluR1 expression was reduced even further following unilateral arthritis with no change to any other subunit (Pellegrini-Giampietro *et al.* 1994).

Kainate receptor mRNA was also seen in the superficial dorsal horn using *in situ* hybridisation. GluR6 was not expressed, GluR5/7 and KA-1 were weakly detectable and KA-2 had a stronger signal (Tolle *et al.* 1993).

Non-NMDA receptor mRNA and immunoreactivity has been seen in DRG cells and peripheral sensory axons (Sato *et al.* 1993; Tachibana *et al.* 1994). There was a marked distribution in unmyelinated sensory axons suggesting a nociceptive role for these receptors, however both small and large DRG cells expressed AMPA receptors whilst kainate subtypes were restricted to small cells. This would imply that AMPA may be involved in the presynaptic regulation of Aβ, Aδ and C fibres whereas kainate might regulate just smaller neurones.

Electrophysiological and behavioural experiments gave a more accurate picture of the role of non-NMDA receptors in sensory processing. By measuring the latency of different components of synaptic transmission it become apparent that AMPA and kainate receptors were involved in monosynaptic pathways in the spinal cord and were therefore believed to mediate fast synaptic transmission (Jahr and Jessell, 1985; Gerber and Randic, 1989). In agreement with this, a variety of electrophysiological and behavioural tests demonstrated a prominent role for these receptors in the transmission of brief noxious inputs. For example, the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) reduced dorsal horn neuronal responses to C-fibre, Aδ and Aβ inputs (Alford and Grillner, 1990; Yoshimura and Jessell, 1990; Paleckova *et al.* 1992), innocuous and noxious mechanical stimulation (Dougherty *et al.* 1992; Neugebauer *et al.* 1993), initial and prolonged firing evoked by peripheral formalin injection (using the antagonist γ-D-glutamylglycine) (Haley *et al.* 1990) and the development of arthritis induced hyperexcitabiliy (Neugebauer *et al.* 1993a). In agreement, peripherally and intrathecally administered non-NMDA agonists (AMPA and kainate) resulted in behaviour indicative of nociception, and CNQX reversed this effect (Aanonsen and Wilcox, 1987; Kitto *et al.* 1992). CNQX also reduced behavioural responses

to formalin-induced irritation (Nasstrom *et al.* 1992), carrageenan-induced hyperalgesia (Ren *et al.* 1992b) and thermal hyperalgesia elicited by peripheral nerve injury (Mao *et al.* 1992a) or by noxious thermal stimulation of the tail. However, there are some reports that non-NMDA receptors do not mediate the transmission of noxious stimulation. Radhakrishnan and Henry (1993a) found that CNQX blocked dorsal horn responses to innocuous hair movement and not noxious thermal stimulation, and in behaving animals Coderre et al. (1992) reported that non-NMDA antagonists did not alter responses in the formalin test.

Non-NMDA ionotropic receptors therefore play a complicated role in spinal sensory transmission, but the majority of evidence has implicated these receptors in the processing of nociceptive information particularly that of rapid onset and short duration.

1.8.3 NMDA Receptors

The NMDA receptor is a complex molecular entity that represents a class of ionotropic receptor with unique functional properties (Figure 1.3).

The pioneering work of Moriyoshi *et al.*(1991), using expression cloning of a rat forebrain library, led to the isolation of the first cDNA clone of an NMDA subunit and this was termed NMDAR1. However, whilst this formed homomeric channels with NMDA receptor properties in *Xenopus* oocytes, when expressed in mammalian cells no functional channels were created. This led to a search for other NMDA receptor subunits, and using low-stringency hybridisation screening, four more NMDAR cDNAs were isolated (Monyer *et al.* 1992). These have been called NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D and when expressed with NMDAR1, functional receptors are formed in mammalian expression systems.

Like the nicotinic acetylcholine receptor, NMDA receptor subunits are believed to consist of four transmembrane domains and form pentameric structures. The subunit stoichiometry of these ion channels is unknown, but natively expressed receptors are likely to comprise of NMDAR1 subunits and at least one member of the NMDAR2 class or occasionally two. The properties of NMDA receptors are altered by different subunit compositions (see below) and indeed the conformation of each subunit may be influenced by other subunits associated with it (for review see Sucher *et al.* 1996). Furthermore, the expression of NMDA mRNAs is different throughout the brain implying that distinct subunit combinations might contribute to altered function between anatomical locations. These distribution studies have also provided evidence that there may be additional NMDA receptor subunits that are undiscovered at this time. Using *in situ* analysis it has been shown that the expression of NMDAR1 and NMDAR2 do not have a complete overlap in some brain areas (Monyer *et al.* 1992) and the recent cloning of a homologous novel NMDA receptor-like subunit (Ciabarra

et al. 1995; Sucher *et al.* 1995) suggests that the molecular structure of this receptor is more complex than was originally thought.

The complexity of the NMDA receptor is also reflected in its pharmacology (Figure 1.3). It is a ligand-gated ion channel, but unlike most other ionotropic receptors, it is permeable to Na^+, K^+ and Ca^{2+} ions (MacDermott *et al.* 1986) and has a number of distinct recognition sites for endogenous and exogenous ligands (for review see Scatton, 1993). These include a recognition site for glutamate, divalent cation binding sites for Mg^{2+} (Nowak *et al.* 1984) and Zn^{2+} (Westbrook and Mayer, 1987), a binding site for dissociative anaesthetics called the PCP site (Anis *et al.* 1983) and modulatory sites for the amino acid glycine (Johnson and Ascher, 1987) and polyamines such as spermine (Scott *et al.* 1993). NMDA receptors also have proton-binding sites (Tang *et al.* 1990), a redox site (Aizenman *et al.* 1989) and consensus phophorylation sites for protein kinase C (PKC) (Greengard *et al.* 1991), Ca^{2+}-calmodulin kinase II (CaM-KII) (Kolaj *et al.* 1994) and tyrosine kinases (Wang and Salter, 1995).

One of the unique properties of the NMDA receptor is its regulation by Mg^{2+} ions. These bind in the channel pore in a voltage-dependent fashion and block ionic entry at resting membrane potential (Nowak *et al.* 1984). This means that NMDA receptors are generally relatively quiescent and can only be activated after strong or repetitive stimuli that cause a relative depolarisation of the nerve cell. It also subserves a function of the receptor as a logical 'and' gate due to its requirement for both presynaptic release of agonist and postsynaptic depolarisation, hence it has been used as molecular evidence for Hebb's (1949) model of synaptic modification (Coan and Collingridge, 1984). This has functional relevance in area CA1 of the hippocampus where activity-dependent synaptic plasticity may underlie long term potentiation (Collingridge *et al.* 1983) and in the developing visual cortex where synaptic modifications strengthen the stability of simultaneously active inputs (Artola and Singer, 1987) and weaken the connections between asynchronously active afferents (Kleinschmidt *et al.* 1987) leading to ocular dominance. Both of these examples of plasticity have been shown to be NMDA-dependent by application of selective antagonists.

It would therefore appear that the Mg^{2+} block would in effect limit NMDA involvement in normal synaptic events, but since Curtis and Watkins (1960) applied NMDA to cat spinal interneurones it has been known that NMDA potently activates most central neurones *in vivo*. If it is assumed that the voltage-dependence remains constant, this may be attributed to neurones *in vivo* having different resting potentials, yet at potentials predicting maximum Mg^{2+} block, NMDA can still evoke activity (Headley and Grillner, 1990). Thus in certain cases, no prior depolarisation is necessary for NMDA-mediated synaptic transmission, although removal of Mg^{2+} would presumably amplify the signal. The importance of the voltage dependence of the block has been further qualified by observations

that intracellular transduction systems can modulate the extent of Mg^{2+} binding. Chen and Huang (1992) reported that in whole cell trigeminal neurone recordings, the action of PKC increased the dissociation constant for Mg^{2+} block by nearly fourfold and consequently NMDA currents were generated at more negative membrane potentials.

Another unusual property of NMDA receptors is their modulation by glycine and this has particular importance for the present study. The glycine site of the NMDA receptor (Gly_{NMDA}) was first discovered by Johnson and Ascher in 1987. In *Xenopus* oocytes expressing NMDA receptors from the mRNA, the NMDA receptor could not be activated by glutamate in a glycine-free solution (Johnson and Ascher, 1987). Kleckner and Dingledine (1988) confirmed that glycine was an essential requirement for NMDA activation and hence it was termed a co-agonist rather than a positive modulator. The mechanism of interaction between the glycine site and the EAA site has remained unclear. Electrophysiological data supports a role in minimising desensitisation of the EAA site, because at low glycine concentrations NMDA responses are very transient (Mayer *et al.* 1989), whereas radioligand binding studies show that a positive allosteric relationship exists (Monahan *et al.* 1990). In support of both of these ideas Kemp and Priestley (1991) demonstrated that the Gly_{NMDA} antagonist 7-chlorokynurenic acid increased NMDA desensitisation in cultured cortical neurones and binding of the partial agonist (+)-HA966 to the glycine site produced an allosteric reduction in the affinity of agonists for the glutamate recognition site. The most recent model reviewed by Corsi *et al.* (1996) agrees with this data and shows how co-agonism can affect the potency as well as the efficacy of agonist and antagonist interactions within the NMDA receptor.

The generation of modulating influences such as $Mg2^{+}$ block and glycine co-agonism at NMDA receptors is not constant throughout the nervous system and it is likely that the subunit conformation regulates these properties. For example, distinct pharmacological effects can be seen for different subunit combinations such that NMDAR1/NMDAR2B-D receptors have significantly higher affinities for glycine than the NMDAR1/NMDAR2A heteromer (Priestley *et al.* 1995). This is also reflected in CNS cells where there are differences in glycine binding affinities between cultured cerebellar granule and cerebral cortex cells (Priestley and Kemp, 1983; O'Shea *et al.* 1991). Indeed, in a recent study by Bovetto *et al.* (1997) it was reported that chronic application of the Gly_{NMDA} partial agonist ACPC induced changes in the subunit expression of NMDA receptor subunit mRNAs in the hippocampus and cerebral cortex, such that NMDA receptors developed reduced affinities for glycine and glutamate. Interestingly, NMDAR1/NMDAR2A-C receptors can be activated by glycine alone without glutamate binding (Kutsuwada *et al.* 1992). However, this may highlight an important discrepancy between recombinant and native NMDA receptors, as this effect has not been reported *in vivo* (Scatton, 1993).

Very few investigations have questioned the release and dynamics of endogenous ligands to the Gly_{NMDA} site. In addition to glycine, D-serine (Hashimoto *et al.* 1992) possesses similar affinity for the site, but both ligands are present at physiological concentrations (μM) that would saturate the Gly_{NMDA} site (nM) (Nagata *et al.* 1994). Nevertheless, there is evidence that the site operates in a dynamic range relevant to normal physiology. In the dorsal horn *in vivo,* application of glycine potentiated NMDA responses (Budai *et al.* 1992), in hippocampal slices NMDA receptors at CA1 synapses were potentiated by increased glycine concentrations (Minota *et al.* 1989) and in the cerebellum, NMDA-dependent increases in cGMP levels were blocked by tetrodotoxin (TTX) in a D-serine-reversible fashion, suggesting an important presynaptic component that exerts its effect through the Gly_{NMDA} site (Southam *et al.* 1991). Furthermore, D-serine-LI was located on glial cells in close proximity to NMDA receptors (Kehlet, 1995; Schell *et al.* 1995) and glutamate was shown to stimulate its release from these cells (Snyder S.H. 1997). These data indicate that endogenous ligands for the Gly_{NMDA} site must therefore be under the close control of local clearance mechanisms to reduce concentrations at the level of the synapse (see section 1.10.2).

1.8.3.1 Location of NMDA Receptors in the Spinal Cord

NMDA receptors are distributed within the superficial dorsal horn from laminae I-III. Thus, radiolabelled ligands for the NMDA receptor bind in this area (Greenamyre *et al.* 1984; Jansen *et al.* 1990; Mitchell and Anderson, 1991; Shaw *et al.* 1991) and Co2+, which can be visualised histochemically, enters laminae I-III cells when NMDA receptors are activated (Nagy *et al.* 1994b). This data is also supported by immunohistochemical experiments, where at the electron microscope level NMDA receptors are localised predominantly on postsynaptic densities and occasionally in presynaptic terminals (Liu *et al.* 1994c). The demonstration that these receptors are also present in DRG cells and peripheral sensory axons indicates that many of these presynaptic receptors arise from primary afferent terminals. Indeed, 70% of these cells contain high concentrations of glutamate and therefore NMDA receptors may be acting as autoreceptors (Liu *et al.* 1994c).

In situ hybridisation studies have also located NMDA receptor mRNA in the dorsal horn. One study found NMDAR1 expressed at low levels throughout the grey matter with stronger hybridisation in the deeper layers (Furuyama *et al.* 1993). However there are many reports of abundant signal in almost every dorsal horn cell with increased expression in laminae I-III (Tolle *et al.* 1993; Watanabe *et al.* 1994). NMDAR2 subunits are expressed differentially in the dorsal horn. Tolle *et al.* (1993) reported detectable levels of NMDAR2D in the rat superficial dorsal horn with no NMDAR2A-C labelling. In the mouse, Watanabe *et al.* (1994) showed that NMDAR2A (ε1) was expressed in all laminae except lamina II and

NMDAR2B (ε2) by cells in only laminae II, whereas NMDAR2C (ε3) was not expressed in the dorsal horn and NMDAR2D (ε4) was present only early in development.
Substantial evidence therefore exists for the presence of NMDA receptors in the superficial dorsal horn and since primary afferents terminate in this area NMDA receptors are likely to play an important role in sensory processing.

1.8.3.2 Role of NMDA Receptors in Sensory Processing

Whereas AMPA and kainate receptors are believed to be essential components of fast synaptic transmission bought about by glutamate release, the latency of NMDA-mediated synaptic transmission suggests that this receptor contributes towards prolonged changes in excitability evoked by polysynaptic inputs (Davies and Watkins, 1983; Peet *et al.* 1983; Schneider and Perl, 1985; Schouenborg and Sjolund, 1986; King *et al.* 1988; Gerber and Randic, 1989; Morris, 1989). Nevertheless, electrophysiological and behavioural experiments have demonstrated a role for NMDA receptors in brief noxious inputs as well as sustained nociception.
From electrophysiological observations, application of NMDA readily excited dorsal horn neurones (Anis *et al.* 1983; King *et al.* 1988; Sher and Mitchell, 1990; Dougherty and Willis, 1991) with a preferential selectivity for nociceptive types (Aanonsen *et al.* 1990) and potentiated neuronal responses to noxious mechanical and thermal inputs (Aanonsen *et al.* 1990; Dougherty and Willis, 1991). Similarly, in behavioural experiments, intrathecal injection of NMDA receptor agonists evoked scratching and biting behaviour evocative of pain (Aanonsen and Wilcox, 1987b; Raigorodsky and Urca, 1987b; Coderre and Melzack, 1991b) and shortened the tail flick withdrawal time to noxious thermal stimulation (Kolhekar *et al.* 1993). NMDA receptor antagonists (2-amino-5-phosphonovalerate (APV), 2-amino-7-phosphonoheptanoic acid (AP7) and 3-(2-carboxypiperazine-4-yl)propylphosphonate (CPP)) also reduced nociceptive responses in a variety of electrophysiological and behavioural tests investigating noxious thermal and mechanical modalities (Cahusac *et al.* 1984; Raigorodsky and Urca, 1990; Nasstrom *et al.* 1992). However, many groups have reported that a sensitising stimulus appears to be necessary to evoke an NMDA receptor component and NMDA receptor antagonists are often ineffective antinociceptive agents during brief noxious stimuli.
Perhaps the most extensively studied electrophysiological model of sensitisation is the "wind-up" phenomenon (Mendell, 1966). This is a progressive increase in the excitability of dorsal horn neurones caused by repeated electrical stimulation of the peripheral receptive field. The stimulus must have frequencies greater than 0.3Hz and be strong enough to excite C-fibres and the sensitisation appears to originate centrally since it occurs in the absence of any increase in the size of the incoming volley. "Wind-up" has at least two components, an

initial transient phase (that is thought to be A-fibre mediated and is NMDA-insensitive) and a longer-lasting second phase (that is evoked by C-fibre activity and is blocked by NMDA antagonists) (Davies and Lodge, 1987; Dickenson and Sullivan, 1990; Thompson *et al.* 1990). Headley and Grillner (1990) have also suggested a third phase and they reported that this was likely to be mediated by another neurotransmitter such as a neuropeptide.

NMDA receptor antagonists are also effective at reducing the responses of other experimental models thought to reflect sustained nociception. Many of these models induce peripheral inflammation and corresponding increases in the sensitivity of dorsal horn neurones. For example, topical application of the algogen mustard oil selectively activates C-fibres and NMDA receptor antagonists blocked reflex facilitation evoked in this model (Woolf and Thompson, 1991). Similarly, subcutaneous injection of the inflammatory agents complete Freund's adjuvant (CFA) or capsaicin brings about hyperexcitability of dorsal horn neurones and this was blocked by both competitive (AP-7) (Dougherty *et al.* 1992) and channel-blocking NMDA receptor antagonists (MK-801) (Ren *et al.* 1992a). Joint inflammation also sensitises dorsal horn neurones and the NMDA receptor antagonists APV reduced increased neuronal responsiveness to innocuous and noxious stimuli of the inflamed knee (Schaible *et al.* 1991a; Neugebauer *et al.* 1994a) and the hyperexcitability induced by acute arthritis (Neugebauer *et al.* 1993a). Finally, subcutaneous injection of formalin causes a biphasic increase in neuronal excitability and APV (Haley *et al.* 1990) did not block the first transient stage but did inhibit the longer-lasting second stage indicative of sustained nociception.

Behavioural experiments support the electrophysiological data. Several similar models are used such as subcutaneous injection of CFA or formalin and behaviours thought to reflect nociceptive responses are monitored. Coderre *et al.* have investigated the effects of agents acting at different sites of the NMDA receptor complex on behavioural responses to formalin. They reported that much more potent analgesia could be produced by combinations of channel blockers (such as MK-801) and polyamine site agonists than application of the same antagonists alone (Coderre and Van Empel, 1994a) and these results help confirm the allosteric nature of interactions occurring between NMDA receptor binding sites.

Meller *et al.* (1993) have suggested that the modality of brief noxious inputs is regulated by different EAA receptors. They reported that brief mechanical nociceptive behaviours were inhibited only by combinations of AMPA and metabotropic antagonists whilst brief noxious thermal stimulation was blocked by NMDA antagonists. Following sustained nociception, such a clear-cut pharmacological definition of these classes of pain is not seen. For example, peripheral inflammation such as that induced by subcutaneous injection of carrageenan or CFA leads to both thermal and mechanical hyperalgesia. Ren *et al.* found that after induction of inflammation, intrathecally applied MK-801 consistently reduced behavioural responses

to noxious heat (Ren *et al.* 1992b) and noxious pinch (Ren and Dubner, 1993). However Smith *et al.* (1994) reported that mechanical hyperalgesia was not affected by the same compound after CFA injection.

NMDA receptors may also play an important role in neuropathic pain resulting from nerve injury. NMDA antagonists were effective at reducing both thermal hyperalgesia (Davar *et al.* 1991; Mao *et al.* 1992) and mechanical allodynia (Smith *et al.* 1994) associated with loose ligation of the sciatic nerve. This form of pain is often insensitive to morphine treatment in humans and the potential effectiveness of NMDA antagonists as analgesics in these situations is currently being explored. The availability of several clinically tested NMDA antagonists such as ketamine (a dissociative anaesthetic) and memantine (an adamantane derivative used for treating spasticity associated with Parkinson's disease) has speeded this process as the safety profile of these drugs has already been evaluated. Indeed, ketamine reduced ongoing and touch-evoked allodynia in neuropathic pain (Felsby *et al.* 1996) and decreased these symptoms in phantom limb pain (Stannard and Porter, 1993; Rawal, 1995; Nikolajsen *et al.* 1996) and neuropathic cancer pain (Mercadante *et al.* 1995). NMDA antagonists were also shown to reduce both primary and secondary hyperalgesia in humans (Ilkjaer *et al.* 1996) and trials have been performed to assess their effectiveness in reducing post-operative pain. These compounds were shown to alleviate pain without narcotic-like side effects (Islas *et al.* 1985; Chung *et al.* 1986) although some groups reported that they produced only marginal and short-term analgesia (Bars and Hanna, 1987; Wong *et al.* 1995). Nevertheless, NMDA antagonists have been used in combination with more conventional opioid treatments where they reduced the requirement of opioids and the danger of respiratory depression (Wong *et al.* 1995; Yang *et al.* 1996).

Most of the NMDA antagonists currently under clinical trials such as ketamine and memantine are non-competitive channel blockers acting at the PCP site. Alternative therapies may perhaps result from the use of antagonists acting at other NMDA binding sites such as the polyamine site or the glycine site; indeed Gly_{NMDA} antagonists are effective analgesics in a number of animal models of nociception.

1.8.3.3 Role of the Gly_{NMDA} site in Sensory Processing

Blockade of the Gly_{NMDA} site alters nociceptive transmission in both electrophysiological and behavioural protocols. The selective Gly_{NMDA} antagonist, 7-chlorokynurenate inhibited repetitive stimulus-induced 'wind-up' and late stage formalin responses in dorsal horn neurones (Dickenson and Aydar, 1991) and reduced the amplification of Aβ fibre-induced responses which occurred after UV burn and was used to model the development of inflammatory allodynia (Chapman and Dickenson, 1994). In behavioural tests, both normal tail flick reflexes to noxious heat and those facilitated by NMDA were reduced by intrathecal

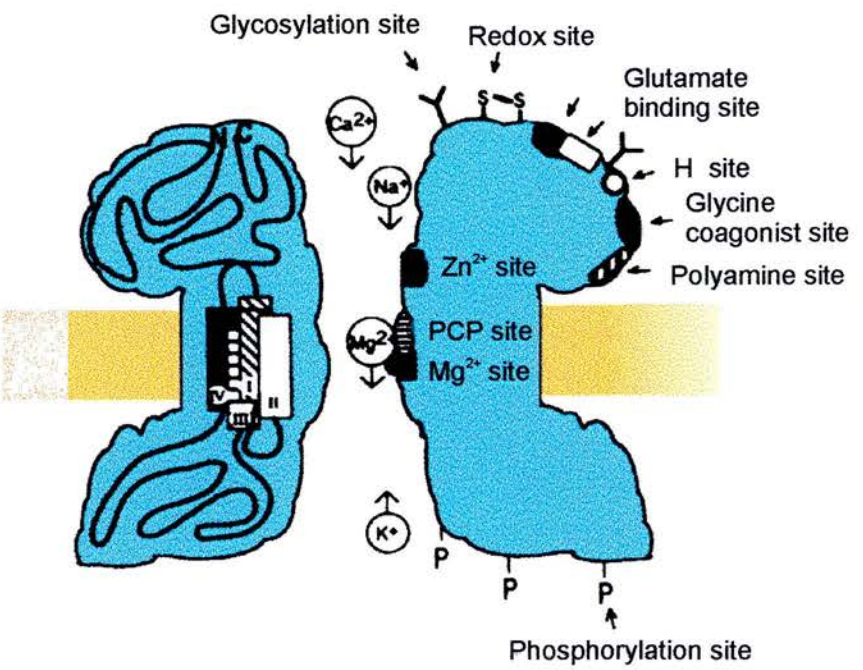
application of the Gly_{NMDA} antagonist ACEA-1021 (Lutfy and Weber, 1996). Partial agonists and antagonists at the Gly_{NMDA} site (L687,414, D-cycloserine, (+)-HA966, ACEA-1021, MDL29951, DCPQ) also inhibited pain induced behaviour induced behaviour induced behaviour induced behaviour induced behaviour induced behaviour induced behaviour evoked during the second stage of the formalin model in mice and rats (Coderre and Van Empel, 1994b; Millan and Seguin, 1994b; Lutfy and Weber, 1996b). Furthermore, Laird *et al.* (1996) have shown that a Gly_{NMDA} partial agonist (L687,414) and an antagonist (L701,324) blocked mechanical hyperalgesia associated with the subcutaneous application of carrageenan.

These observations provide evidence that the Gly_{NMDA} site is occupied *in vivo* and serves as an essential modulator of NMDA mediated events in the dorsal horn of the spinal cord.

FIGURE 1.3

Schematic representation of a NMDA receptor

Schematic representation of a NMDA receptor, indicating sites for various agonists, antagonists, modulators for modifying its activity. The sub-unit drawn on the left schematically indicates the coiling of the protein required to produce four transmembrane domains as well as extracellular N- and C-termini. Only two of the five subunits required for ion channel formation are shown. (From Hollmann and Heinemann, 1994).


Glycosylation site
Redox site
Glutamate binding site
H site
Glycine coagonist site
Polyamine site
Ca2+
Na+
Zn2+ site
Mg2+
PCP site
Mg2+ site
K+
P
P
P
Phosphorylation site
S
S
V
III
II

1.9 TACHYKININS

The tachykinins are a family of structurally related peptides characterised by their common carboxyl terminal sequence Phe-X-Gly-Leu-Met (Figure 1.4). The family includes substance P (SP), neurokinin A (NKA, also known as substance K), neurokinin B (NKB, also known as neuromedin K), neuropeptide K (NPK) and neuropeptide γ (NPγ). These ligands are distributed widely in the CNS and peripheral tissues and evoke a variety of biological activities including neuronal excitation, vasodilatation and smooth muscle contraction.

The mammalian tachykinins are derived from two peptide precursor genes designated preprotachykinin A (PPT-A) and PPT-B which are likely to have evolved from a common ancestor gene (Nawa and et al. 1983; Kotani *et al.* 1986; Krause *et al.* 1987). Alternative RNA processing gives rise to multiple forms of mRNA for both genes (Nawa and et al. 1984). PPT-A produces 3 variants termed α-PPT-A, β-PPT-A and γ-PPT-A. SP is encoded by all three genes, NKA by β and γ-PPT-A, NPK by β-PPT-A and NPγ by γ-PPT-A (Nawa and et al. 1983). PPT-B gives rise to two variants, both of which code for NKB (Kotani *et al.* 1986). Since the proportion of α-PPT-A expression in the CNS is very low (0.5% of total PPT-A expression), SP should therefore almost always be expressed in combination with other β and γ-PPT-A gene products.

The tachykinins exert their effects through three receptors called the neurokinin$_1$ (NK_1), NK_2 and NK_3 receptors. Radioligand binding displacement assays (Yokota *et al.* 1989; Shigemoto *et al.* 1990) have shown that SP preferentially activates the NK_1 receptor at two orders of magnitude higher than NKA and NKB. Similarly, the NK_2 receptor is activated by NKA one order of magnitude higher than NKB and two orders of magnitude higher than SP and the NK_3 receptor shows preferential binding of NKB two orders of magnitude higher than NKA and SP. This evidence implies that there is a degree of interaction between different ligands and receptors. However in most physiological situations, NK_1 receptors will be activated by SP, NK_2 receptors by NKA and NK_3 receptors by NKB.

Tachykinin receptors were initially isolated in 1987 using expression cloning of the bovine NK_2 receptor (Masu *et al.* 1987). This led to the cloning of cDNAs for rat NK_1 and NK_3 receptors using a similar functional assay combined with cross-hybridisation to the bovine NK_2 receptor (Sasai and Nakanishi, 1989; Yokota *et al.* 1989; Shigemoto *et al.* 1990). These three receptors show considerable sequence homology, share the common structure of seven transmembrane domains and are linked via G proteins to phosphoinositide metabolism (Watson and Downes, 1983; Nakajima *et al.* 1992) (Figure 1.4). They also have significant sequence similarities to other G protein-coupled receptors such as muscarinic acetylcholine receptors and are therefore members of this receptor superfamily.

FIGURE 1.4

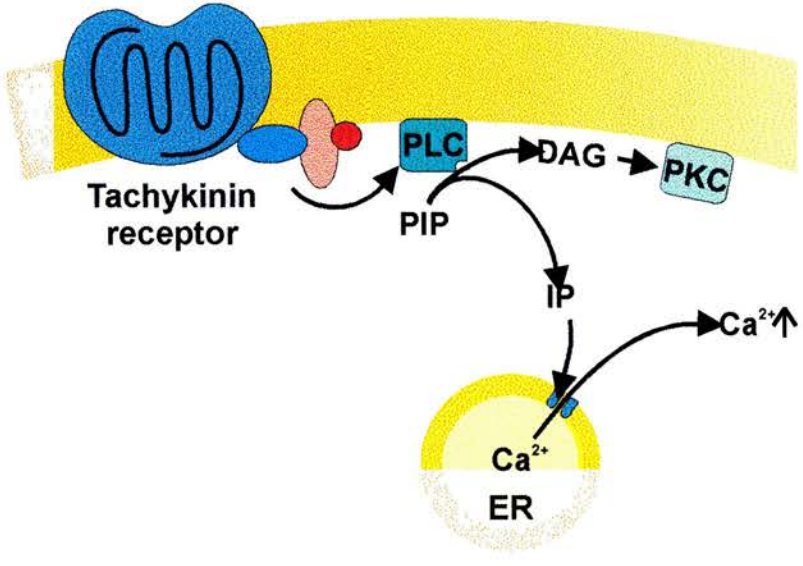
Tachykinins and receptors

The mammalian tachykinin system consists of three distinct peptides: substance P, neurokinin A and neurokinin B, that bind to NK_1, NK_2 and NK_3 receptors respectively and share the common carboxyl-terminal sequence, Phe-X-Gly-Leu-Met-NH_2 (A). Tachykinin receptors possess seven transmembrane domains with extracellular amino termini and cytoplasmic carboxyl termini and are linked via G proteins to phosphoinositide metabolism (B). Red circles in (C) indicate the conserved amino acids of the three tachykinin receptors (adapted from Nakanishi, 1991).

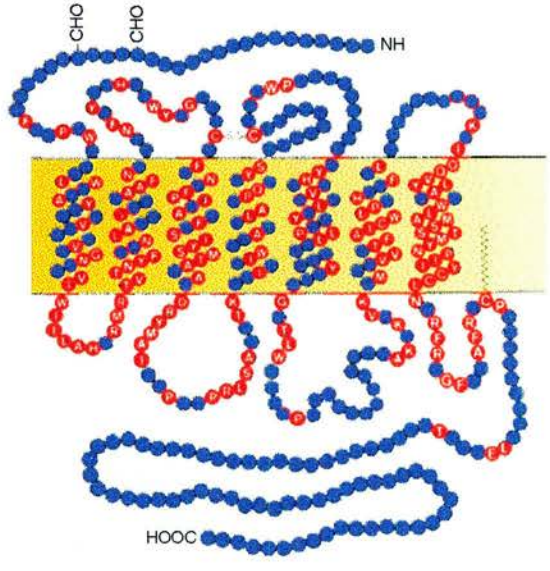
A.

Ligands	Receptors
Substance P : Arg-Pro-Lys-Pro-Gln Gln Phe Phe-Gly-Leu-Met-NH	Neurokinin
Neurokinin A: His-Lys Thr-Asp-Ser Phe-Val-Gly-Leu-Met-NH	Neurokinin
Neurokinin B: Asp-Met-His-Asp-Phe Phe-Val-Gly-Leu-Met-NH	Neurokinin

B. Signal Transduction



C. Receptor Homology



1.9.1 Substance P and NK_1 Receptors

SP has traditionally been regarded as the classical spinal pain transmitter and there is abundant evidence supporting this role. However, despite the availability of selective NK_1 receptor antagonists, the therapeutic value of this receptor to analgesia has not been realised and this is reflected in the experimental data. Investigations into the precise nature of SP and NK_1 receptor involvement in nociceptive processing have revealed varied and sometimes inconsistent results emphasising the complexity of SP and NK_1 receptor function in sensory transmission.

1.9.1.1 Location of SP and NK_1 receptors in the Spinal Cord

SP-LI and PPTA mRNA has been detected in dorsal root fibres and laminae I, II, V-VII and X using immunohistochemistry, immunocytochemistry and *in situ* hybridisation (Hokfelt *et al.* 1975; Hokfelt *et al.* 1977; Hokfelt *et al.* 1980; Gibson *et al.* 1981; Warden and Young, 3d, 1988; Harlan *et al.* 1989). Nerve section and dorsal rhizotomy largely but not entirely reduced this distribution, hence it is likely that the SP-LI was derived from primary afferents, intrinsic neurones and descending fibres (Jessell *et al.* 1979; Ogawa *et al.* 1985). In agreement with this, neonatal capsaicin application destroyed most C-fibres and also reduced SP-LI by approximately 55% (Moussaoui *et al.* 1992).

At the electron microscope level, Weiya *et al.* (1996) have analysed the types of neurone that SP-LI boutons are apposed to. They reported that high threshold dorsal horn neurones received most SP-LI appositions followed by WDR and then low threshold neurones and that one third of all appositions displayed a synaptic contact. This data indicates that a direct correlation exists between the amount of SP-LI input and the nociceptive nature of the cell and importantly suggests that SP can act at NK_1 receptors close to the site of release.

Changes in dorsal horn and primary afferent SP-LI and mRNA have been observed following peripheral injury. For example, nerve injury decreased PPT-A gene expression (Marchand *et al.* 1994; Sommer and Myers, 1995; Munglani *et al.* 1996) whereas increases in mRNA were seen after subcutaneous formalin or CFA injection (Minami *et al.* 1989; McCarson and Krause, 1995). Similarly, adjuvant-induced arthritis increased the number of SP-immunoreactive fibres in the dorsal horn (Kar *et al.* 1994). In agreement with this data, studies investigating the release of SP have also reported increases following peripheral stimulation.

Experiments using spinal cord perfusion as a method of measuring SP release have shown that it was released following electrical stimulation of high threshold primary afferent fibres and noxious heat applied to the hind limbs (Go and Yaksh, 1987). However this method has limitations in its accuracy and a clearer picture can be seen from studies using push-pull

cannulae and microdialysis. With this technique it was found that noxious mechanical and severe thermal stimuli increased SP release (Kuraishi *et al.* 1989), but greater increments in SP levels were measured following stimuli that caused damage to peripheral tissue. Accordingly, injection of formalin also increased release of SP in the dorsal horn (Kuraishi *et al.* 1989). However, McCarson and Goldstein (1991) have reported decreases in SP-immunoreactivity using this model and they suggested that this was due to intrinsic antinociceptive systems inherent to more sustained nociception.

SP release has also been measured in the spinal cord using the antibody microprobe technique to give both spatial and temporal discrimination. SP was detected in the superficial dorsal horn following peripheral noxious heat, mechanical or chemical stimulation or electrical stimulation at C-fibre strength (Duggan *et al.* 1988). However, it was again apparent that tissue damage is a much more effective stimulus and after the development of peripheral inflammation such as that produced by arthritis (Schaible *et al.* 1990), compression or flexion of the injured area produced a massive release of SP over a wide area. Interestingly, a similar distribution of SP-LI was also reported after microinjection of the peptidase inhibitor kelatorphan (Duggan *et al.* 1992) or of calcitonin gene related peptide (CGRP) (Schaible *et al.* 1992), which can both slow the degradation of SP. This highlights an important component of SP function. If it can spread away from its release site after sustained nociception, possibly under the endogenous control of CGRP, then both the gain and the duration of incoming signals might be increased. Furthermore, neurones receiving inputs from adjacent tissue may also be affected and these could potentially contribute to secondary hyperalgesia. Therefore it is likely that under certain conditions, the primary function of SP is not to transmit brief noxious information, but to alter the response properties of dorsal horn neurones to subsequent inputs.

The distribution of NK_1 receptors has also been studied in detail. Radiolabelled SP bound to postsynaptic cells in laminae I and II (Quirion *et al.* 1983; Helke *et al.* 1986; Yashpal *et al.* 1991; Näsström *et al.* 1992) and this was altered by peripheral stimulation. Brief noxious inputs decreased the binding after 1 minute due to competition by the endogenous ligand (Yashpal *et al.* 1994) whereas sustained forms of nociception such as inflammation or peripheral nerve injury raised levels of bound SP presumably as a result of postsynaptic receptor upregulation (Yashpal *et al.* 1991a; Aanonsen *et al.* 1992a; Kar *et al.* 1994a).

Immunohistochemical observations of NK_1 receptor distribution do not agree with the autoradiographic data. In these studies, NK_1 receptor-LI was located in laminae I and III-V but not in laminae II (Bleazard *et al.* 1994; Liu *et al.* 1994; Nakaya *et al.* 1994). This lack of staining was quite striking, but is supported by physiological and *in situ* hybridisation observations where few lamina II cells respond to or make the NK_1 receptor (Kiyama *et al.* 1993; Bleazard *et al.* 1994). Nevertheless, at the electron microscope level, NK_1 receptor-LI

outlines somata and major dendrites of dorsal horn neurones in laminae III, IV and to some extent V, which have dendrites that extend into laminae II and presumably act as targets for SP released in this area (Brown *et al.* 1995; Naim *et al.* 1997). Furthermore, many of these cells are likely to project via the STT to the thalamus and may be nociceptive in nature (Marshall *et al.* 1996). Liu *et al.* (1994a) reported that these heavily labelled membranes make direct synaptic contact with SP terminals in only about 15% of cases indicating that much of the transmission in this area could be due to diffusion of the ligand. However Naim *et al.* (1997) observed a higher proportion of synaptic contact and they suggested that whilst some of this might be important in maintaining a degree of secure synaptic contact with deeper neurones, much could be attributed to glutamatergic transmission as glutamate is often co-localised with SP.

Upregulation of NK_1 receptors after inflammation and nerve injury was also seen with immunohistological techniques (Abbadie *et al.* 1996). Interestingly, this did not necessarily correlate with alterations in SP levels which were reduced after nerve injury. Furthermore, increases in NK_1 receptor-LI occurred over a wide area and extended into the contralateral side of the cord (Abbadie *et al.* 1996). NK_1 receptor upregulation may therefore contribute to the central sensitisation of dorsal horn neurones under conditions of persistent pain.

The activation of NK_1 receptors by endogenous ligand, or exogenous agonist has recently been visualised *in vivo* using confocal microscopy. This is dependent upon the phenomenon of receptor endocytosis, exhibited by all G protein-linked receptors. Activation of the receptor leads to rapid internalisation from the membrane into endosomes with a resulting desensitisation during which time the receptor is recycled. Mantyh *et al.* (1995a) first demonstrated this phenomenon for NK_1 receptor immunoreactive receptors in rat striatum *in vivo*. They found that injection of SP bought about massive internalisation of NK_1 receptor-LI and reorganisation of the morphological characteristics of NK_1 receptor immunoreactive dendrites. This occurred after one minute and recovered within sixty minutes. The technique has also been applied to the dorsal horn where noxious stimuli evoked a marked endocytosis on dendrites in laminae II that was graded with respect to the intensity of the stimulus (Pook *et al.* 1993; Mantyh *et al.* 1995). Furthermore, peripheral inflammation increased the degree of receptor internalisation such that near-maximal endocytosis was induced in cells in laminae I and III-VI following mechanical stimulation after inflammation (Abbadie *et al.* 1997).

In situ hybridisation localisation of mRNA for NK_1 receptors showed a low distribution in the superficial dorsal horn and lamina III (Elde *et al.* 1990; Kiyama *et al.* 1993). However CFA or formalin-induced inflammation caused a considerable upregulation of NK_1 receptor mRNA throughout the superficial and deeper layers (Schäfer *et al.* 1993; McCarson and

Krause, 1994) that was blocked by prior administration of opioid agonists (McCarson and Krause, 1995).

Evidence concerning the location of NK_1 receptors on primary afferent is contradictory. NK_1 receptor-LI did not decrease in the dorsal horn after dorsal rhizotomy, and DRG cells did not label for NK_1 receptor-LI, NK_1 mRNA or radioactive SP binding (Brown *et al.* 1995). However, a significant number of cutaneous sensory axons were immunolabelled for the NK_1 receptor (Carlton *et al.* 1996b). This contradiction has not been resolved (for review see Coggeshall and Carlton, 1997).

1.9.1.2 Role of SP and NK_1 receptors in Sensory Processing

There is a large body of both behavioural and electrophysiological evidence implicating SP and NK_1 receptors in sensory processing in the dorsal horn.

Ionophoresis of SP into the dorsal horn evoked neuronal activity (Henry, 1976; Zieglgansberger and Tulloch, 1979) and selectively activated high threshold and WDR laminae I and II neurones (Randic and Miletic, 1977) resulting in a characteristic slow postsynaptic depolarisation (Urban and Randic, 1984; Randic *et al.* 1988). In agreement, intrathecal application of SP was shown to evoke scratching and biting behaviour indicative of pain in a number of studies (Hayes and Tyers, 1979; Hylden and Wilcox, 1981; Piercey *et al.* 1981). However, NK_1 antagonists were generally not effective at reducing responses to brief noxious stimuli (Fleetwood-Walker *et al.* 1987b; Fleetwood-Walker *et al.* 1990b). They did not alter baseline nociceptive reflex activity (CP96345 and RP67580) (Laird *et al.* 1993), single C-fibre volleys (CP96345) (De Koninck and Henry, 1991; Chapman and Dickenson, 1993) and in some cases sustained C-fibre stimulation in normal animals (CP96345 and RP67580) (Thompson *et al.* 1994). Similarly, behaviour induced by SP and neuronal and behavioural responses to more natural stimuli, such as brief noxious pinch and acute noxious thermal stimulation of the tail or paw, were generally not affected by NK_1 antagonists (CP96345, CP99994, RP67580, SR140333, WIN51708 and WIN62577) (Malmberg and Yaksh, 1992; Yamamoto and Yaksh, 1992; Couture *et al.* 1993; Garces *et al.* 1993; Picard *et al.* 1993; Seguin *et al.* 1995).

Nevertheless some groups do report effective NK_1 mediated analgesia in these tests. For example, Radhakrishnan and Henry (1991) and De Koninck and Henry (1991) found that CP96345 reduced dorsal horn responses to brief noxious stimuli and Holland and Goldstein (1994) showed that NK_1 receptor desensitisation (through administration of high doses of SP) reduced phasic but not tonic pain behaviours. In these situations though, the difficulties in maintaining standardised experimental procedures is highlighted. For instance the intensity and duration of brief noxious stimuli may differ between groups, and antagonist

specificity is often questioned, especially as CP96345 has Ca^{2+} channel blocking effects that could interfere with any direct NK_1 receptor antagonism (Seguin *et al.* 1995).

It has become apparent that SP and NK_1 receptors may have a more prominent role in sustained nociception. From behavioural experiments, a number of models have been used to test for NK_1 receptor involvement. These include the measurement of pain induced behaviours evoked by intraperitoneal injection of acetic acid (Garret *et al.* 1991; Seguin *et al.* 1995) and intraplantar injection of capsaicin (Sakurada *et al.* 1993a; Sakurada *et al.* 1993a), carrageenan (Nagahisa *et al.* 1992; Yamamoto *et al.* 1993; Traub, 1996) or formalin (Charriaut-Marlangue *et al.* 1991; Murray *et al.* 1991; Yamamoto and Yaksh, 1991; Yashpal *et al.* 1993; Seguin *et al.* 1995). NK_1 antagonists (CP96345, CP99994, PR67580, SR140333, WIN51708, WIN62577, GR82334 and D-pro-SP) were usually antinociceptive in these more sustained tests. However, these antagonists were found to be ineffective at increasing paw withdrawal latencies to noxious mechanical and thermal stimuli following chronic constrictive nerve injury (Yamamoto and Yaksh, 1992) or carrageenan-induced hyperalgesia (Young *et al.* 1995a).

Sluka *et al.* (1997a) and Traub (1996) investigated the differential contribution of NK_1 receptors to the development and maintenance of hyperalgesia. Using intraarticular injection of carrageenan and kaolin as a model, Sluka *et al.* (1997a) demonstrated that spinal NK_1 receptors were essential for the maintenance of thermal hyperalgesia whereas peripheral receptors were involved in the development of this state. However, Traub (1996) reported that spinal NK_1 receptors were also important during the initial stages of thermal and mechanical hyperalgesia evoked by subcutaneous carrageenan or, spontaneous pain behaviours induced by formalin. He suggested that NK_1 receptors could contribute to the generation of hyperalgesia but the additional presence of NMDA receptor activation was needed to maintain the sensitisation. The variability between these studies probably reflects the differences in the models used.

In electrophysiological protocols, SP enhanced the responses of WDR and high threshold neurones to repetitive C-fibre strength stimulation (Kellstein *et al.* 1990) and paralleled the increase in duration and magnitude of the flexor reflex evoked by C-fibre conditioning stimuli (Wiesenfeld-Hallin, 1986). Furthermore, NK_1 antagonists ([D-Pro^2,D-$Trp^{7,9}$]-SP, CP96345 and RP67580) blocked both the SP-induced (Wiesenfeld-Hallin, 1986; Kellstein *et al.* 1990) and electrically-conditioned elevated responses of these tests (De Koninck and Henry, 1991; Xu *et al.* 1992; Laird *et al.* 1993), reduced an NK_1 receptor-mediated component of wind-up (Kellstein *et al.* 1990b; Thompson *et al.* 1993b) and blocked the high frequency, high intensity induction of C-fibre evoked LTP (Liu and Sandkühler, 1997). Similarly, Thompson *et al.* (1994) reported that whilst CP96345 did not affect ventral root

potentials in an *in vitro* hemisected spinal cord preparation taken from normal rats, it did reduce this response in animals sensitised by a UV burn to the cutaneous receptive field.

NK_1 receptor antagonists are also effective at reducing neuronal responses to inflammatory stimuli. Dorsal horn activity evoked during the second stage of response to subcutaneous formalin injection was blocked by NK_1 antagonists (RP97580) (Chapman and Dickenson, 1993) as was the *in vivo* sensitisation of primate STT neurones by peripheral application of capsaicin (GR82334 and CP96234) (Dougherty *et al.* 1994). However NK_1 antagonists did not reduce capsaicin-induced sensitisation in an *in vitro* spinal cord preparation (CP96234) (Urban *et al.* 1993) or neuronal responses to cutaneous application of the algogen mustard oil (GR82334 and L668169) (Munro *et al.* 1993). Therefore, like the behavioural data, both the intensity and mode of nociceptive model may be crucial in defining an NK_1 receptor involvement in sustained nociception.

In addition to the above pharmacological approaches of addressing the role of spinal NK_1 receptors in nociception, two recent studies have used new methods to explore NK_1 receptor function. Hua *et al.* (1998) investigated the effects of intrathecally administered NK_1 receptor antisense oligonucleotides on nociception and Mantyh *et al.* (1997) assessed nociceptive responses in rats following ablation of lamina I neurones expressing SP receptors. Hua *et al.* (1998) reported that there was no reduction of pain behaviour or immunostaining of NK_1 receptors after repeated intrathecal treatment with antisense oligonucleotides. However, spinal application of SP in these animals caused a reduction in the behavioural response to formalin and in NK_1 receptor immunoreactivity. These results suggested that under quiescent conditions the half-life of NK_1 receptors was relatively long and unless the receptors were challenged by a stimulus of sufficient strength to cause internalisation (and subsequent degradation) then reduction in the ability to synthesise new protein by antisense oligonucleotides would not be apparent. Similarly, Mantyh *et al.* (1997) demonstrated that by infusing a conjugate of SP and the ribosome-inactivating protein saporin into the spinal cord, receptor internalisation allowed the toxin to enter cells and inactivate and ultimately kill lamina I neurones expressing SP receptors. This treatment left responses to mild noxious stimuli unchanged but markedly attenuated responses to highly noxious stimuli and thermal and mechanical hyperalgesia induced by capsaicin application. Both of these investigations therefore support a role for NK_1 receptors in the transmission of sustained or severe stimuli and show that mild pain can be dissociated from severe or hyperalgesic pain by the apparent lack of NK_1 receptor involvement.

Much of the reviewed evidence implicates the NK_1 receptor as a sensitising agent rather than a pain transmitter. Dirig and Yaksh (1996) directly tested this assumption by examining the change in nociceptive threshold under three different thermal stimuli after intrathecal administration of SP. They found that an NK_1 receptor antagonist increased paw withdrawal

latencies to noxious thermal stimulation at all intensities, but that this change remained directly proportional to the withdrawal latency of untreated animals for each stimulus intensity investigated. They therefore suggested that SP was not acting through an additive mechanism via a fixed increase in excitation, but elevated the gain of spinal nociceptive neurones and thus functioned as a sensitising agent.

SP and agonists at NK_1 receptors have also been demonstrated to have inhibitory affects. Ionophoresis of SP or the NK_1 agonist SP methyl ester (SPOMe) into the superficial dorsal horn inhibited non-nociceptive responses of deeper dorsal horn SCT cells whilst leaving nociceptive responses unaffected (Fleetwood-Walker *et al.* 1988b; Fleetwood-Walker *et al.* 1990b). Davies and Dray (1980) reported that administration of SP into laminae II initially inhibited and then enhanced responses to noxious thermal stimuli in two thirds of laminae I, IV and V neurones whilst depressing the activity of the remaining neurones and not affecting innocuous stimulation. Mixed effects were also reported in primate STT cells where SP caused excitation and inhibition of glutamate driven activity, background activity and noxious pinch stimuli (Willcockson *et al.* 1984), and in rat and cat laminae IV-V WDR neurones where a GABAergic inhibitory interneurone was suggested to mediate SP inhibitory actions (Ryall and Pini, 1987).

Intracellular recordings from *in vitro* spinal cord slice preparations support the *in vivo* data. Murase and Randic (1984) have shown that bath-applied SP evoked a hyperpolarising response in one third of dorsal horn neurones that was blocked with either TTX or high Mg^{2+}, low Ca^{2+} solutions. This suggests that the action of SP was either indirect though an inhibitory interneurone or involved presynaptic inhibition of primary afferents, however the lack of anatomical evidence for presynaptic NK_1 receptors supports the former hypothesis.

An alternative mechanism for SP inhibition has been proposed by Larson *et al.* (1994). They reported that a metabolite of SP, the N-terminal fragment (1-7) ($SP_{(1-7)}$), had inhibitory actions on dorsal horn neuronal responses to nociceptive stimuli. This compound had opposite properties to SP and was demonstrated to inhibit C fibre evoked-flexor reflexes (Goettl *et al.* 1994), reduce acetic acid-evoked writhing behaviour (Kreeger *et al.* 1994), inhibit amino acid release (Skilling *et al.* 1990) and initially decrease and then facilitate NMDA receptor-evoked activity (Hornfeldt *et al.* 1994). Furthermore, $SP_{(1-7)}$ down-regulated NK_1 receptor binding via an mechanism not directly associated with NK_1 receptor activation (Yukhananov and Larson, 1994). $SP_{(1-7)}$ is a potential candidate for SP induced inhibition, but the ability of selective NK_1 agonists to also inhibit neuronal responses suggests that the mechanism probably occurs directly via NK_1 receptor activation.

The main role of SP and NK_1 receptors in sensory processing therefore appears to be as a modulator of nociceptive transmission. A wide variety of studies including behavioural, electrophysiological, anatomical, and investigations into the release of SP, support this

function and provide little evidence for a direct role in the transmission of brief noxious inputs. However, it is not yet known how the NK_1 receptor sensitises neurones to subsequent inputs and what mechanisms limit its involvement to this process. This study is concerned with those mechanisms and they will be discussed with respect to positive interactions between NK_1 and NMDA receptors and inhibitory regulation via strychnine-sensitive glycine receptors.

1.9.2 Other Tachykinins

There is only a small body of evidence on the distribution and role of other tachykinins in the dorsal horn and this is effectively limited to investigations on NKA and NKB and their respective receptors, NK_2 and NK_3.

NKA and NKB have both been located in the spinal cord. NKA coexists with SP (Dabgaard *et al.* 1985) but concentrations of NKA have been shown to be significantly higher in the central terminals of primary afferents than SP (Bakhle and Bell, 1995). Furthermore, levels were reduced by half following neonatal capsaicin treatment supporting its role as a primary afferent neurotransmitter (Moussaoui *et al.* 1992).

The release of NKA has been investigated using the antibody microprobe technique. Duggan *et al.* (1990) reported that NKA was released following noxious thermal and mechanical stimuli but unlike SP, NKA was relatively resistant to enzyme degradation. Therefore it was detected widely in the spinal cord and persisted for sixty to ninety minutes following peripheral nerve stimulation (Hope *et al.* 1990a). Additionally, NKA has been detected immediately after joint injection with carrageenan and kaolin when it again had a widespread presence in the spinal cord (Hope *et al.* 1990b).

Very few studies have examined the precise localisation of NKB in the dorsal horn. It is not present in DRG cells and the distribution of PPT-B mRNA is restricted to laminae I-IV and X (Ogawa *et al.* 1985; Moussaoui *et al.* 1992). Furthermore, neonatal capsaicin or sectioning of the dorsal roots did not alter NKB-LI suggesting that it is not a primary afferent neurotransmitter (Ogawa *et al.* 1985; Meldrum, 1992).

NK_2 and NK_3 receptors are also present in the spinal cord and are located postsynaptically from primary afferents (Yashpal *et al.* 1991a). Radioligand binding studies have located NK_2 receptors in a thin band across the dorsal part of the dorsal horn (Yashpal *et al.* 1990) and NK_3 receptors in laminae I-III (Ninkovic *et al.* 1984). MRNA for NK_2 receptors was detected in spinal cord using blot hybridisation analysis but as yet, no results on NK_2 receptor expression have been reported from *in situ* hybridisation studies (Takeda and Krause, 1991). In contrast, mRNA for NK_3 receptors has been localised in spinal cord using *in situ* hybridisation (Tsuchida *et al.* 1990) and this was reported to increase following adjuvant or formalin induced nociception (McCarson and Krause, 1994).

Ionophoresis of NKA into the dorsal horn evoked a slow and prolonged increase in the firing rate of all types of dorsal horn neurones including low threshold cells (Salter and Henry, 1991). NKA also depolarised spinal neurones in *in vitro* preparations (Matsuto *et al.* 1984) and Nagy *et al.* (1993) reported that only capsaicin-sensitive laminae II-IV neurones were affected by NK_2 agonists. Furthermore, capsaicin-induced responses in these neurones were inhibited by NK_2 antagonists (MEN10376) (Nagy and Hunt, 1982; Nagy *et al.* 1993). Thresholds of behavioural responses to noxious thermal stimuli were also reduced by intrathecal application of NK_2 agonists (Picard *et al.* 1993a) and Yashpal *et al.* (1996) demonstrated that this elevation was blocked by antagonists of NK_2 (SR4896) but not NK_1 receptors and similarly, SP-evoked behavioural changes were only blocked by NK_1 antagonists (CP96345). Therefore NKA appears to act only at NK_2 receptors in the spinal cord and SP is specific for NK_1 receptors.

Experiments with NK_2 antagonists (MEN10376, MEN10207, GR103537 and R368) suggest that these receptors mediate both brief and sustained nociceptive transmission, especially that of a thermal nature (Santucci *et al.* 1993a; Yamamoto *et al.* 1993a; Seguin *et al.* 1995a; Sluka *et al.* 1997a). Many studies have investigated the effects of NK_2 antagonists on electrically-evoked activity. Otsuka *et al.* (1995) and Nagy *et al.* (1994a) showed that slow prolonged ventral root potentials induced by C-fibre stimulation were sensitive to NK_2 antagonists and Thompson *et al.* (1993a and 1994) reported that unlike the NK_1 receptor, NK_2 antagonists reduced C-fibre potentials both before and after a UV-induced burn to the cutaneous receptive field. The nociceptive flexor reflex has also been studied extensively as a model for NK_2 receptor involvement and it has been reported that adjuvant-induced increases in the reflex were reduced by NK_2 antagonists (Jia and Seybold, 1997) as was the development of mechanical allodynia caused by muscle (and not cutaneous) C-fibre conditioning stimuli (Ma and Woolf, 1995).

NK_2 receptors are also important in the spinal transmission of more natural stimuli. For example NK_2 antagonist (SR48968) reduced dorsal horn neuronal responses to noxious and innocuous stimulation of the normal and inflamed joint (Neugebauer *et al.* 1996).Furthermore NKA facilitated (Fleetwood-Walker *et al.* 1990a), and an NK_2 antagonist (L659874) inhibited (Fleetwood-Walker *et al.* 1993) activity evoked by cutaneous noxious heat and not mechanical stimulation of the skin. Unlike NK_1 antagonists these antagonists were also effective at blocking neuronal responses to peripheral application of mustard oil (Munro *et al.* 1993).

Behavioural experiments support the electrophysiological evidence and NK_2 receptors have been implicated in models exploring thermal nociception (Fleetwood-Walker *et al.* 1990a), the second phase of formalin evoked behaviour and responses induced by intraperitoneal acetic acid (Seguin *et al.* 1995). Additionally, Sluka *et al.* (1997a) showed that whilst NK_1

receptors were involved in the maintenance of heat hyperalgesia caused by intraarticular injection of kaolin and carrageenan, spinal NK_2 receptors were essential for its development. This evidence supports a role for NKA and NK_2 receptors in nociceptive processing in the dorsal horn, but unlike SP that may act as a sensitising agent, NK_2 receptors are likely to mediate the transmission of brief noxious inputs.

1.10 Glycine as a Neurotransmitter

Glycine is an amino acid with many metabolic functions throughout the nervous system. It is also a neurotransmitter in the vertebrate CNS where it serves two distinct functions, an excitatory modulatory role via NMDA receptors (section 1.8.3) and an inhibitory action at the strychnine-sensitive glycine receptor.

This inhibitory function was first demonstrated in the late 1960s by Curtis et al. (1967) who showed that glycine depressed spinal neurones when applied ionophoretically. Initial disagreement as to the specificity of glycine and the inhibitory amino acid γ-aminobutyric acid (GABA) for their selective receptors came for the work of Davidoff and Aprison (Davidoff and Aprison, 1969; Davidoff *et al.* 1969). However Curtis *et al.* (1969) maintained that glycine bound specifically at the strychnine-sensitive receptor. It has since been confirmed that glycine is released in a calcium-dependent manner (Hopkin and Neal, 1970) and acts at postsynaptic sites to generate IPSPs through an increased Cl^- conductance that can be antagonised competitively by strychnine (Barker and Nicoll, 1973).

1.10.1 Glycine Transporters

Like the EAA glutamate, rapid termination of the synaptic actions of glycine is achieved by the uptake of transmitter into surrounding presynaptic terminals and glial cells.

Two types of glycine transporter belonging to the GABA transporter gene super-family have been cloned to date (Liu *et al.* 1993): GLYT1, which exists as two variants GLYT1a and GLYT1b, and GLYT2 that shares approximately 50% amino acid homology with GLYT1. Both transporters share similar mechanisms of glycine uptake which involves the transfer of Na^+ and Cl^- ions with a stoichiometry of 2 Na^+:1 Cl^-:1 glycine (Aragon *et al.* 1987). This transfer can also be modulated by PKC, which leads to a decrease in glycine uptake (Gomeza *et al.* 1995; Sato *et al.* 1995).

Interestingly, GLYT1 and GLYT2 have different distributions throughout the CNS. GLYT1 is located in glial cells in the spinal cord, brainstem, cerebellum, thalamus, hypothalamus and hippocampus (Zafra *et al.* 1995a; Zafra *et al.* 1995a) whereas GLYT2 is expressed in presynaptic terminals and glia exclusively in the spinal cord, brainstem and cerebellum (Luque *et al.* 1995; Zafra *et al.* 1995; Zafra *et al.* 1995). Furthermore, GLYT1 protein has

been localised with NMDA receptors in the hippocampus and is often found in areas devoid of inhibitory glycinergic transmission (Smith *et al.* 1992; Zafra *et al.* 1995; Zafra *et al.* 1995). This raises the possibility that GLYT1 regulates glycine concentrations at NMDA receptors and GLYT2 is associated with inhibitory strychnine-sensitive receptors.

1.10.2 Strychnine-Sensitive Glycine Receptors

The strychnine-sensitive glycine receptor (GlyR) is a pentameric structure consisting of two types of transmembrane subunit, the 48KDa α subunit and the 58KDa β subunit. These subunits have significant sequence homology with those of the nicotinic acetylcholine receptor and consequently the GlyR is classified as a member of the superfamily of ligand gated ion channels (Betz *et al.* 1994).

To date, three different genes encoding α subunits of this receptor have been cloned in rats with a variety of splice variants and these form functional receptors in *Xenopus* oocytes of variable stoichiometries. In contrast, incorporation of the β subunit usually generates a stoichiometry of 3α:2β (Kuhse *et al.* 1993) which may reflect the endogenous situation *in vivo*. It is proposed that the α subunit contributes to the ligand binding site of GlyRs (Langosch *et al.* 1994) whereas the β subunit may have more of a prominent role in ion channel function (Pribilla *et al.* 1992). Furthermore, β subunits have been shown to co-distribute with the protein gephyrin in the CNS (Kirsch *et al.* 1993a). This protein is believed to anchor the GlyR to both microfilaments and microtubules (Kirsch and Betz, 1995), and its association with the β subunit suggests that both polypeptides may interact *in vivo*. Indeed inhibition of gephyrin expression by antisense oligonucleotides prevented the formation of GlyR clusters (Kirsch *et al.* 1993b) and this correlates with the mouse mutant *spastic* that lacks synaptically-located receptors due to incorrectly spliced β mRNA (Becker, 1990). Therefore the β subunit and gephyrin may be essential for the correct placement of glycine receptors in the neuronal membrane. However it has become apparent that gephyrin is not exclusively associated with GlyRs and may also localise with GABA receptors at non-glycinergic synapses (Todd *et al.* 1995).

GlyRs can be modulated by a number of other agents as well as glycine. Zn^{2+} ions have been shown to have a biphasic effect and potentiate Cl^- entry at low concentrations and inhibit at high concentrations (Bloomenthal *et al.* 1994). GlyRs are also phosphorylated by a number of compounds including enhancement of response by protein kinase A (PKA) (Vaello *et al.* 1994) and (CaM-KII) (Wang and Randic, 1996) and inhibition by PKC (Vaello *et al.* 1994). This underlies the plasticity of these receptors in many physiological and pathological processed including spinal nociceptive transmission.

1.10.2.1 Location of Glycine and GlyRs in the Spinal Cord

Glycine is involved in metabolic as well as transmitter functions in the CNS. However it appears to be present in higher concentrations in those areas important to synaptic transmission, especially in the spinal cord, strengthening evidence for its role as a neurotransmitter.

In the rat dorsal horn, glycine-LI has been located in laminae I-III with predominant staining in laminae III (Todd, 1990). At the subcellular level glycine-LI was observed, co-localised with GABA (Spike and Todd, 1992; Mitchell *et al.* 1993; Todd *et al.* 1996) presynaptically at axodendritic and axosomatic synapses and also at axo-axonic synapses where the postsynaptic boutons appeared to be on myelinated primary afferents (Todd, 1990; Todd *et al.* 1991). Furthermore, many immunoreactive dendrites were postsynaptic to type II glomeruli that are usually associated with myelinated axons (Todd, 1996). This evidence implies that glycine may be involved in monosynaptic processing of Aβ type afferents.

In the monkey however, the situation appears to differ slightly. Carlton *et al.* (1996a) have demonstrated that glycine-LI is distributed mainly in laminae III-VII with some light staining in laminae I and II. Importantly, they also found no evidence of axo-axonic type glycinergic synapses indicating that whilst primary afferents can activate glycine systems, there is little presynaptic control by glycine on monkey primary afferents.

Radioligand binding studies indicate that GlyRs are distributed throughout the CNS but are concentrated in the spinal cord and brainstem. In the dorsal horn, binding occurs in laminae II, III and V (Zarbin *et al.* 1981). Immunocytochemical observations broadly agree with the binding data and GlyR-LI has been reported in laminae II, III, IV and V with a noticeable absence in the predominantly nociceptive laminae I (Araki *et al.* 1988; Basbaum, 1988). At the electron microscope level, GlyR-LI is almost always confined to synaptic locations associated with axodendritic and axosomatic synapses. Interestingly, and in contrast to reports of axo-axonic distribution of glycine-LI (Todd, 1990; Todd *et al.* 1991), GlyR labelling has not been observed on axo-axonic connections or on primary afferent membranes (Mitchell *et al.* 1993) indicating that is does not appear to function as an inhibitory auto-receptor on these neurones.

In situ hybridisation studies have localised mRNA for α and β subunits of GlyRs in laminae II-VIII (Fujita *et al.* 1991; Malosio *et al.* 1991; Sato *et al.* 1991) and much of this hybridisation occurs on glial cells (Kirchhoff *et al.* 1996). In contrast with binding and immunocytochemical studies, GlyR mRNA has also been detected in DRG cells. These neurones are mostly large diameter and also contain mRNA for GABA receptors (Furuyama *et al.* 1994).

Physiological and pathological alterations to dorsal horn neuronal morphology have been associated with glycinergic transmission. For example, noxious stimulation evoked by

peripheral subcutaneous formalin injection evoked increases in c-fos immunoreactivity in many glycinergic neurones (Todd *et al.* 1994), and trans-synaptic degeneration of the superficial dorsal horn associated with peripheral nerve injury was increased by strychnine application (Sugimoto *et al.* 1989; Sugimoto *et al.* 1990). Furthermore, ALS induced motorneurone cell death dramatically decreased GlyR binding in the anterior grey matter and this may further accelerate the symptoms of this disease (Hayashi *et al.* 1981).

1.10.2.2 The role of Glycine and GlyRs in Sensory Processing

In addition to its excitatory effect at NMDA receptors, ionophoretic application of glycine is known to reduce spinal neuronal responses *in vivo* (Curtis *et al.* 1967) and *in vitro* (Barker and Nicoll, 1973). Consistent with this data, intrathecal application of strychnine, a specific inhibitory glycine receptor antagonist, evoked scratching and biting behaviour indicative of pain in rats (Beyer *et al.* 1985) and strychnine poisoning in humans is known to evoke intense pain that is paralleled by significant increases in sensitivity to innocuous stimuli (Perper, 1985).

An increased sensitivity to innocuous mechanical stimuli, or allodynia, forms the basis of a model of hyperalgesia developed by Sosnowski and Yaksh (1990) where intrathecal administration of strychnine brings about allodynia and receptive field expansion in rats. Characterisation of this model revealed that strychnine caused an enhanced neuronal response to hair deflection, enlargement of the low threshold receptive field and an increased neuronal afterdischarge. Moreover, these changes only occured in dorsal horn cells activated by both hair deflection and by high intensity mechanical stimuli, suggesting that the inhibitory modulation was associated with large fibre-mediated nociceptive states which resulted from removal of tonic inhibition of hair afferent input onto convergent neurones (Yaksh, 1989; Sherman and Loomis, 1995; Sherman and Loomis, 1996; Sorkin and Puig, 1996).

The pharmacology of strychnine induced allodynia (and presumably of the convergent neurones that receive glycinergic input) has been investigated extensively. Importantly, this model of hyperalgesia was insensitive to morphine and μ-opiate receptor agonists (Yaksh, 1989; Sorkin and Puig, 1996) but it was blocked by NMDA and AMPA antagonists, nitric oxide synthase inhibitors, guanylate cyclase inhibitors and adenosine agonists (Sosnowski and Yaksh, 1989; Onaka *et al.* 1996). The lack of effect of conventional analgesic agents on this form of nociception has important implications for the therapy of morphine-intractable pain and suggests that removal of inhibitory circuits may contribute to these states.

Glycine has also been implicated in other nociceptive systems. For example, Sivilotti and Woolf (1994) have demonstrated that intrathecal strychnine increased the nociceptive flexor reflex in a similar way to C-fibre conditioning stimuli, suggesting that the reflex was under

the control of segmental inhibitory mechanisms. Furthermore, Peng *et al.* (1996) and Lin *et al.* (1994) have shown that periaquaductal grey-induced descending inhibition of STT neurones involves a glycinergic mechanism that was reduced during central sensitisation by PKC-mediated phosphorylations (Lin *et al.* 1997).

Inhibitory control may also be an important component of neuropathic pain. Autotomy, a behavioural response that is associated with injury discharges from damaged sensory fibres was significantly increased by application of strychnine (Seltzer *et al.* 1991) and following sciatic nerve constriction injury, glycine levels (measured by HPLC) increased in the dorsal horn (Satoh and Omote, 1996). Intrathecal application of glycine also attenuated mechanical and thermal hyperalgesia in this model (Simpson *et al.* 1996; Simpson *et al.* 1997) whereas strychnine enhanced responses (Yamamoto and Yaksh, 1993; Sorkin and Puig, 1996). Interestingly, Simpson *et al.* (1994 and 1997) have reported that inhibition of thermal and mechanical hyperalgesia by glycine was differentially blocked by NMDA antagonists. They have shown that NMDA antagonists failed to block the antinociceptive effects of glycine on mechanical allodynia whereas they did block the influence of glycine on thermal responses.

Inhibitory glycine receptors may therefore be important regulators of excitatory input during normal transmission. However, removal of this tonic inhibition can unmask inappropriate connections and contribute to an increase in spinal cord excitability.

1.11 Receptor Interactions and Spinal Sensory Processing

Interactions between different receptor systems and their signalling mechanisms form the basis of our current understanding of plasticity in somatosensory processing. Anatomical studies on the co-localisation of different agents and behavioural and electrophysiological analysis of these distributions has revealed that both negative and positive influences modulate incoming signals and exert rapid and profound effects on the excitability of the dorsal horn. The present study has addressed the role of NK_1 receptors in spinal nociception and their participation in a series of interactions involving the release of glycine. The actions of this amino acid at either inhibitory GlyRs or excitatory NMDA receptors may therefore differentially regulate the role of NK_1 receptors during nociceptive events.

1.11.1 Interactions Between NK_1 Receptors and Glycine

Most evidence on the role of NK_1 receptors in sensory transmission suggests that they play little part in mediating responses to brief inputs but act as sensitising agents to increase the gain of dorsal horn neurones after sustained nociception (see section 1.9.1.2). It is not yet known if a specific mechanism exists to exclude their involvement from acute nociception or whether this latency of action can be attributed to slow degradation or slow spread of SP to

NK_1 sites some way from the release site. However, brief inputs were not enhanced by the application of agonists directly onto dorsal horn cells (Fleetwood-Walker *et al.* 1987; De Koninck and Henry, 1991; Malmberg and Yaksh, 1992; Yamamoto and Yaksh, 1992; Couture *et al.* 1993) indicating that NK_1 receptors might be under some form of regulation. This is also supported by observations that NK_1 agonists often inhibited neuronal responses both *in vivo* (Davies and Dray, 1980; Willcockson *et al.* 1984; Ryall and Pini, 1987; Fleetwood-Walker *et al.* 1988; Fleetwood-Walker *et al.* 1990) and *in vitro* (Murase and Randic, 1984) and that intrathecally-applied SP was actually antinociceptive at low doses (Masuyama *et al.* 1996). The apparent lack of NK_1 receptor involvement in acute responses may therefore be due to the co-activation of an inhibitory mechanism by SP.

Littlewood *et al.* (1995) have investigated the extent of GABA and glycine-LI in neurones expressing NK_1 receptors. They reported that in lamina I, none of these cells were GABA or glycine-immunoreactive although some cells in laminae III-V did stain for both receptor and inhibitory ligand. They concluded that most neurones that were activated by SP were excitatory in the dorsal horn. However, this evidence does not preclude the existence of an inhibitory mechanism activated by SP. Indeed increased glycine release was detected following bath application of SP *in vitro* (Kangrga and Randic, 1990; Maehara *et al.* 1995) and after microdialysis of SP into the spinal cord *in vivo* (Smullin *et al.* 1990b). Furthermore, intrathecal administration of glycine has been shown to inhibit SP-evoked scratching and biting behaviour (Beyer *et al.* 1989). Taken together this data indicates that an inhibitory interneurone may well be activated at some point down line of NK_1 receptor activation, presumably after SP acts on an excitatory neurone.

This model therefore predicts that NK_1 receptor activation of a glycine interneurone would initially obscure an NK_1 excitatory input. However, the accumulation of lasting intracellular changes such as phosphorylations and NMDA receptor activation would eventually overcome the acute inhibitory modulation. Consequently, effects of NK_1 antagonists would not be apparent following brief noxious stimuli but in those situations where the stimulus was more severe and led to sensitisation, the combination of intracellular changes and increased SP release would eventually lead to a direct, measurable NK_1 receptor effect.

1.11.2 Interactions Between NK_1 Receptors and NMDA Receptors

NMDA and NK_1 receptors have been shown to play important individual roles in sensory processing, but there is also evidence that during sustained nociception a synergistic interaction develops between them. It is probably the most extensively studied receptor interaction in the dorsal horn and stems from observations that glutamate and SP coexist in DRG cells (Battaglia and Rustioni, 1988b) and primary afferent terminals in the superficial laminae (De Biasi and Rustioni, 1988). This led to suggestions that they are co-released

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following noxious stimulation and act together at NK_1 and NMDA receptors to contribute to neuronal sensitisation.

A facilitation of glutamate-evoked activity in dorsal horn neurones by SP was first reported by Zieglgansberger and Tulloch in 1979 (1979). Randic *et al.* (1990) demonstrated a similar phenomenon in isolated dorsal horn neurones *in vitro* and showed that SP both reduced and potentiated NMDA responses without affecting AMPA or kainate current. Furthermore, Dougherty and Willis (1991a and 1995) found that NMDA-induced activity in identified STT cells was facilitated by SP administration *in vivo* for a number of hours and this paralleled an enhancement of responses to mechanical stimulation of the skin indicative of sensitisation. Later studies by the same group (Dougherty *et al.* 1993a) also reported excitation and inhibition of NMDA and AMPA responses by SP and whilst NMDA alterations could be blocked by NK_1 antagonists, reductions in AMPA-evoked activity were unaffected by these antagonists.

Experiments investigating behavioural responses have also described an interaction between NMDA and NK_1 receptors. For example, kainate, AMPA and NMDA all evoked caudally directed biting and scratching when applied intrathecally, but this was significantly increased by co-administration with SP, especially responses to NMDA (Mjellem-Joly *et al.* 1991). Similarly NK_1 receptor agonist-induced facilitation of the tail flick reflex was blocked by NMDA antagonist (APV) (Yashpal *et al.* 1991b), although Okano *et al.* (1993) reported that NMDA-evoked biting and scratching behaviour was not inhibited by NK_1 antagonists (CP96345) even though NK_1 receptor agonist evoked behaviour was reduced by NMDA antagonists (APV).

Several studies have addressed the role of the NMDA and NK_1 receptor interaction in a number of different models of nociception. For example, behavioural responses to a variety of stimuli including subcutaneous formalin (Murray *et al.* 1991; Mjellem-Joly *et al.* 1992) or capsaicin injection (Okano *et al.* 1994), and hyperalgesia caused by repeated cold stress (Okano *et al.* 1995) were blocked more significantly by co-administration of NMDA (APV) and NK_1 receptor antagonists ([D-Pro^2,D-Trp^{7,9}]-SP, [D-Pro^4,D-Trp^{7,9,10},Phe^{11}]-$SP_{(4-11)}$ and CP96345) than application of these antagonists alone. Furthermore, subthreshold doses of NK_1 (CP96345) and NMDA (MK-801) receptor antagonists reduced the electrically-evoked flexor reflex and in the same study SP facilitation of this reflex was blocked by NMDA antagonists (Xu *et al.* 1992a). Co-administration of NMDA and SP also facilitated Aδ-evoked dorsal horn responses and the development of wind-up (Chapman *et al.* 1994) and slow nociceptive ventral root potentials have been shown to rely on both NMDA and NK_1 receptors to generate a full response (Woodley and Kendig, 1991).

There is also evidence against a specific synergistic NMDA/NK_1 interaction and some investigations have reported only an additive effect. Cumberbatch *et al.* (1995a) addressed

this question by cycling the ionophoresis of selective NK_1 and EAA agonists into spinal cord. They reported that application of an NK_1 agonist (GR76362) enhanced responses to AMPA, kainate and NMDA equally whilst an NK_2 agonist (GR64349) reduced AMPA evoked activity and did not change NMDA responses. It was suggested that the NK_1 receptor mediated facilitation was simply due to a general increase in excitability (hence the similar increase in AMPA-evoked activity). Nevertheless, they argued that in a physiological situation, co-release of NKA with SP would reduce AMPA receptor activity and bias transmission towards NMDA receptors. However, the data they presented showed that NK_1 agonist facilitation of NMDA responses was still clearly greater than facilitation of AMPA and kainate activity indicating that the NMDA/NK_1 interaction could be fully accounted for by a simple additive process. Cumberbatch *et al.* (1995) also investigated the effects of ionophoreses of NK_1 receptor antagonists on EAA responses to assess the importance of tonic facilitation. They found that NK_1 receptor antagonists (CP99994 and GR82334) preferentially reduced kainate, AMPA and then NMDA responses whilst the NK_2 receptor antagonist GR159897 increased kainate evoked activity. These results implied that there was a tonic release of endogenous tachykinins that could modulate glutamatergic transmission in the spinal cord, furthermore the effects of the NK_1 antagonists appeared to resemble the actions of NK_2 agonists on EAA-evoked responses. It was suggested that this effect could be attributed to the limited selectivity of tachykinin receptors for their ligands. Since SP and NKA could theoretically act at both NK_1 and NK_2 receptors, by blocking the NK_1 component, synaptically released SP and NKA might have acted at the NK_2 receptor and mimicked the effects of NK_2 agonists.

Baranauskas *et al.* (1995) have reported a similar additive interaction between NMDA and NK_1 receptors in the ventral horn. Recording intracellularly from motorneurones following dorsal root stimulation they showed that in this population of cells, NK_1 receptor antagonist (SR140333) reduction of NMDA receptor activity could only be attributed to excitability changes and not to a specific co-operation. Behavioural investigations by Ren *et al.* (1996) also supported this data. They used isobolographic analysis of drug effects where a theoretical additive line for a combination of the two drugs was calculated and compared with individual and combined administrations. It was found that co-application of NMDA and NK_1 antagonists reduced thermal hyperalgesia induced by CFA but that this was no different from the predicted additive value.

These experiments have demonstrated that the NMDA/NK_1 interaction may not always be a synergistic process. However there is a large body of evidence on the mechanisms of this interaction that indirectly support co-operation between the receptors. Much of this data involves the possible participation of intracellular signalling systems and the phosphorylation of NMDA receptors. One known consequence of activating an NK_1 type

receptor is the activation of PKC and both Rusin *et al.* (1993a) and Urban *et al.* (1994a) have shown that this 2nd messenger activated enzyme contributes to NK_1 receptor agonist-induced facilitation of NMDA receptor activity. Furthermore PKC has been implicated as a sensitising agent for dorsal horn neurones (Munro *et al.* 1994; Peng *et al.* 1997; Sluka and Willis, 1997) emphasising the important physiological consequences of co-activation of NMDA and NK_1 receptors. Other 2nd messengers may also be involved in the NMDA/NK_1 interaction. Blockers of nitric oxide production (Radhakrishnan and Henry, 1993b; Bjorkman *et al.* 1994b; Coderre and Yashpal, 1994b) and arachidonic acid (Malmberg and Yaksh, 1992b; Coderre and Yashpal, 1994b) reduced both behavioural and electrophysiological responses to co-administration of NMDA and NK_1 agonists.

Many investigators have considered enhanced release of ligand as a mechanism for receptor co-operation and SP has been shown to increase concentrations of glutamate both *in vitro* (Kangrga *et al.* 1990; Kangrga and Randic, 1990) and *in vivo* (Smullin *et al.* 1990a) where release is further enhanced following nerve injury (Skilling *et al.* 1992). Liu (1997) have investigated NMDA receptor regulation of SP release from primary afferents. They demonstrated that injection of NMDA into the cerebrospinal fluid enhanced NK_1 receptor internalisation, which was significantly reduced by an NK_1 receptor antagonist. They therefore suggested that activation of presynaptic NMDA receptors and increased release of SP was the mechanism for co-operation between NMDA and NK_1 receptors. In support of this hypothesis, Marvizon *et al.* (1997) showed that NK_1 receptor internalisation produced by high frequency stimulation of the dorsal roots was also blocked by NMDA receptor antagonist whilst AMPA and kainate antagonists had no effect. Nevertheless, much of this evidence is circumstantial. For example, SP release could be the result of an indirect action of NMDA receptors and there is little anatomical evidence for the existence of functional presynaptic NMDA receptors on primary afferent nociceptors. Indeed, in a recent study by Aicher *et al.* (1997) it was reported that the NMDA receptor was usually postsynaptic to SP-containing axons (252/324 neurones). Furthermore a postsynaptic locus of interaction is supported by the experiments of Randic *et al.* (1990) who demonstrated a facilitation of NMDA activity by SP in *isolated* single postsynaptic dorsal horn neurones.

This study has addressed the role of the Gly_{NMDA} site in the NMDA/NK_1 interaction. It is proposed that an NK_1 induced increase in glycine concentration (see section 1.11.1) may activate the Gly_{NMDA} site and this hypothesis and the effects of PKC-mediated phosphorylations on Gly_{NMDA} function have been tested.

A potential role for glycine in the NK_1/NMDA receptor interaction has previously been investigated using anatomical, behavioural and electrophysiological protocols. Chapman *et al.* (1996) showed that inflammation induced increases in spinal C-fos expression were reduced by co-application of NK_1 (RP67580) and Gly_{NMDA} antagonists ((+)-HA 966) and in

behavioural responses to subcutaneous injections of formalin, the Gly_{NMDA} partial agonist (+)-HA 966, promoted the antinociceptive effects of NK_1 receptor antagonists RP 67580 and CP99994 (Seguin and Millan, 1994). Furthermore, Rusin *et al.* (1993c) reported that in isolated dorsal horn neurones the presence of glycine greatly enhanced NMDA responses and this prevented a SP facilitation of NMDA evoked inward currents. This data is consistent with the idea that SP potentiation of NMDA receptors may be dependent on the status of the Gly_{NMDA} site and emphasises the potential importance of this site in the development and maintenance of central sensitisation.

1.12 Leukaemia Inhibitory Factor

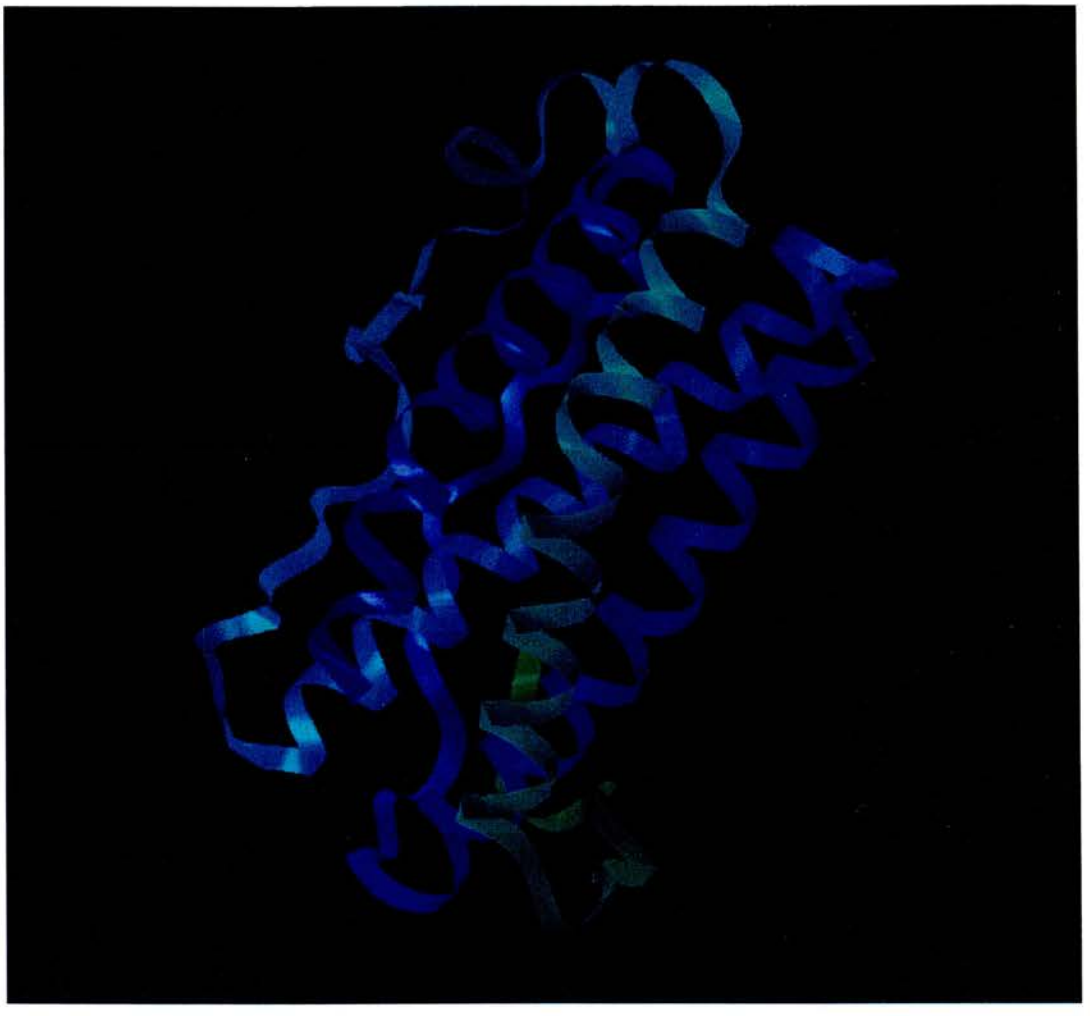
Leukaemia inhibitory factor (LIF) is a hematopoietic cytokine that has recently been shown to function in neuronal tissue. Initially characterised on the basis of its capacity to induce the differentiation and death of a mouse myeloid leukaemic cell line M1 (Metcalf *et al.* 1988), LIF is now known to exert a variety of effects on immune system and nervous system cells including actions in development, hematopoiesis, bone metabolism, inflammation and cachexia (for review see Gearing, 1993). This functional diversity is reflected in the number of different names ascribed to LIF and through discovery and rediscovery it has been known as D-factor, differentiation-inducing factor, cholinergic nerve differentiation factor (CDF) and hepatocyte stimulating factor III amongst others. However, the purification of murine LIF in 1987 (Hilton *et al.* 1988) and the subsequent isolation of its encoding cDNA (Gearing *et al.* 1987) has now revealed that all of these molecules are identical to LIF.

Due to its many functions in the immune and nervous systems, LIF has been grouped with a number of similar proteins termed the neuropoietic cytokines. This family includes interleukin-6 (IL-6), IL-11, ciliary neurotrophic factor (CNTF), oncostatin M and cardiotrophin-1 and whilst these proteins have little sequence homology they share a common secondary structure (Bazan, 1991; Rose and Bruce, 1991) (Figure 1.5) and exert similar actions on a variety of tissues. Indeed there is a large functional overlap between members of the neuropoietic cytokine family that is revealed by gene knockout studies (Stewart *et al.* 1992) and these have shown that this redundancy is most easily accounted for by a shared receptor framework.

FIGURE 1.5

Leukaemia inhibitory factor

Despite the limited homology of neuropoietic cytokines they are all predicted to share a four antiparallel helix bundle secondary structure, a prediction that is borne out by the structure of LIF as determined by single crystal X-ray diffraction (from the Brookhaven protein databank at ftp://ftp.pdb.bnl.gov (Robinson *et al.* 1994)).



1.12.1 LIF Signal Transduction

The first neuropoietic cytokine receptor to be identified was the IL-6 receptor (IL-6R) (Yamasaki *et al.* 1988). However, transfection with the cDNA for IL6-R conferred mostly low-affinity IL-6 binding, while native IL-6 responsive cells showed both high and low affinity sites (Hibi *et al.* 1990). This led to the search for a molecule associated with IL-6R and immunoprecipitation of IL-6 stimulated cells revealed this protein to be the 130-kDa membrane glycoprotein gp130 (Taga *et al.* 1989).

It has now become apparent that gp130 is critical for the signal transduction of IL-6 and the other members of the neuropoietic cytokine family, with IL6-R conferring specificity to IL-6 and IL-11, LIF-receptor (LIF-R) binding LIF and cardiotrophin-1, CNTF-receptor (CNTFR) binding CNTF, and oncostatin M-receptor (OSM-R) binding oncostatin M. These subtypes then form gp130 receptor complexes either by inducing homodimerisation of two gp130 subunits in the case of IL-6R or by forming heterodimers consisting of the relevant low-affinity receptor associated with gp130 (for review see Taga and Kishimoto, 1997).

Dimerisation initiates signal transduction via activation of a Janus kinase (Jak). This phosphorylates tyrosine residues on the distal part of gp130 and provides a docking site for a transcription factor termed STAT3 (Signal Transducer and Activator of Transcription 3). Tyrosine phosphorylated STAT3 proteins form homodimers and are translocated to the nucleus where they initiate transcription through a cytokine response element (CRE) (Taga, 1996; Taga and Kishimoto, 1997) and induce the expression of a number of peptides including VIP, somatostatin, CGRP, SP and Glial Fibrillary Acidic Protein (GFAP) (Frank and Greenberg, 1996). Additionally, gp130 has been demonstrated to activate a RAS/MAPK (mitogen activated protein kinase) pathway and this also induces an upregulation of transcription, particularly of acute phase proteins (Taga, 1996; Taga and Kishimoto, 1997).

The mechanism that underlies cytokine redundancy therefore appears to lie in the nature of the receptor. This is reflected in experiments examining knockout of the gp130 receptor, where removal of this element is lethal early in development (Ware *et al.* 1995; Yoshida *et al.* 1996). However, specific biological functions of neuropoietic cytokines can still be maintained *in vivo*. A combination of regulated expression of the individual cytokine and limited cellular expression of the ligand-specific receptor component will serve to define the actions of the cytokine.

1.12.2 Location of LIF in the Nervous System

Most investigations on neuronal LIF have addressed its location in the peripheral nervous system where it was primarily regarded as a sympathetic neuronal factor, however more recent studies have also demonstrated actions of LIF in sensory neuronal function.

Expression of LIF in the uninjured nervous system is normally very low (Yamamori, 1991) but this is dramatically upregulated by axotomy or explantation. In the sympathetic system the process occurs in Schwann cells at the site of injury and in the ganglion, whilst in sensory nerves it is restricted to the nerve trunk both proximal and distal to the injury (Banner and Patterson, 1994; Curtis *et al.* 1994; Kurek *et al.* 1996; Sun *et al.* 1996). Nevertheless, sensory fibres retrogradely transport LIF and a study by Thompson *et al.* (1997) defined the population of fibres that undertook this task. They reported that biotinylated LIF (and not IL-6) was transported by peptide and non-peptide small diameter neurones, many of which were immunopositive for the high affinity nerve growth factor (NGF) receptor tyrosine kinase A (trk A). They therefore suggested that this subpopulation was associated with predominantly nociceptive innervation and reflected a role for LIF in pain signalling.

Few studies have investigated the distribution of LIF in the central nervous system. Using reverse transciption-polymerase chain reaction (RT-PCR), Yamamori (1991) detected LIF mRNA in the visual cortex and superior cortex. However spinal cord expression was not examined. More investigators have addressed the central location of the related cytokine IL-6. This is upregulated in the spinal cord by both central injury such as HTLV-I-associated myelopathy (Umehara *et al.* 1994) and by peripheral injury such as sciatic cryoneurolysis neuropathy where it produced mechanical and thermal behavioural hypersensitivity (DeLeo *et al.* 1996). These data support a role for the neuropoietic cytokines in central neuronal function.

1.12.3 Role of LIF in the Nervous System

LIF has important functions in both the developing and adult nervous systems. There is evidence that LIF promotes the differentiation of sensory fibres from progenitor cells of neural crest origin (Murphy *et al.* 1991) and later in development (from E14 and E15), acts as a target derived survival factor in gut and skin for a small population of DRG neurones (Murphy *et al.* 1993). More relevant to this study are its effects in the adult nervous system where it rescues both motorneurones (Cheema *et al.* 1994b) and sensory neurones (Cheema *et al.* 1994a) from axotomy-induced cell death and induces alterations in the chemical phenotype of axotomised sympathetic and sensory fibres (Zigmond *et al.* 1996).

Neuronal injury such as axotomy or explantation brings about changes in the DRG of injured nerves that are collectively called the cell body reaction (Zigmond *et al.* 1996). This was originally characterised as a morphological reaction that reflected a change in neuronal function from synaptic transmission to regeneration (Grafstein and McQuarrie, 1978). Thus the expression of structural proteins such as tubulin, actin and GAP-43 increased in axotomised nerves whilst proteins associated with neurotransmission such as tyrosine

hydroxlase in sympathetic neurones decreased (Koo *et al.* 1988; Hoffman, 1989). This was accompanied by alterations in peptide synthesis such that SP, galanin and VIP were upregulated whilst NPY was decreased in sympathetic neurones (Hyatt-Sachs *et al.* 1993; Schreiber *et al.* 1994) and in sensory neurones, galanin, neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) expression were increased whilst SP and CGRP were decreased (Hokfelt *et al.* 1994). Initial experiments to find the induction factor that caused this process revealed that non-neuronal cells were essential mediators and by pretreating the conditioned medium with an antiserum that immunoprecipitated LIF, the active agent was removed (Shadiack *et al.* 1993; Sun *et al.* 1994). It has now become apparent that LIF is involved in the induction of a number of peptides in both sympathetic and sensory neurones.

From *in vitro* studies, LIF has been demonstrated to increase levels of substance P, VIP, galanin and NK_1 receptor in sympathetic neurones (Rao *et al.* 1993; Shadiack *et al.* 1993; Fann and Patterson, 1994; Sun *et al.* 1994; Ludlam *et al.* 1995). Furthermore, knockout of the LIF gene prevents axotomy-induced upregulation of galanin in sensory neurones (Corness *et al.* 1996; Sun and Zigmond, 1996) and reduces the expression of galanin, neurokinin A and VIP in sympathetic neurones *in vivo* (Rao *et al.* 1993). This process appears to be dependent upon the activation of STAT proteins, which have been demonstrated to act at the cytokine response element in the promoter region of VIP, SP and other peptides and activate gene transcription (Symes *et al.* 1994; Rajan *et al.* 1995; Frank and Greenberg, 1996). The evidence therefore places LIF as an essential mediator in the cascade of events leading to increased gene expression. Indeed it has been reported that axotomy induced changes in neuropeptide expression involve the sequential induction of tumour necrosis factor (TNF) followed by IL-1 and then LIF (Shadiack *et al.* 1993; Ding *et al.* 1995; Ludlam *et al.* 1995). However, Zigmond *et al.* (1994a) have recently identified a novel 50kDa protein that also leads to LIF induction. They have called this factor LIF-induction factor (LIF-IF) and its release could represent one of the earliest biochemical changes associated with axotomy.

Peripheral nerve axotomy brings about changes in the structure of damaged neurones. Postganglionic sympathetic fibres are known to sprout into the DRG and form noradrenergic pericellular basket structures around damaged sensory neurones (McLachlan *et al.* 1993). Thompson *et al.* (1998) have recently shown that LIF may induce this process. Following intrathecal administration of LIF, varicose tyrosine hydroxylase-immunoreactive (TH-IR) sprouts penetrated deeply into the parenchyma of the DRG and formed TH-IR baskets in the absence of peripheral injury. It was suggested that this might lead to a coupling between sympathetic and sensory activity and contribute to sympathetically-maintained neuropathic pain. Indeed, systemic or local injection of LIF brought about an enhanced mechanical

sensitivity in behaving rats (Thompson *et al.* 1996) indicative of the allodynia seen following nerve injury.

A potential consequence of LIF-induced increases in neuropeptide synthesis by primary sensory neurones is a facilitated release of these substances at their central terminals and enhanced spinal neuronal excitability. However, changes in gene expression within central nervous system cells also contribute to spinal hyperexcitability and the factors that regulate these central changes are unknown. The present study investigated the role of centrally applied LIF on gene expression in the spinal cord. Using *in situ* hybridisation, alterations in spinal LIF expression evoked by peripheral nerve injury and inflammation were examined. Since this may have led to alterations in the synthesis of factors involved in central sensitisation, the effects of intrathecally-applied LIF on dorsal horn gene expression were then examined. An important component of central sensitisation is the co-activation of neuropeptide and EAA neurotransmitters. Therefore as a marker for neuropeptide expression, NK_1 receptors were chosen as they are upregulated by both nerve and tissue injury (Abbadie *et al.* 1996) and for the EAA system, glutamate transporter EAAT2 was considered. EAA transmission can occur via ionotropic AMPA, kainate and NMDA receptors or metabotropic receptor subtypes, therefore changes in expression of glutamate transporter mRNA could reflect altered EAA activity at any of these receptor types. Furthermore, the distribution of EAAT2 has been extensively characterised in the spinal cord where it is located on glial cells in laminae I-III and in the ventral horn (Rothstein *et al.* 1994) suggesting that it may regulate glutamatergic transmission in this area. Finally the effects of intrathecally-applied LIF on behavioural sensitivities to thermal and mechanical stimulation were considered.

These experiments addressed for the first time the role of LIF in the central mediation of spinal neuronal hyperexcitability and established its function as a regulatory factor in the spinal cord.

CHAPTER 2: FUNCTIONAL INTERACTIONS INVOLVING NK_1 RECEPTORS AND GLYCINE

2.1 AIMS

There is little evidence to support a direct role of spinal NK_1 receptors in mediating brief noxious cutaneous inputs. These experiments have addressed that lack of action and tested a novel mechanism that describes an apparently covert role for NK_1 receptors during acute nociceptive events. It was proposed that an inhibitory interneurone might mask the NK_1 component during acute nociception. Since SP is known to increase concentrations of glycine in the dorsal horn, the actions of this amino acid at its inhibitory receptor (GlyR) and subsequent regulation of NK_1 receptor activity were investigated.

2.2 METHODS

2.2.1 Animals

Wistar rats were purchased from Charles River UK Ltd, Margate, Kent, UK. They were housed in groups of 3 or 4 in standard rat cages on a 12 hour light/12 hour dark cycle, with a room temperature of 19-23°C. Food and water were available *ad libitum*.

2.2.2 Surgical Procedures

62 Male Wistar rats (210g-400g) (Charles River) were anaesthetised with halothane (Zeneca Pharmaceuticals, Macclesfield, UK) followed by intravenous α-chloralose ($60mgkg^{-1}$) (Sigma, Poole, UK) and urethane ($1.2gkg^{-1}$) (Sigma). Subsequent doses of α-chloralose were given when required (as determined by testing the blink reflex). The trachea was cannulated and a light flow of oxygen passed over the cannula. Body temperature was maintained at 37° C using a thermostatically controlled heat blanket.

The animal was placed in a stereotaxic frame, with stability provided by jaw and ear bars. The spinal column was exposed and supported with 3 pairs of swan-necked clamps and a laminectomy (L_1-L_4) was performed. Agar was injected under the most rostral vertebra and over the exposed area to provide stability. This was removed from the recording area and the dura was cut. Paraffin at 37°C was poured onto the exposed surface.

2.2.3 Electrophysiological Recording and Ionophoresis of Drugs

Extracellular recordings were made from single multireceptive lamina III-V neurones in the exposed dorsal horn using the central barrel of a 4-5μm tipped 7-barrelled glass microelectrode filled with 4M NaCl (pH 4.0-4.5) (Figure 2.1). Recording locations were assessed from micro-manipulator depth readings which were adjusted in 2 or 6μm steps via a microdrive and were well correlated with the position of Pontamine Sky Blue dye spots when ejected from a side barrel.

Recordings were amplified though a recording amplifier with a bandwidth of 1Hz to 7KHz, discriminated through a D.130 spike processor and monitored on an oscilloscope (Tektronix). Discriminated neuronal firing rate was continuously plotted on line on an IBM PS/2-70-121 computer using SCAP90, a customised analysis programme (M. Dutia, Department of Physiology, University of Edinburgh) that allowed event markers to be added as data was collected.

Drugs were ionophoresed from the side barrels of the electrode. A retention current of 10nA to 15nA was applied to each barrel and resistance was monitored regularly; electrodes were rejected if values exceeded 35MΩ. One of the remaining barrels contained 1M NaCl (pH4.0-4.5) for automatic current balance using a Neurophore BH2 Ionophoresis system (Medical systems Corporation) and for current controls. Ionophoresis of vehicle (0.5% DMF, pH4.5 or pH8) or 1M Na^+ had no detectable effect on any responses. Wherever investigated in detail, the magnitude of drug effects was dependent on ionophoretic currents.

2.2.3.1 Drugs for Ionophoresis

Glycine receptor antagonists: phenylbenzene-ω-phophono-α-amino acid (PMBA) (Research Biochemicals International), 1mM in 77mM NaCl, pH8.5 (Saitoh *et al.* 1994); strychnine (Sigma), 1mM in 77mM NaCl and 0.05% dimethylformamide (DMF), pH 4.5.

NK_1 receptor antagonist: GR82334, 1mM aqueous, pH 4.5 (Hagan *et al.* 1991).

NMDA channel blocker: MK-801 maleate (Tocris Cookson), 200μM aqueous, pH 4.65 (Wong *et al.* 1986).

All compounds were ejected with positive current except for PMBA, which was ejected at negative current.

2.2.4 Quantification of Neuronal Responses to Cutaneous Sensory Stimuli

Neurones were located by simultaneously lowering the electrode into the spinal cord and manually brushing the hind limb. Once an appropriate electrode position had been found, spikes were carefully discriminated and the location of neuronal receptive fields was checked by light brushing of the ipsilateral hind limb. The multireceptive nature of the cell

was then established by assessing responses to noxious pinch and 48°C heat. Recording was started to determine the basal firing level.

2.2.5 Quantification of Neuronal Responses to Application of Mustard Oil

Following location and identification of a neurone, mustard oil was applied to the receptive field. Mustard oil is a chemical irritant that can be applied topically, intramuscularly or intra-articularly to selectively activate C-afferent fibres (Woolf and Wall, 1986; Harris and Ryall, 1988). It induces a 2-3°C increase in skin temperature, oedema and an increase in plasma extravasation at the site of application (Lippe *et al.* 1993) and sensitises central neurones to subsequent inputs (Woolf and Wall, 1986). Application of mustard oil therefore brings about enhanced nociceptive responses such that it causes an intense burning sensation in man (Woolf, 1984), prolongs nociceptive reflexes in the rat (Woolf and Wall, 1986) and when applied repetitively, evokes a sustained and incremental level of C-fibre activation analogous to electrically evoked wind-up (Woolf and King, 1990).

In this study, mustard oil (7.5-10% in paraffin) was applied topically to the receptive field at 5 minute intervals until a steady elevated neuronal firing rate (5-30Hz) was maintained (usually after 5-7 applications). The NK_1 receptor antagonist GR82334 was then ejected at a current of 50nA for 2 minutes. Following a 2-minute recovery time, glycine receptor antagonists strychnine or PMBA were applied alone at 30nA for greater than 1 minute and then with 50nA of GR82334. The ejection current of the glycine antagonists was then increased by increments of 10nA at 1-minute intervals. When a reduction in the firing rate occurred, application of GR82334 was stopped and the cell was tested for recovery in the absence of the NK_1 receptor antagonist.

To investigate the specificity of this inhibitory mechanism to NK_1 receptor activation, the protocol was repeated with the NMDA channel blocker MK801 and like the NK_1 receptor antagonist GR82334, this was tested alone and in combination with increasing ejection currents of glycine antagonists.

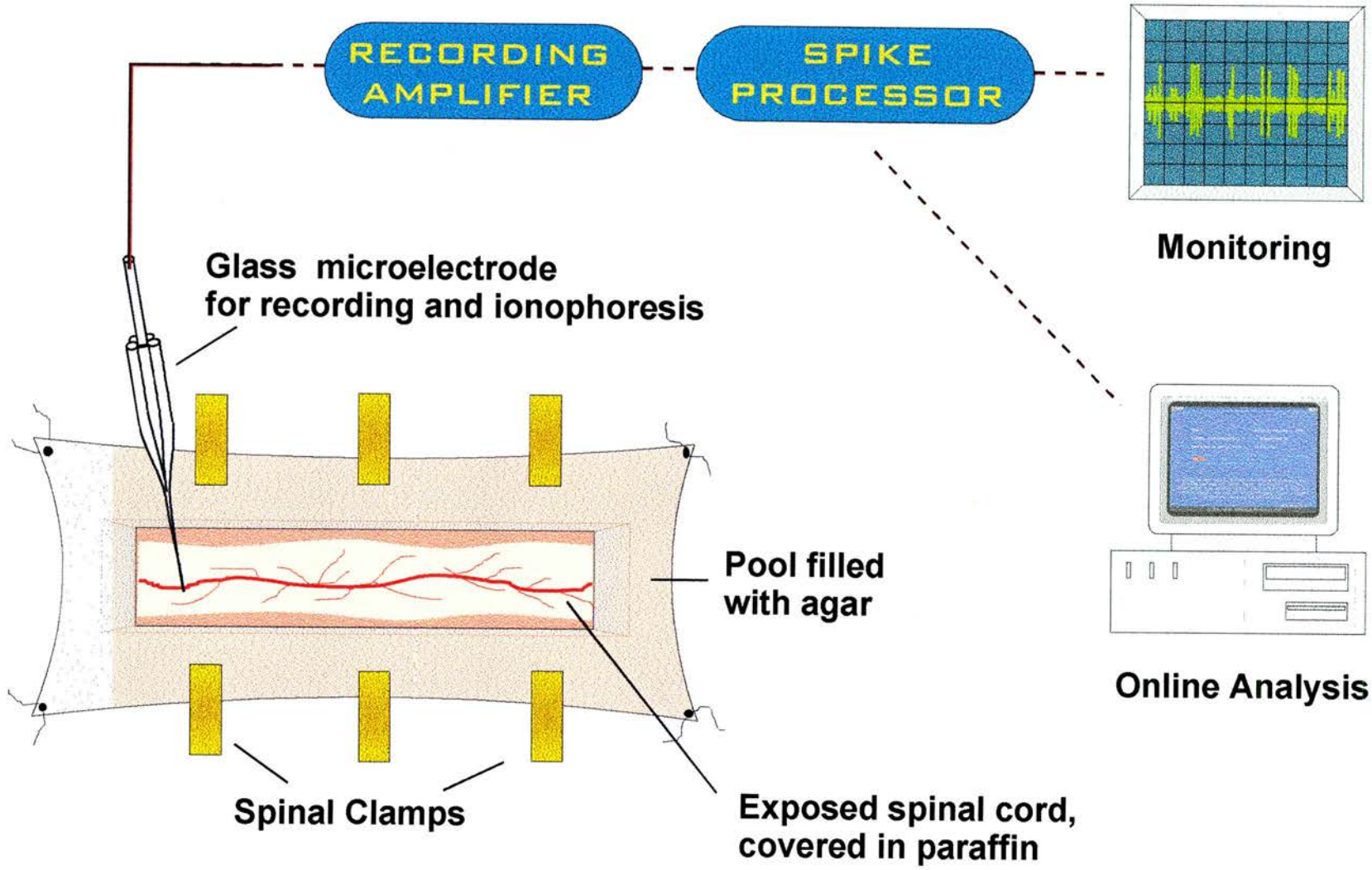
2.2.6 Analysis of Results

Analysis was carried out off-line using the SCAP90 programme. The mean evoked activity was assessed for each drug and corresponding control over a constant period of 30 seconds of stable representative activity before and after application. The change in activity was expressed as a percentage for each cell and then pooled to find the mean±SEM. The statistical significance of differences was determined using a one way repeated measures analysis of variance.

FIGURE 2.1

Schematic representation of the protocol utilised in electrophysiological recording experiments

Schematic representation of the spinal cord exposed in the region of L1-L4 and stabilised using 3 pairs of swan necked clamps. The surrounding area was covered with agar except for the recording region which was protected with paraffin at 37°C. Extracellular recordings were made via a 7-barrelled glass microelectrode and discriminated spikes plotted onliine as actions potentials per second.


RECORDING AMPLIFIER
SPIKE PROCESSOR
Monitoring
Glass microelectrode for recording and ionophoresis
Pool filled with agar
Online Analysis
Spinal Clamps
Exposed spinal cord, covered in paraffin

2.3 RESULTS

In agreement with previous data (Munro *et al.* 1993) the NK_1 antagonist GR82334 did not alter the activity evoked by mustard oil (100±4% of control) in any of the 19 cells tested (Figures 2.2 and 2.4). Similarly, at the ejection currents used the glycine receptor antagonists were without significant effects (strychnine: 96±4%, PMBA: 113±9%) (Figures 2.2 and 2.4). However, when GR82334 was ejected together with strychnine or PMBA it caused a significant ($P<0.05$) inhibition of the mustard oil activity to 43±7% and 68±7% respectively until application of the glycine receptor antagonists was stopped at which time values returned to baseline (Figures 2.2 and 2.4).

In contrast to the NK_1 receptor antagonist, the NMDA channel blocker MK801 readily inhibited mustard oil evoked activity when ejected alone (69±4%) and importantly, strychnine did not alter this reduction (69±14%) (Figures 2.3 and 2.4).

Changes brought about in neuronal firing which were within 15-20% of pre-drug controls were not considered to be of likely functional importance as they were within (or close to) the range of natural variability brought about by vehicle or current controls.

2.4 DISCUSSION

These results demonstrate new evidence for an endogenous inhibitory control over influences of the NK_1 receptor and describe a mechanism where this receptor is prevented from having a direct excitatory effect during acute nociception.

Topical application of mustard oil to the cutaneous receptive field was used as an *in vivo* model of short term but sustained activation of C-fibres. This led to an elevated-firing rate of dorsal horn neurones to which antagonist responses could be tested. Mustard oil application is known to sensitise dorsal horn neurones to subsequent inputs and this is often accompanied by an increase in the receptive field size (Woolf and King, 1990). A similar change was observed in this study indicating that mustard oil might be inducing central sensitisation. However, there was a degree of variability in the time and extent of neuronal "wind-up" that may reflect a lack of access of mustard oil to pertinent sites. Harris and Ryall (1988) demonstrated that application of mustard oil to glabrous skin resulted in poor penetration and no neuronal activation. Observations from the present study are in agreement with this and accordingly, topical application was restricted to receptive fields of multireceptive neurones located over hairy skin.

The current evidence from both electrophysiological and behavioural studies implicates a role for NK_1 receptors in spinal pain transmission only after sustained or prolonged

conditioning stimuli. For example NK_1 receptor antagonists were ineffective at reducing responses to single C-fibre volleys (De Koninck and Henry, 1991) or brief noxious stimuli (Garces *et al.* 1993) but did inhibit electrically-evoked activity and behaviours evoked by more severe models such as subcutaneous formalin injection (Charriaut-Marlangue *et al.* 1991) or sensitisation of STT neurones by capsaicin (Dougherty *et al.* 1994). Indeed Dirig and Yaksh (1996) proposed that SP is a sensitising agent and not a pain transmitter and functions to lower nociceptive thresholds rather than directly evoke pain induced behaviours. The precise nature of the stimulus required to elicit an NK_1 mediated response is not clear, but it is apparent from this experiment and previous data (Munro *et al.* 1993) that topical application of mustard oil does not evoke a direct NK_1 component in the activation of dorsal horn neurones. In this model, mustard oil application reflects a relatively brief form of sustained nociception that does not require NK_1 receptor activation. However in agreement with Dirig *et al.* (1996) the sensitisation of dorsal horn neurones by mustard oil may be NK_1 receptor dependent (S.M. Fleetwood-Walker, unpublished observations). When neuronal responses to brief noxious thermal and mechanical stimulation of the receptive field were tested during mustard oil application, the NK_1 antagonist RP67580 was effective at reducing sensitised activity supporting a role for NK_1 receptors in central sensitisation but not acute nociceptive transmission.

The mechanism that prevents NK_1 receptors from engaging in brief noxious inputs is not yet known. It is possible that a large release of SP, such as that seen after sustained nociception, is required to enable spreading to distant receptors. However direct application of NK_1 agonists onto dorsal horn neurones did not enhance responses to brief inputs (Fleetwood-Walker *et al.* 1987; De Koninck and Henry, 1991; Malmberg and Yaksh, 1992; Yamamoto and Yaksh, 1992; Couture *et al.* 1993) but often inhibited evoked activity (Davies and Dray, 1980; Willcockson *et al.* 1984; Ryall and Pini, 1987; Fleetwood-Walker *et al.* 1988; Fleetwood-Walker *et al.* 1990). Murase and Randic (1984) have investigated this inhibitory phenomenon using an *in vitro* spinal cord slice preparation and they reported that a SP-evoked hyperpolarising response could be blocked with TTX or high Mg^{2+} and low Ca^{2+} solutions indicative of an indirect mechanism. Similarly, Masayama *et al.* (1996) addressed an antinociceptive effect of low doses of SP on behavioural responses to intrathecally-applied NMDA and proposed that this occurred via an opioidergic inhibitory interneurone. However, SP is known to increase levels of glycine in the dorsal horn and this has inhibitory effects via its strychnine-sensitive receptor. It is therefore possible that the activation of an inhibitory glycine interneurone precludes the actions of NK_1 receptors during brief nociceptive transmission.

Glycine has been previously implicated in nociception. Intrathecal application of the glycine receptor antagonist strychnine increased the nociceptive flexor reflex (Sivilotti and Woolf,

1994) and induced allodynia and receptive field expansion (Sosnowski and Yaksh, 1990). Indeed glycinergic inhibitory modulation may be an essential mechanism for regulating spinal neuronal hyperexcitability and removal of this tonic inhibition could unmask inappropriate connections and contribute to pathological pain states (Sosnowski and Yaksh, 1990).

In this study, ionophoresis of strychnine or the selective glycine receptor antagonist PMBA did not alter mustard oil-evoked activity at the current used. Since the aim of these experiments was to unmask an inhibitory regulation of NK_1 receptor activity, very low concentrations of these antagonists were ejected at currents just below the threshold required to potentiate ongoing activity. Therefore, when the NK_1 antagonist was co-applied any excitatory effects of strychnine would not mask potential GR82334 inhibition.

Glycinergic inhibition of NK_1 excitatory inputs has recently been considered. Littlewood *et al.* (1996) investigated the anatomical arrangement of a NK_1/glycine circuit and reported that very few cells were doubled labelled for NK_1 receptors and glycine-LI. However, glycine levels were increased in the dorsal horn following SP application (Kangrga and Randic, 1990b; Smullin *et al.* 1990b; Maehara *et al.* 1995b) and intrathecally-administered glycine inhibited SP-evoked biting and scratching behaviour (Beyer *et al.* 1989) indicating that NK_1 receptors could still activate a glycinergic inhibitory mechanism, albeit indirectly. Data from the present study shows that co-application of GR82334 and glycine receptor antagonists significantly reduced activity evoked by mustard oil and that this inhibition increased with incrementing ejection currents of glycine receptor antagonist and recovered when application of these antagonists was stopped. It is important to note that at the same ejection current GR82334 alone did not reduce mustard oil activity and that the additional presence of the glycine receptor antagonists caused the inhibition. One possible interpretation of these results is that by blocking the glycine receptor, inhibitory regulation of NK_1 receptors was removed. Therefore antagonism of the glycine receptor may have unmasked a *covert* contribution by NK_1 receptors to mustard oil-evoked activity and allowed NK_1 antagonists to exert their inhibitory effects (Figure 2.5).

In contrast, ejection of the NMDA antagonist MK801 in the presence of strychnine had a quite different effect. Ionophoresis of MK801 inhibited the mustard oil activity to 69±4% of control and then this was not altered by the addition of strychnine. Thus dorsal horn neuronal activity evoked by mustard oil application had a direct measurable NMDA receptor component as shown by the action of MK801 and there was no regulatory glycinergic inhibitory mechanism as demonstrated by the lack of effect of strychnine. This would imply that the inhibitory control of NK_1 receptors by strychnine/PMBA-sensitive glycine receptors is not a general phenomenon but is specific to the NK_1 receptor.

Data from this study helps explain the apparent lack of action of NK_1 receptors in brief nociception. NK_1 antagonists are known to be ineffective at reducing neuronal responses to acute stimuli (Charriaut-Marlangue *et al.* 1991; De Koninck and Henry, 1991; Garces *et al.* 1993; Dougherty *et al.* 1994; Dirig and Yaksh, 1996) but the mechanism that precludes their involvement from short-term nociception yet allows them to act as sensitising agents after prolonged input is not known. The present study demonstrates that NK_1 receptor activation may be under the control of a regulatory mechanism where increased release of glycine obscures any direct NK_1 mediated excitatory input. However during more sustained nociception the accumulation of other factors such as increased SP release and intracellular phosphorylations will outweigh this inhibitory modulation and contribute to the sensitising effect of NK_1 receptors (Figure 2.5). Therefore the actions of NK_1 receptors in spinal nociception may occur via a series of time-dependent interactions that help define the physiological response to the stimulus. NK_1 mediated sensitisation will only occur if the stimulation is severe enough to outweigh the inhibitory control. This would suggest that if NK_1 receptors are essential for the initiation of central sensitisation, this balance of inhibitory versus excitatory input should serve as a dynamic regulator of dorsal horn neuronal excitability.

FIGURE 2.2

Effects of ionophoretically applied NK_1 receptor and glycine receptor antagonists on a single dorsal horn neurone.

Individual records of ongoing firing frequency are displayed as action potentials per second (A/s) plotted against time for a single neurone. Record A shows the lack of effect of GR82334 when applied alone or with strychnine vehicle compared to its inhibitory action when ejected in combination with strychnine. Record B shows a similar lack of effect of GR82334 when applied alone compared to inhibition when ejected with an alternative glycine receptor antagonist PMBA.

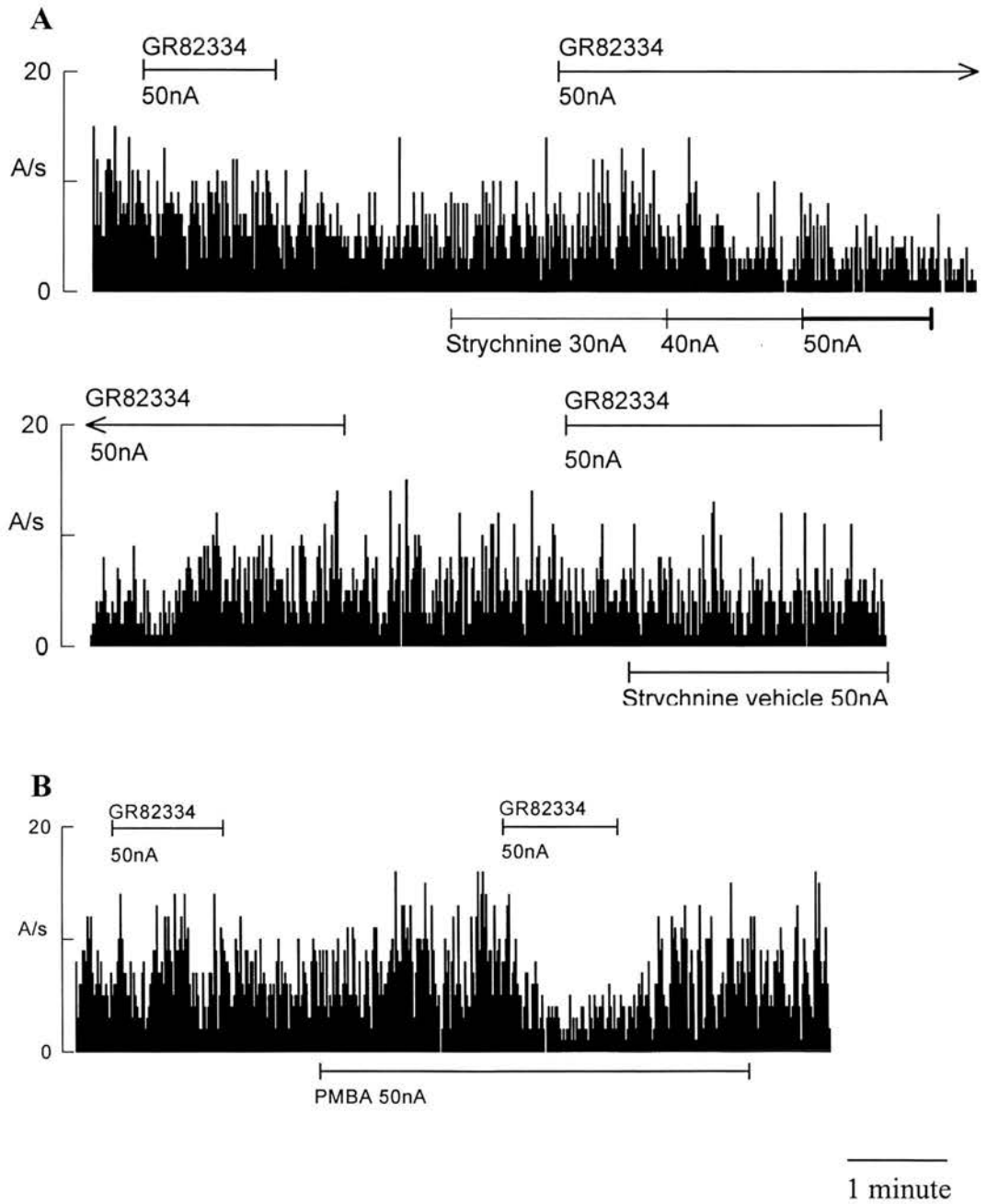
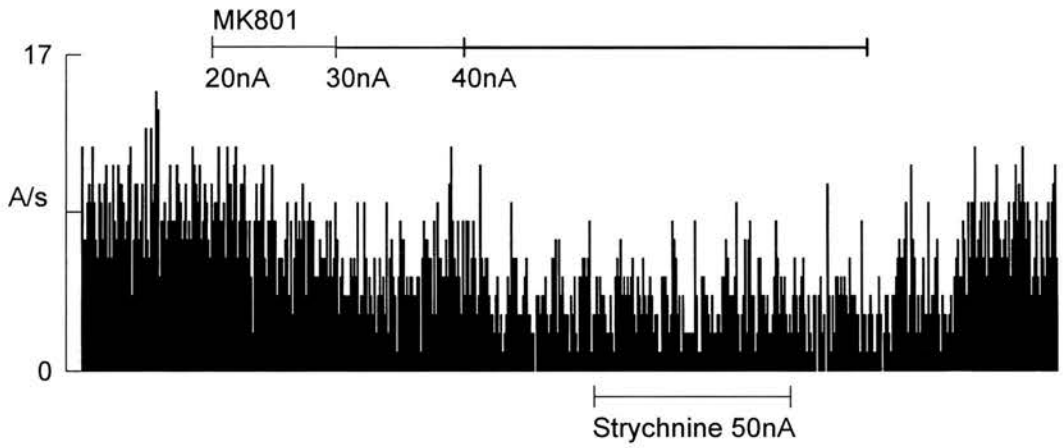

A
GR82334
50nA
GR82334
50nA
20
A/s
0
Strychnine 30nA
40nA
50nA
GR82334
50nA
GR82334
50nA
20
A/s
0
Strychnine vehicle 50nA
B
GR82334
50nA
GR82334
50nA
20
A/s
0
PMBA 50nA
1 minute

FIGURE 2.3

Effects of ionophoretically applied NMDA and glycine antagonists on a single dorsal horn neurone

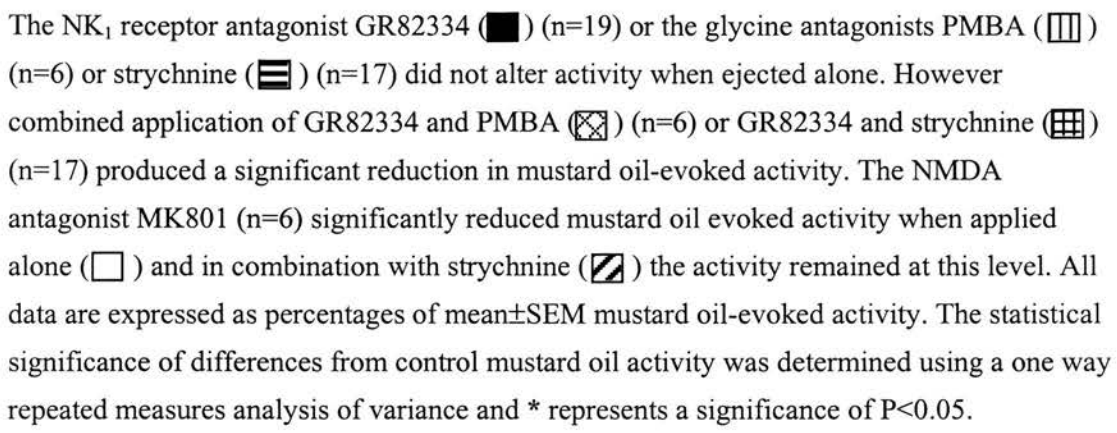
Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s) plotted against time for a single neurone. The record shows the inhibitory effects of MK801 when applied alone and when co-ejected with strychnine.



1 minute

FIGURE 2.4

Effects of NK_1 and NMDA receptor antagonists tested alone and in combination with glycine receptor antagonists on mustard oil-evoked activity



The NK_1 receptor antagonist GR82334 (■) (n=19) or the glycine antagonists PMBA (▥) (n=6) or strychnine (▤) (n=17) did not alter activity when ejected alone. However combined application of GR82334 and PMBA (▨) (n=6) or GR82334 and strychnine (▦) (n=17) produced a significant reduction in mustard oil-evoked activity. The NMDA antagonist MK801 (n=6) significantly reduced mustard oil evoked activity when applied alone (□) and in combination with strychnine (▨) the activity remained at this level. All data are expressed as percentages of mean±SEM mustard oil-evoked activity. The statistical significance of differences from control mustard oil activity was determined using a one way repeated measures analysis of variance and * represents a significance of $P<0.05$.

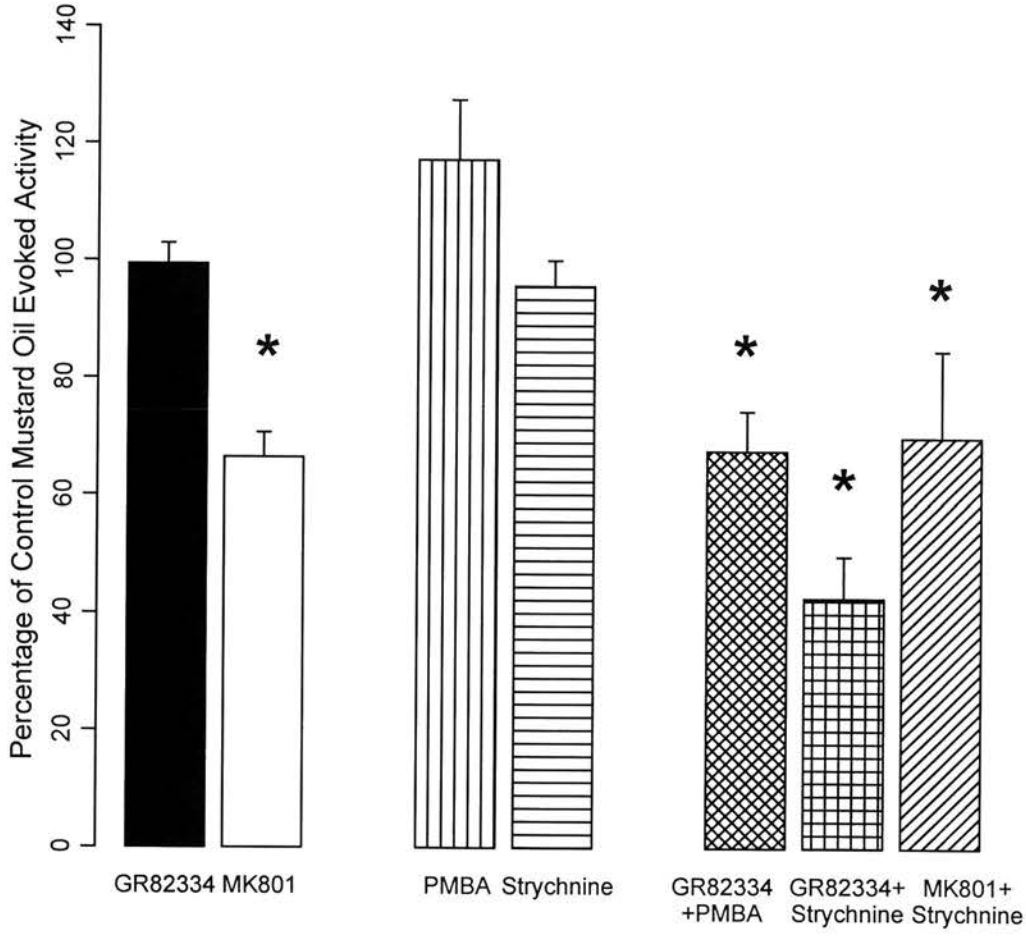
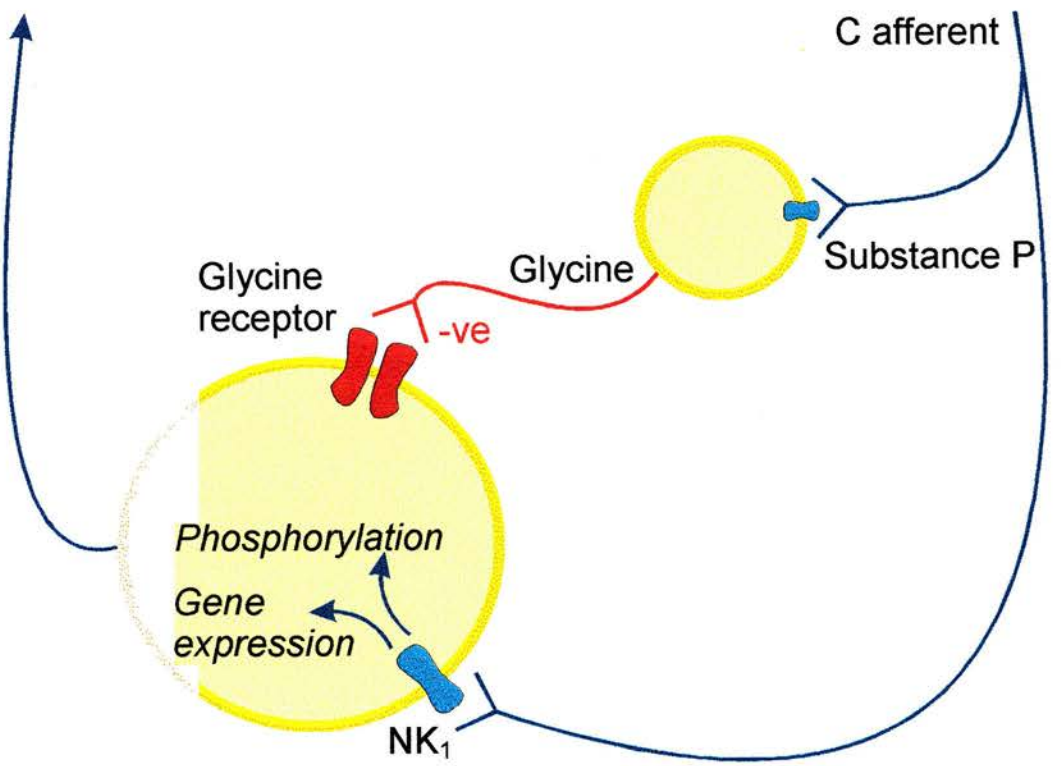

Percentage of Control Mustard Oil Evoked Activity
0
20
40
60
80
100
120
140
*
*
*
*
GR82334
MK801
PMBA
Strychnine
GR82334
+PMBA
GR82334+
Strychnine
MK801+
Strychnine

FIGURE 2.5

A hypothetical model of the actions of SP during acute nociception

SP is known to increase concentrations of glycine in the dorsal horn. During brief noxious inputs this may act via a network of interneurones at the inhibitory glycine receptor and mask any direct effects of NK_1 receptors. However, in more prolonged situations, a combination of increased SP release coupled with an accumulation of intracellular phosphoylation events would overcome the inhibitory modulation.


C afferent
Substance P
Glycine
Glycine receptor
-ve
Phosphorylation
Gene expression
NK1

CHAPTER 3: FUNCTIONAL INTERACTIONS INVOLVING NK_1 RECEPTORS, NMDA RECEPTORS AND GLYCINE

3.1 AIMS

This series of experiments examined the role of the Gly_{NMDA} site in the NMDA/NK_1 receptor interaction. Since SP increases concentrations of glycine in the dorsal horn, it was proposed that this could act at the Gly_{NMDA} site and facilitate NMDA receptor-evoked activity. The possible contribution of PKC mediated phosphorylations to this interaction was also assessed using selective PKC inhibitors.

3.2 METHODS

3.2.1 General Methods

Please refer to section 2.2 for methods regarding animals, surgical procedures, electrophysiological recording techniques, quantification of neuronal responses to cutaneous sensory stimuli and analysis of results.

3.2.2 Drugs for Ionophoresis

AMPA receptor agonist: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 10mM aqueous, pH8 (Krogsgaard-Larsen *et al.* 1980).

Gly_{NMDA} site agonist: 1-aminocyclopropanecarboxylic acid (ACPC), 150μM in 0.5% DMF, pH8 (Nadler *et al.* 1988).

NK_1 receptor agonist: acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-SP_{6-11} (Sar^9-SP), 100μM in 0.5% DMF, pH4.5 (Regoli *et al.* 1988).

NMDA receptor agonist: 1-aminocyclobutane-*cis*-1,3-dicarboxylic acid (ACBD), 100μM aqueous, pH8 (Allan *et al.* 1990).

AMPA receptor antagonist: 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX), 100μM in 0.5% dimethylformamide (DMF), pH8.5 (Sheardown *et al.* 1990).

Gly_{NMDA} site antagonists: 2-carboxy-4,6-dichloro-(1H)-indole-3-propanoic acid (MDL 29951), 100μM in 0.5% DMF, pH4.5 (Salituro *et al.* 1992).

7-chloro-3-(cyclopropylcarbonyl)-4-hydroxy-2(1H)-quinoline (L701,252), 100μM in 0.5% DMF, pH4.5 (Rowley *et al.* 1993).

5,7-dinitroquinaxoline-2,3-dione (MNQX), 100μM in 0.5% DMF, pH4.5 (Sheardown *et al.* 1989).

7-chlorothiokynurenic acid (7-CTK), 50μM aqueous, pH5.5 (Moroni *et al.* 1991) .

Non-competitive NMDA receptor antagonist: MK801 maleate, 200μM aqueous, pH 4.65 (Wong *et al.* 1986).

PKC inhibitors: chelerythrine, 1mM aqueous, pH4.5 (Herbert *et al.* 1990).

GF109203X, 200μM in 0.2%DMF, pH4.5 (Toullec *et al.* 1990).

Polyamine site antagonist: arcaine sulphate, 25mM aqueous, pH4.3 (Reynolds, 1990).

All compounds were from Tocris Cookson (Bristol, UK) except Sar^9-SP from Sigma and the PKC inhibitors which were from Calbiochem (Nottingham, UK). ACBD, ACPC, AMPA, MDL 29951 and NBQX were ejected with positive current and all others were ejected with negative current.

3.2.3 Quantification of Neuronal Responses to Ionophoresis of Agonists

3.2.3.1 NMDA/NK_1 Interaction

In order to determine the effects of Gly_{NMDA} site antagonists and PKC inhibitors on the NMDA/NK_1 receptor interaction, NMDA and NK_1 receptor agonists were ionophoresed to establish facilitated activity.

NK_1 receptor agonist Sar^9-SP was initially ionophoresed at 20nA for 2 minutes. Then following a recovery time of 5 minutes, the NMDA receptor agonist ACBD was applied at 15nA. After a response the ejection current was decreased until a steady firing rate of approximately 15Hz had been reached. Following 3 minutes of regular activity, Sar^9-SP was applied at 5nA or greater and the firing was allowed to stabilise at a constant rate.

3.2.3.2 Effects of Gly_{NMDA} site antagonists

The status of the Gly_{NMDA} site during the NK_1/NMDA interaction was investigated by comparing the effects of Gly_{NMDA} antagonists on the facilitated activity occurring in the joint presence of both NMDA and NK_1 receptor agonists with antagonism of activity evoked by an NMDA receptor agonist alone.

Gly_{NMDA} site antagonists MDL29951, 7-CTK, MNQX or L701,252 were tested on the ACBD and Sar^9-SP evoked activity at a range of currents from 2nA to 60nA and then ionophoresis was discontinued to check for recovery. In 33 cases, following a recovery time of 10 minutes, the compounds were tested on ACBD-evoked activity in the absence of Sar^9-SP. Sar^9-SP was also applied alone at 20nA for 4 minutes before ACBD (n=10) to assess its direct action. A recovery time of 10 minutes was allowed before ACBD was tested.

3.2.3.3 *Effects of a Gly_{NMDA} site agonist*

Substituting Sar9-SP with the Gly_{NMDA} site agonist ACPC further assessed the importance of the Gly_{NMDA} site in the NK_1/NMDA interaction. Sar^9-SP was applied at 15-30nA, superimposed on stable ACBD-evoked activity (n=24) and then when a steady firing rate had been attained L701, 252 was ejected (n=12). ACPC was also applied alone at 30nA for 4 minutes (n=11) with 10 minutes recovery time before further experiments.

3.2.3.4 *Specificity of the NMDA/NK_1 receptor interaction and the influence of Gly_{NMDA} site antagonists*

To demonstrate the specificity of the NMDA/NK_1 interaction and of blockade of the Gly_{NMDA} site, effects of the antagonists were examined on control AMPA-evoked activity. AMPA was applied at 15nA (n=10) and the current then decreased to maintain a stable firing rate. For five of these neurones, Sar^9-SP was ejected at 20nA. The remaining cells were tested with the selective AMPA receptor antagonist NBQX (20-40nA) and Gly_{NMDA} site antagonist L701,252 (20-50nA). Following a recovery time of 5 minutes, activity was evoked from these cells by the combined application of ACBD and Sar^9-SP and then NBQX was applied at a range of currents from 20nA to 60nA.

3.2.3.5 *Effects of other NMDA antagonists*

Controls for the involvement of other regulatory sites on the NMDA receptor complex were carried out by applying the non-competitive NMDA receptor antagonist MK801 (n=5) and the polyamine site antagonist arcaine (n=5) during activity evoked by ACBD alone and (following a recovery time of 10 minutes) to facilitated Sar^9-SP/ACBD-evoked activity.

3.2.3.6 *Effects of PKC inhibitors*

The role of PKC in the NMDA/NK_1 interaction was investigated by testing the effects of PKC inhibitors on facilitated and non-facilitated NMDA receptor-evoked activity. The PKC inhibitors chelerythrine and GF109203X were ionophoresed at a range of currents from 2nA to 60nA and inhibitions of activity evoked by NMDA and NK_1 receptor agonists versus activity evoked by NMDA receptor agonist alone were compared.

3.3 RESULTS

3.3.1 Effects of NMDA and NK_1 receptor agonists and Gly_{NMDA} site antagonists

The majority of tested neurones had basal firing rates lower than 1Hz and responded vigorously to ACBD at an ejection current of 15nA. A steady rate of firing was maintained

by decreasing the current to less than 5nA until a stable activity was observed over a 3 minute time period. The mean firing rate for this stable ACBD-evoked activity was 12±1Hz. Sar^9-SP (which had no effect alone (Figure 3.1A)) was then applied, causing the ACBD response to be facilitated in 80 out of 89 cells by an average of 227±37% (Figure 3.1B); the remaining cells remained unaffected by Sar^9-SP. The facilitation usually occurred over 5 to 10 minutes at a current of 5nA for Sar^9-SP and took up to 15 minutes to fully stabilise. A steady firing rate was attained and this was then resistant to further increases in the current of Sar^9-SP. Application of the Gly_{NMDA} site antagonist MDL 29951 produced an inhibition of the Sar^9-SP plus ACBD-evoked activity in 14 out of 19 cells; completely reversing the facilitation due to Sar^9-SP and inhibiting activity to 49±21% of that evoked by ACBD alone. MDL 29951 was active at a range of 5-20nA and inhibition occurred within 20 seconds to 5 minutes. The activity of 7 out of 14 cells recovered to that previously seen in the presence of ACBD plus Sar^9-SP 15 minutes after ionophoresis of the antagonist was stopped. L701,252 had a similar action with the activity of 19 out of 22 cells being inhibited to 111±30% (5-35nA) of ACBD-evoked control activity (Figure 3.2A) and 13 out of 19 of these showed recovery following discontinuation of L701,252 ionophoresis. MNQX and 7-CTK reduced the Sar^9-SP-enhanced ACBD-evoked activity in 7 out of 11 cells (2-50nA) and 16 (7-65nA) out of 26 cells respectively to 129±62% and 102±29% of ACBD-evoked activity. 4 out of 7 and 13 out of 16 cells of these respective populations recovered within 15 minutes. Figure 3.3 illustrates the mean reduction of Sar^9-SP potentiation of ACBD-evoked activity by the Gly_{NMDA} site antagonists and also shows that at currents matching those used to demonstrate marked inhibition of Sar^9-SP plus ACBD-evoked activity, none of the Gly_{NMDA} site antagonists caused reductions in activity evoked by ACBD alone, although they were effective when ejected at significantly higher currents (see below). The small (and not statistically significant) depression of Sar^9-SP and ACBD-evoked activity below the level due to ACBD alone that was apparently caused by MDL 29951, was not reproduced when the antagonist was tested on ACBD alone at the same current.

12 neurones were tested with MDL 29951 (n=4), L701,252 (n=4) (Figure 3.2B) or 7-CTK (n=4) on activity evoked by ACBD alone following at least 15 minutes recovery from inhibition in the presence of Sar^9-SP and showed only marginal inhibition at currents up to double those required to inhibit ACBD plus Sar^9-SP-evoked activity. At higher currents, the ACBD-evoked activity was reduced in all cases by the Gly_{NMDA} site antagonists. A further 61 separate cells were also tested with MDL 29951 (n=14), L701,252 (n=19), 7-CTK (n=16) or MNQX (n=12) solely on ACBD-evoked activity and the current range for inhibition (MDL 29951: mean 30±7nA, L701,252: mean 38±7nA, MNQX: mean 41±6nA, 7-CTK: mean 44± 6nA) was again significantly higher ($P<0.05$) than that required for inhibition in the additional presence of Sar^9-SP (MDL 29951: mean 14±1nA, L701,252: 16±2nA, MNQX:

18±6nA, 7-CTK: 25±4nA). These results indicate that structurally diverse Gly_{NMDA} site antagonists do not uniformly depress all evoked activity at the currents effective on Sar^9-SP facilitation of ACBD activity and the effect is therefore likely to be specific. Furthermore they underline the point that the facilitation due to Sar^9-SP is outstandingly sensitive to blockade of the Gly_{NMDA} site.

For the 10 neurones first tested with Sar^9-SP alone, 8 out of 10 were not excited by Sar^9-SP (20nA) and of these cells, all showed a potentiation of ACBD-evoked activity by the NK_1 receptor agonist. 2 out of 10 cells were directly excited by Sar^9-SP (20nA) from a basal level of less than 1Hz to 15-20Hz; this activity was further potentiated in the additional presence of ACBD (1-10nA), but L701,252 (5-40nA) or MDL 29951(50-50nA) had no inhibitory effect.

3.3.2 Effects of the Gly_{NMDA} Site Agonist ACPC

The Gly_{NMDA} site agonist ACPC (15-30nA) had no effect on basal firing rates when applied alone (n=25) (Figure 3.4A). Following a recovery time of 10 minutes, ACBD was applied, and ACPC at the same current range (15-30nA) significantly increased the firing rate by 88± 11% in 24 out of 25 neurones (Figure 3.4B) ($P<0.05$). The facilitation consistently took less than 5 minutes to stabilise at this level and did not show any further increase (up to 35nA). Application of L701,252 (n=12) reduced this activity to 79±17% of ACBD-evoked control activity for all 12 neurones (Figure 3.4C) and the average current for inhibition was 18±4nA. This current was significantly lower ($P<0.05$) than that required to inhibit activity evoked by ACBD alone and resembled the low currents required to inhibit the Sar^9-SP facilitation of ACBD-evoked activity.

3.3.3 Effects of AMPA and the AMPA Receptor Antagonist NBQX

Application of AMPA caused excitation in 10 out of 10 neurones and this was maintained at a stable rate (15-30Hz) by reducing the ejection current to below 5nA (Figure 3.5A). Sar^9-SP (20nA) (Figures 3.5A amd 3.5B) or L701,252 (50nA) (Figure 3.5C) caused only minimal alteration in the steady AMPA-evoked activity in 5 out of 5 of the tested neurones (107±5% and 104±5% respectively) whereas NBQX (n=5) significantly inhibited the firing rate in all cases to an average of 52±13% ($P<0.05$) (Figure 3.5D). Following a recovery time of 5 minutes, the combined application of Sar^9-SP and ACBD evoked a steady activity of 34± 7Hz and ejection of NBQX at up to 50nA did not alter the firing rate of these 5 neurones (101±21% of control) (Figure 3.5E).

3.3.4 Effects of MK801 and the Polyamine Site Antagonist Arcaine

MK801 (n=5) and arcaine (n=5) (Figures 3.6A and 3.6B) significantly reduced both the Sar^9-SP facilitated ACBD-evoked activity (to 63±4% and 73±9% respectively) and ACBD-evoked activity alone (to 66±7% and 70±9%) to similar levels. Unlike the Gly_{NMDA} site antagonist and PKC inhibitors, facilitated activity was not more sensitive to antagonism by these compounds than activity evoked by ACBD alone (Figure 3.7): Mean effective currents for 50% inhibition were 31±6nA and 19±5nA respectively for MK801 and 46±7nA for both activities with arcaine.

3.3.5 Effects of PKC Inhibitors

The PKC inhibitors, chelerythrine and GF109203X (Figure 3.8A), significantly reduced the Sar^9-SP facilitation (411±81%) of ACBD-evoked activity to 111±26% (10 out of 14 neurones) and 76±34% (8 out of 10 neurones) of control respectively ($P<0.05$). When applied to ACBD-evoked activity alone, the PKC inhibitors had only marginal effects in all tested neurones; chelerythrine reduced the ACBD-evoked activity to 87±9% (n=12) and GF109203X increased the activity to 114±24% of control (n=7) (Figure 3.8B); these small changes were not significant.

3.4 Discussion

The present investigation provides further evidence for an NK_1 receptor-mediated facilitation of NMDA receptor agonist-evoked activity in the dorsal horn. Furthermore this facilitation was blocked by the application of several different Gly_{NMDA} site antagonists and PKC inhibitors and the blockade occurred more potently than blockade of activity evoked by NMDA receptor agonist alone. This is further support for a key role of the Gly_{NMDA} site in sensory processing in the dorsal horn and implicates its occupation as a crucial regulatory mechanism in the influence of NMDA receptors on dorsal horn neurones. In particular the Gly_{NMDA} site and PKC appear to be important mediators of the interaction between the NK_1 and NMDA receptors and these may therefore serve as important components in sensitisation of dorsal horn neurones following sustained input.

An essential requirement for NMDA-agonist evoked activity is occupation of the Gly_{NMDA} site. This is supported by data from the experiments where Gly_{NMDA} antagonists were applied to ACBD-evoked activity and reduced the firing rate. The effects are consistent with evidence that glycine binding to its allosteric site on NMDA receptors is a critical regulator of their function (Kleckner and Dingledine, 1988). In Xenopus oocytes and in cortical neurones *in vitro,* the presence of added glycine seems to be an absolute requirement for the function of NMDA receptors (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988)

and although cerebellar granule cells and spinal cord neurones *in vitro* and cerebral cortex neurones in slices do show responses to NMDA in the absence of added glycine, these are greatly enhanced by glycine addition (Fletcher and Lodge, 1988; Murase *et al.* 1989; Thomson *et al.* 1989; D'Angelo *et al.* 1990). The incomplete reversal of the ACBD-evoked activity by the Gly_{NMDA} antagonists may reflect failure to reach adequate concentrations, non-selective actions of the drugs or residual channel activity continuing in the absence of glycine occupation. The present results do however confirm that NMDA receptor agonist responses of dorsal horn neurones can be inhibited by Gly_{NMDA} site antagonists, indicating an endogenous partial tone due to glycine.

For the Gly_{NMDA} site to be functionally utilised in SP modulation of the NMDA receptor complex, ambient concentrations of glycine must presumably be below saturation levels for this site. Application of the selective Gly_{NMDA} site agonist ACPC facilitated ACBD-evoked activity clearly whilst having no effect when applied alone. This is consistent with other studies which showed that exogenously applied glycine potentiated NMDA receptor responses (Larson and Beitz, 1988; Salt, 1989; Budai *et al.* 1992; Rusin *et al.* 1992), suggesting that the resting concentration of glycine is low. Maehara *et al.* (1993) provided evidence that SP increases the release of glycine in the rat spinal cord by two fold and may therefore increase extracellular glycine concentrations enough to contribute to promoting activation of the NMDA receptor. On balance it appears that most NMDA receptors *in vivo* appear to be normally subjected to a partial tone from glycine, corresponding to the μM concentrations thought to be present in the extracellular space in the CNS *in vivo* (Skilling *et al.* 1988). However, the extent of this tonic influence is likely to vary at different locations because of differences in local glycine clearance mechanisms (Priestley and Kemp, 1983; O'Shea *et al.* 1991) or in glycine sensitivity of NMDA receptor complexes which may correspond to the presence of different subunit configurations (Nakanishi, 1992). Our results confirm that the NMDA receptor agonist responses of dorsal horn neurones can be inhibited by Gly_{NMDA} site antagonists and be potentiated by Gly_{NMDA} agonists, indicating an endogenous partial tone but not complete saturation of the Gly_{NMDA} site.

Ionophoretically-applied Sar^9-SP facilitated ACBD-evoked activity in dorsal horn neurones. This occurred gradually over several minutes, stabilised within 15 minutes and continued at this level even after application of Sar^9-SP was stopped. Furthermore, application of Sar^9-SP alone at greater than 5 times the potentiating current generally had no effect on background activity demonstrating that the amplification of ACBD responses was dependant on NMDA receptor function. By applying Sar^9-SP after ACBD had evoked increased activity it can be assumed that potentiation was not due to changes in membrane potential directly altering the extent of Mg^{2+} block (although activation of PKC may account for a relative lifting of the block) and this is further supported by the observation that at the ejection currents used, Sar^9-

SP elicited no recorded increase in electrical activity. This data also precludes the possibility of summation of the 2 activities and consequently the facilitation might therefore be due to a direct alteration of the conductance of the NMDA channel, perhaps via the Gly_{NMDA} site.

In support of this, Sar^9-SP had no effect on AMPA-evoked firing, implying that under the present circumstances, the facilitation was specific to NMDA receptor-mediated firing and not due to a general increase in subthreshold excitability. This accords with other *in vivo* investigations of the interaction. Dougherty and Willis (1990) found that SP facilitated the action of quisqualic acid in only two out of eight macaque spinothalamic tract neurones whereas combined application of SP and NMDA enhanced activity in six out of eight cells and to a far greater extent. Cumberbatch *et al.* (1995b) reported facilitations of AMPA, Kainate and NMDA receptor mediate responses by the NK_1 agonist GR7362, but the facilitations of the NMDA receptor responses appeared clearly greater than those to AMPA and Kainate. The selective facilitation by NK_1 agonist of NMDA but not AMPA responses under the present conditions therefore verifies the specificity of the interaction apparently mediated by the Gly_{NMDA} site. Furthermore, the Gly_{NMDA} antagonist L701,252 did not inhibit AMPA-evoked activity and the AMPA antagonist NBQX did not affect NMDA plus Sar^9-SP-evoked activity. As well as demonstrating the specificity of the compounds these data also confirm that responses were not due to any artefactual current coupling effects between electrode barrels.

The present experiments suggest that the Gly_{NMDA} site may additionally play a very specific role in the facilitation of NMDA receptor-evoked activity due to NK_1 receptors, since the facilitation of ACBD-evoked activity brought about by Sar^9-SP was selectively blocked by Gly_{NMDA} site antagonists more potently than their inhibition of ACBD-evoked activity alone. Four structurally different antagonists produced similar results in support of the idea that this site was indeed the mediator of their common action. This data is also supported by previous evidence on the role of the Gly_{NMDA} site in nociceptive transmission. Both electrophysiological and behavioural protocols have shown that blockade of spinal Gly_{NMDA} sites reduces dorsal horn neuronal activity (Dickenson and Aydar, 1991; Chapman and Dickenson, 1994) and pain induced behaviours (Coderre and Van Empel, 1994; Millan and Seguin, 1994; Laird *et al.* 1996; Lutfy and Weber, 1996) evoked by sustained nociceptive stimuli.

Furthermore, Rusin *et al.* (1992) have shown that the Gly_{NMDA} site is an important mediator in the NMDA/NK_1 interaction. They reported that SP facilitation of NMDA-induced inward currents in isolated dorsal horn neurones was not observed in cells continuously perfused with glycine, i.e. saturation of the Gly_{NMDA} site led to occlusion of any further effect of SP. However, they also observed a restoration of SP facilitation of NMDA responses in the presence of continuously perfused glycine plus Gly_{NMDA} site antagonist (7-chlorokynurenic

acid) which does not accord with the present results. The experimental conditions differ greatly between the two studies of course, and additional sites of action may well be present in the *in vivo* rather than the isolated neurone preparation. Nevertheless one possible interpretation of their results is a partial reversal by 7-chlorokynurenic acid of the Gly_{NMDA} site-mediated occlusion of SP effect, allowing the reappearance of some SP facilitation perhaps as further glycine released by SP action overwhelms the influence of the Gly_{NMDA} site antagonist.

The greater sensitivity to Gly_{NMDA} site antagonists of the NK_1 receptor agonist-induced facilitation of ACBD-evoked activity (rather than ACBD-evoked activity alone) in the present experiments accords closely with recent behavioural observations. Kolhekar *et al.* (1994) reported that the reduction in tail flick latency caused by the Gly_{NMDA} site agonist, D-serine, was blocked more potently and fully by 7-chlorokynurenate than the reduction caused by NMDA, although both were equally sensitive to MK-801. This suggests that phenomena directly elicited by stimulation of the Gly_{NMDA} site are somewhat more sensitive to Gly_{NMDA} site antagonists than those elicited by stimulation of the excitatory amino acid site of the NMDA receptor and an analogous situation could arise here if Sar^9-SP was acting by increasing glycine release. This hypothesis was tested using two approaches. Firstly the effects of antagonists at other sites on the NMDA receptor were investigated and secondly Sar^9-SP was substituted with a Gly_{NMDA} agonist ACPC to potentiate NMDA activity. From the first protocol it became apparent that inhibitions by MK801 (acting as a non-competitive channel blocker) and arcaine (a polyamine site antagonist) were no different on Sar^9-SP facilitated versus ACBD-evoked activity alone and that increased modulation of the NMDA receptor complex by NK_1 receptor activation only involved functional changes in the influence of the Gly_{NMDA} site. Secondly, by mimicking the presumed action of Sar^9-SP with ACPC a similar facilitation was observed and this was also much more sensitive to blockade by Gly_{NMDA} antagonists than ACBD-evoked activity alone. These data would therefore suggest that the allosteric nature of interactions between the Gly_{NMDA} and EAA recognition sites may produce a situation where activation of the channel by increasing glycine in the face of a constant EAA level would be more sensitive to Gly_{NMDA} site antagonists than activation resulting from increased EAA levels in the face of a constant glycine level. Furthermore, since this phenomenon appears to occur when NK_1 agonist is co-applied with NMDA agonist, it supports the hypothesis that NK_1 receptors may facilitate NMDA receptor function via the Gly_{NMDA} site.

The data is also consistent with a postsynaptic effect of Sar^9-SP. Dendrites of nociceptive laminae III-V neurones have been shown to receive SP-immunoreactive terminals (De Koninck *et al.* 1992) and a subpopulation of neurones, including spinothalamic tract cells, in this region express immunoreactivity for the NK_1 receptor (Bleazard *et al.* 1994; Liu *et al.*

1994; Nakaya *et al.* 1994; Marshall *et al.* 1996). Additionally, mRNAs for NMDA receptor subtypes are widely expressed throughout dorsal horn neurones (Tolle *et al.* 1993; Luque *et al.* 1994; Watanabe *et al.* 1994) consistent with the hypothesis of a postsynaptic interaction between NK_1 and NMDA receptors occurring here. The possibility of a substantial postsynaptic effect of Sar^9-SP was directly investigated by applying Sar^9-SP and ACPC simultaneously to stable ACBD-evoked activity at currents that would not facilitate that activity when applied individually. Although an excitation was observed when ejected together, it was not possible to configure experimental conditions that enabled definitive analysis of whether or not synergistic interactions were occurring. Other evidence however, strongly suggests that functional responses to NMDA in isolated dorsal horn neurones can be facilitated by activation of protein kinase C (Watson and Downes, 1983; Chen and Huang, 1992; Nakajima *et al.* 1992). One known consequence of activating an NK_1-type receptor is the activation of PKC (Rusin *et al.* 1993c), so the modulation of site-site interactions within the NMDA receptor complex by a NK_1 receptor-induced event may occur via PKC-mediated phosphorylation. Indeed PKC has been implicated as a mediator of spinal nociceptive transmission. It augments neuronal activity when activated by phorbol esters (Cerne *et al.* 1992; Palecek *et al.* 1994), is translocated to the membrane compartment after sustained noxious inputs (Mao *et al.* 1993; Munro *et al.* 1994; Yashpal *et al.* 1995) and PKC inhibitors have been shown to reduce activity evoked by mustard oil (Munro *et al.* 1994) or capsaicin (Sluka *et al.* 1997b and 1997c) as well as behaviours evoked by subcutaneous application of formalin (Coderre, 1992; Coderre and Yashpal, 1994; Yashpal *et al.* 1995) and capsaicin (Sluka and Willis, 1997). Furthermore, an isoform of PKC, PKCγ is upregulated in the spinal cord after peripheral inflammation (whilst NMDA receptor subunit expression remains constant) (Berthele *et al.* 1996) and gene knockout of this variant produces mice that do not display behavioural hyperalgesia following nerve injury or inflammation (Malmberg *et al.* 1997).

PKC has also been demonstrated to have an important function in the NMDA/NK_1 interaction. PKC inhibitors block the SP-mediated facilitation of NMDA responses in acutely isolated dorsal horn neurones (Rusin *et al.* 1993b), reduce ventral root potentials evoked by subthreshold applications of NK_1 receptor agonist and NMDA (Urban *et al.* 1994a) and inhibit enhanced behavioural responses to subcutaneous formalin injection induced by the co-application of SP and NMDA (Coderre, 1992; Coderre and Yashpal, 1994).

In agreement with these studies, we report that the PKC inhibitors chelerythrine and GF109203X inhibited Sar^9-SP facilitation of ACBD-evoked activity and propose that this would be consistent with a postsynaptic mechanism of the NMDA/NK_1 interaction. Such an event does not preclude the previous evidence on the role of the Gly_{NMDA} site as this may

occur additionally to any Sar^9-SP-induced increase in glycine release. It is also possible that changes in the sensitivity of the Gly_{NMDA} site seen during NK_1 and NMDA activation are caused by a site specific PKC mediated phosphorylation initiated by the NK_1 receptor. This is further supported by the observation that Gly_{NMDA} site agonist does not facilitate ACBD-evoked activity to the same extent as Sar^9-SP, so a postsynaptic increase in the sensitivity of the Gly_{NMDA} site coupled with an increase in glycine access to the NMDA receptor complex could explain the present results (Figure 3.9).

This investigation has addressed the role of NK_1 receptors in sustained nociception and focused on their involvement in the NMDA/NK_1 interaction. By examining the effects of antagonists at the Gly_{NMDA} site on neuronal activity evoked by combined application of NMDA and NK_1 agonists and comparing this to antagonism of NMDA activity alone, it has become apparent that activation of NK_1 receptors results in an increased sensitivity of the Gly_{NMDA} site. This has been discussed with reference to SP-induced increases in local glycine concentrations and PKC mediated phosphorylations of the NMDA receptor complex. The relative importance of such a mechanism in the context of acute versus sustained nociception will be considered in Chapter 5.

FIGURE 3.1

Effects of ionophoretically-applied NK_1 and NMDA receptor agonists on a single dorsal horn neurone

Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s), integrated over 700 millisecond bins, plotted against time for a single neurone. Record A shows the typical lack of effect of Sar^9-SP on background activity. Record B shows the facilitation of ACBD-evoked activity by Sar^9-SP.

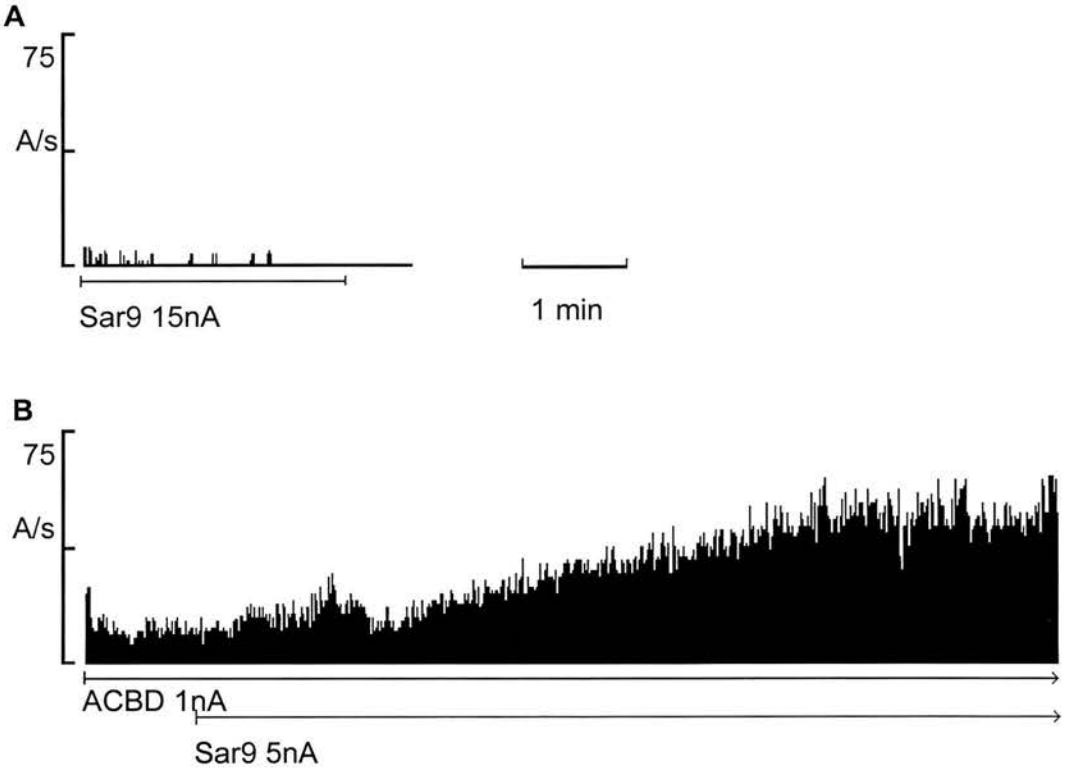

A
75
A/s
Sar9 15nA
1 min
B
75
A/s
ACBD 1nA
Sar9 5nA

FIGURE 3.2

Effect of ionophoretically applied Gly_{NMDA} antagonist on activity evoked by NMDA plus NK_1 receptor agonists compared to that evoked by NMDA receptor agonist alone

Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s), integrated over 700 millisecond bins, plotted against time for a single neurone. Record A shows the inhibitory effects of Gly_{NMDA} antagonist L701,252 on ACBD plus NK_1 receptor agonist-evoked activity and record B shows the lack of effect of L701,252 on ACBD evoked activity alone until higher ejection currents.

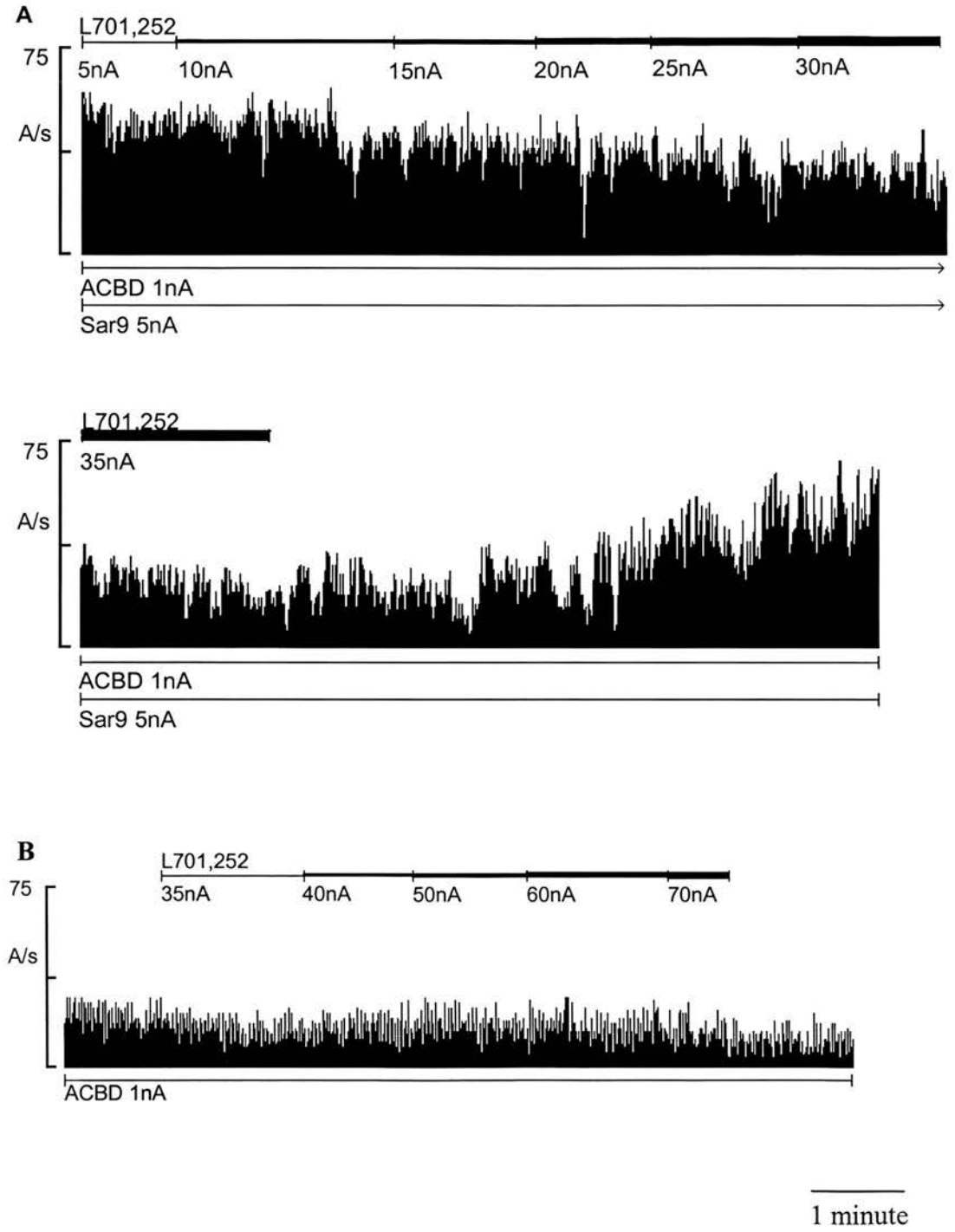

A
L701,252
75
5nA
10nA
15nA
20nA
25nA
30nA
A/s
ACBD 1nA
Sar9 5nA
L701,252
75
35nA
A/s
ACBD 1nA
Sar9 5nA
B
L701,252
75
35nA
40nA
50nA
60nA
70nA
A/s
ACBD 1nA
1 minute

FIGURE 3.3

Effects of Gly_{NMDA} site antagonists on neuronal activity evoked by NMDA receptor agonists or by NMDA plus NK_1 receptor agonists

Activity due to ACBD (□) and ACBD-evoked activity in the additional presence of Sar^9-SP (■). Effects of the Gly_{NMDA} site antagonists were tested on either ACBD-evoked activity (▥,▨) or ACBD- and Sar^9-SP -evoked activity (▦): MDL29951 at 14±1nA in ▥ and ▦ at 30±7nA in ▨. L701,252 at 16±2nA in ▥ and ▦ and at 38±7nA in ▨. MNQX at 18±6nA in ▥ and ▦ and at 41±6nA in ▨. 7-CTK at 25±4nA in ▥ and ▦ and at 44±6nA in ▨. All data is expressed as percentage of mean control ACBD-evoked activity (for individual drugs) and each value is the mean ± SEM. The statistical significance of differences was assessed using the Mann-Whitney U-test (* represents a significant difference from ACBD plus Sar^9-SP - evoked levels at $P<0.05$.)

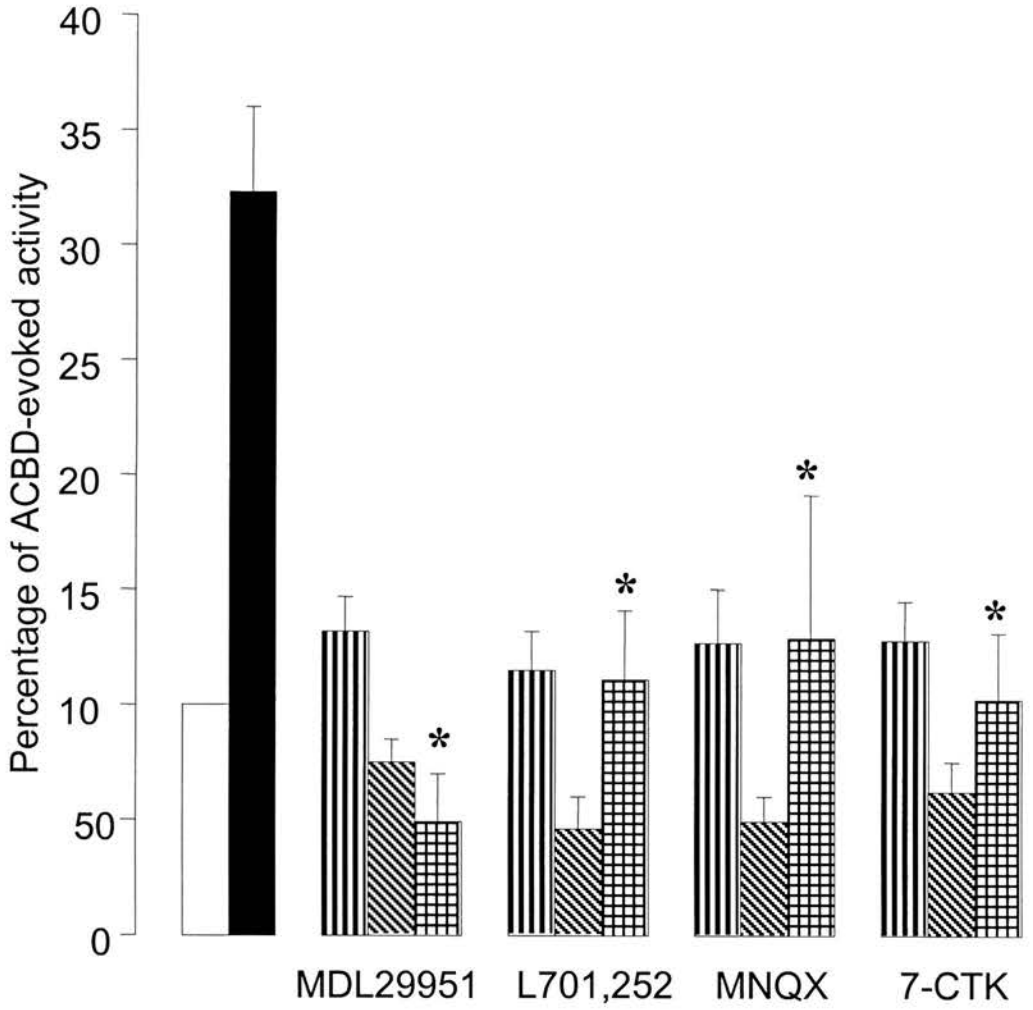

Percentage of ACBD-evoked activity
40
35
30
25
20
15
10
50
0
*
*
*
*
MDL29951
L701,252
MNQX
7-CTK

FIGURE 3.4

Effects of ionophoretically applied Gly_{NMDA} site and NMDA receptor agonists and a Gly_{NMDA} site antagonist on a dorsal horn neurone.

Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s), integrated over 700 millisecond bins, plotted against time. Record A shows the typical lack of effect of ACPC when applied alone. Record B shows the facilitatory effect of the Gly_{NMDA} agonist ACPC on ACBD-evoked activity ($P<0.05$, Mann-Whitney U-test) on the same neurone following a sufficient recovery time. Record C is from a separate neurone and shows the inhibitory effect of L701,252 on ACBD plus ACPC-evoked activity.

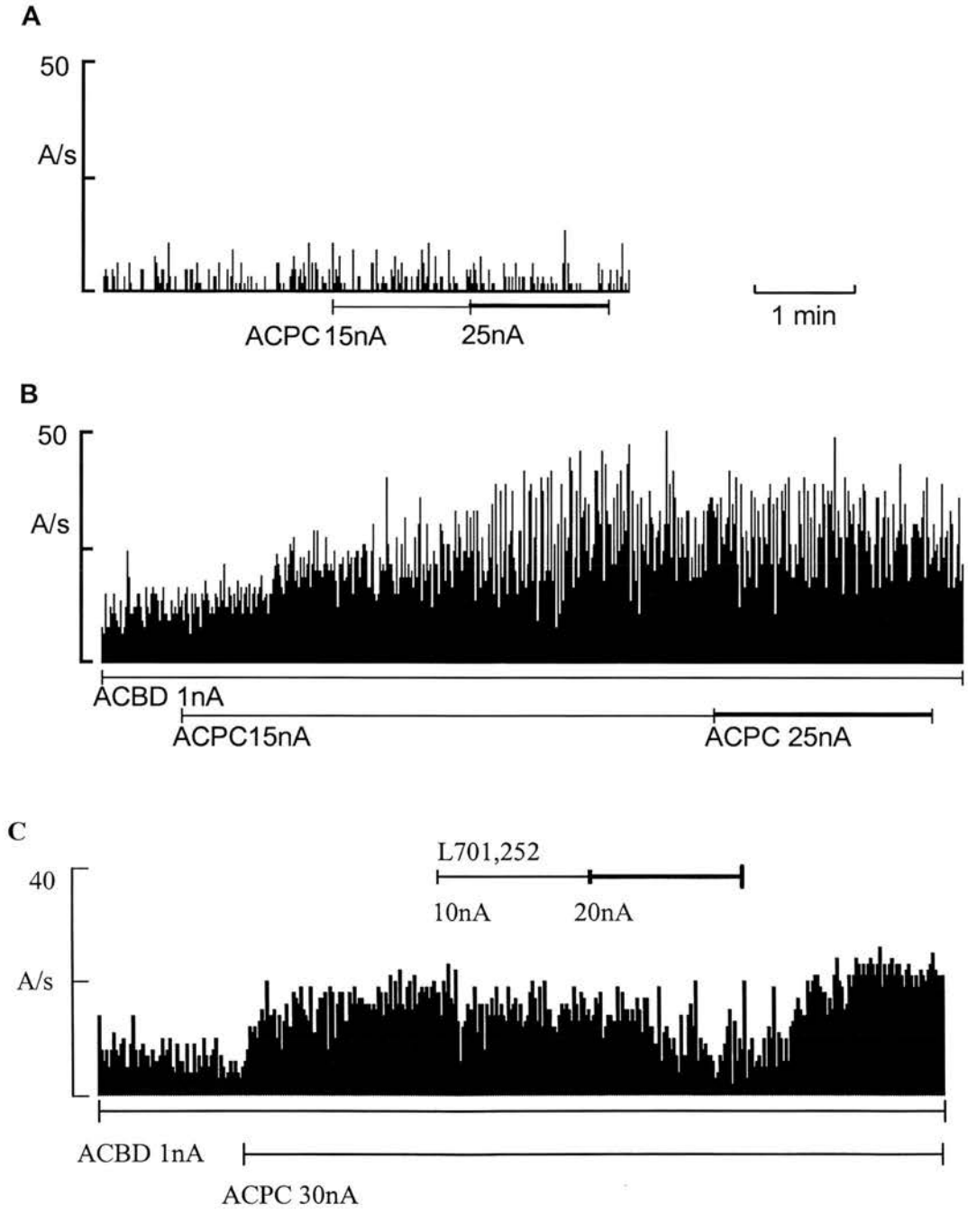
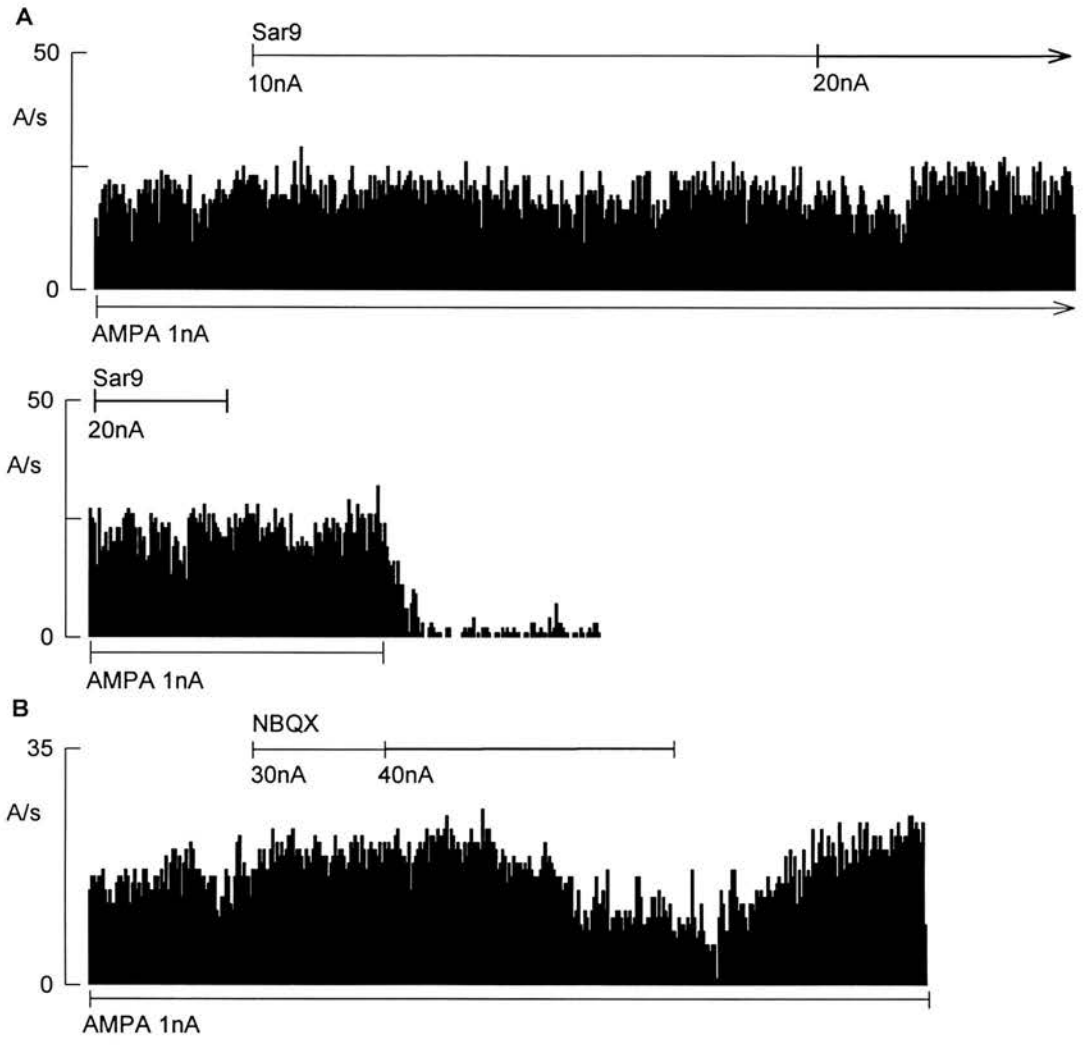
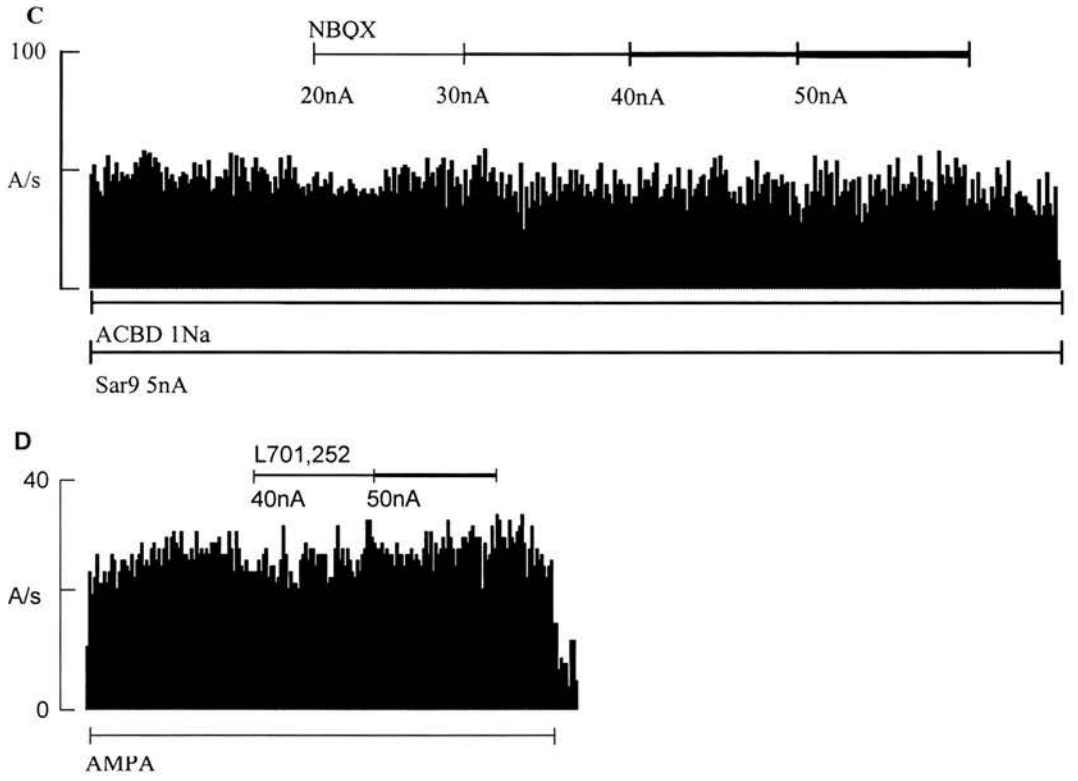

A
50
A/s
ACPC 15nA
25nA
1 min
B
50
A/s
ACBD 1nA
ACPC15nA
ACPC 25nA
C
L701,252
40
10nA
20nA
A/s
ACBD 1nA
ACPC 30nA

FIGURE 3.5

Effects of ionophoretically applied AMPA, NMDA and NK_1 receptor agonists and Gly_{NMDA} site and AMPA receptor antagonists on a dorsal horn neurone

Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s), integrated over 700 millisecond bins, plotted against time. Record A shows the typical lack of effect of Sar^9-SP when applied to AMPA-evoked activity. Record B shows the inhibitory effect of NBQX on AMPA-evoked activity, record C shows the lack of effect of NBQX on activity evoked by Sar^9-SP and ACBD and record D shows the lack of effect of L701,252 on AMPA-evoked activity ($P<0.05$, Mann-Whitney U-test).


A
50
A/s
0
Sar9
10nA
20nA
AMPA 1nA
Sar9
50
20nA
A/s
0
AMPA 1nA
B
35
A/s
0
NBQX
30nA
40nA
AMPA 1nA


C
100
NBQX
20nA
30nA
40nA
50nA
A/s
ACBD 1Na
Sar9 5nA
D
40
L701,252
40nA
50nA
A/s
0
AMPA

1 minute

FIGURE 3.6

Effects of the polyamine site antagonist arcaine on activity evoked by NMDA plus NK_1 receptor agonists and on NMDA receptor agonist-evoked activity alone

Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s), integrated over 700 millisecond bins, plotted against time for a single neurone. Record A shows the inhibitory effects of the polyamine site antagonist arcaine on ACBD plus NK_1 receptor agonist evoked activity and record B shows the inhibition of ACBD evoked activity alone at similar ejection currents.

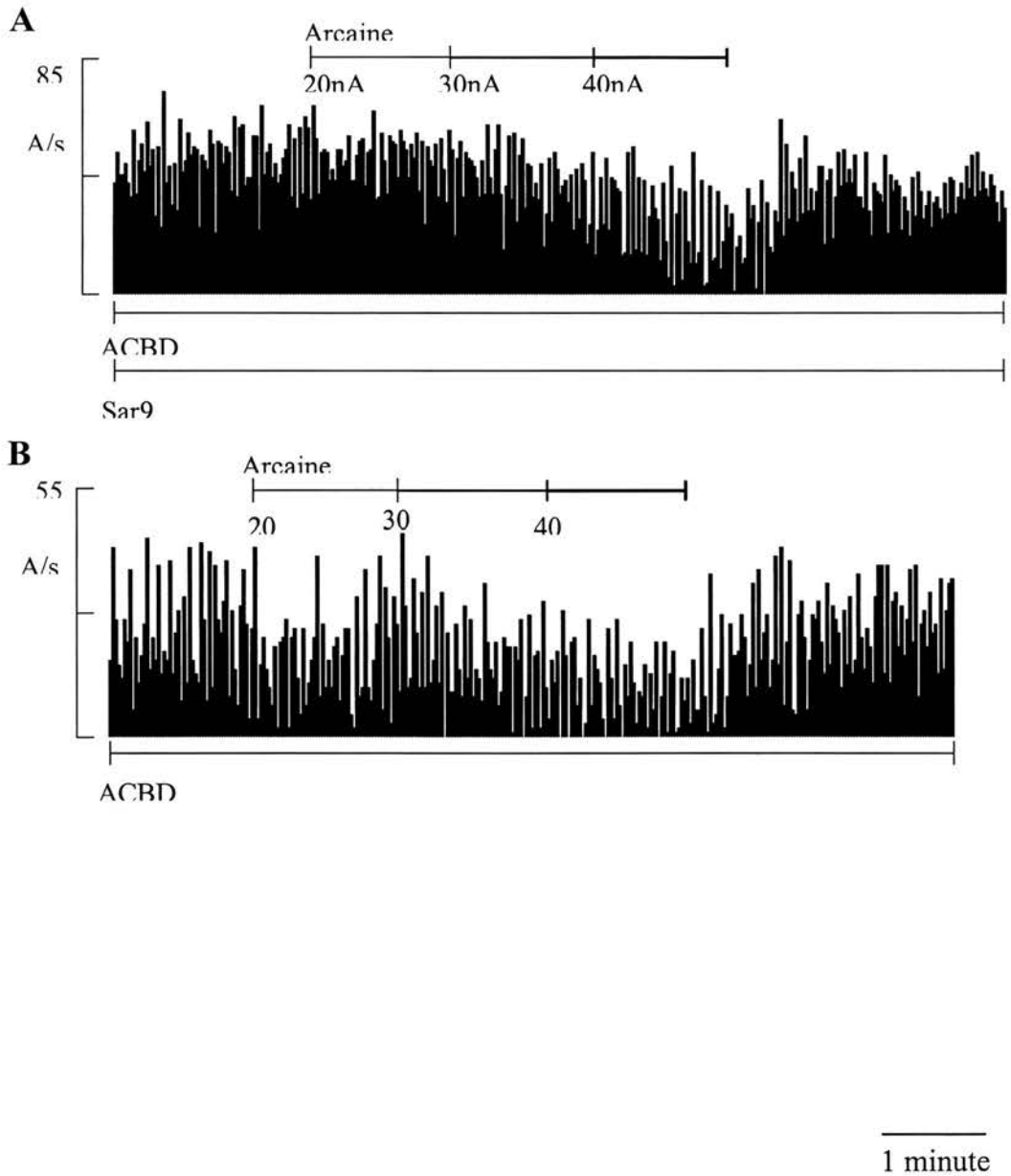

A
Arcaine
85
20nA
30nA
40nA
A/s
ACBD
Sar9
B
Arcaine
55
20
30
40
A/s
ACBD
1 minute

FIGURE 3.7

Effectiveness of Gly_{NMDA} site and other NMDA receptor antagonists at reducing neuronal activity evoked by NMDA receptor agonist alone compared to activity evoked by NMDA plus NK_1 receptor agonists.

Ejection current required to inhibit ACBD evoked activity alone by greater than 20 percent (▦) compared to that required to inhibit ACBD plus Sar^9-SP evoked activity (▨). Four compounds acting at different sites on the NMDA receptor complex were considered: Gly_{NMDA} site antagonists L701,252 and 7-CTK, non-competitive channel blocker MK801 and polyamine site antagonist arcaine. The statistical significance of differences was assessed using the Mann-Whitney U-test (* represents a significant difference from ACBD plus Sar^9-SP - evoked levels at $P<0.05$).

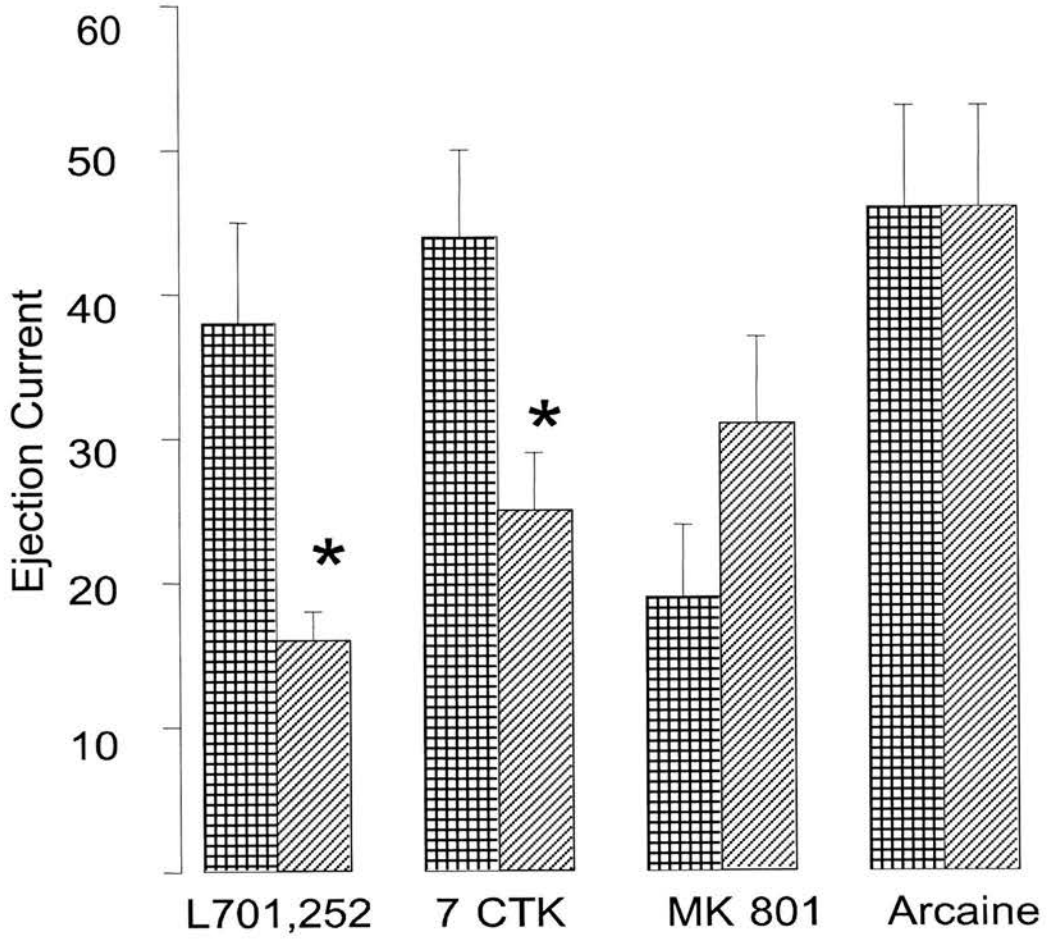

Ejection Current
60
50
40
30
20
10
*
*
L701,252
7 CTK
MK 801
Arcaine

FIGURE 3.8

Effect of ionophoretically applied PKC inhibitor on NMDA plus NK_1 receptor evoked activity compared to NMDA receptor-evoked activity alone

Individual records of ongoing firing frequency are displayed as the action potentials per second (A/s), integrated over 700 millisecond bins, plotted against time for a single neurone. Record A shows the inhibitory effects of PKC inhibitor GF109203X on ACBD plus NK_1 receptor-evoked activity and record B shows the lack of effect of GF109203X on ACBD-evoked activity alone until higher ejection currents.

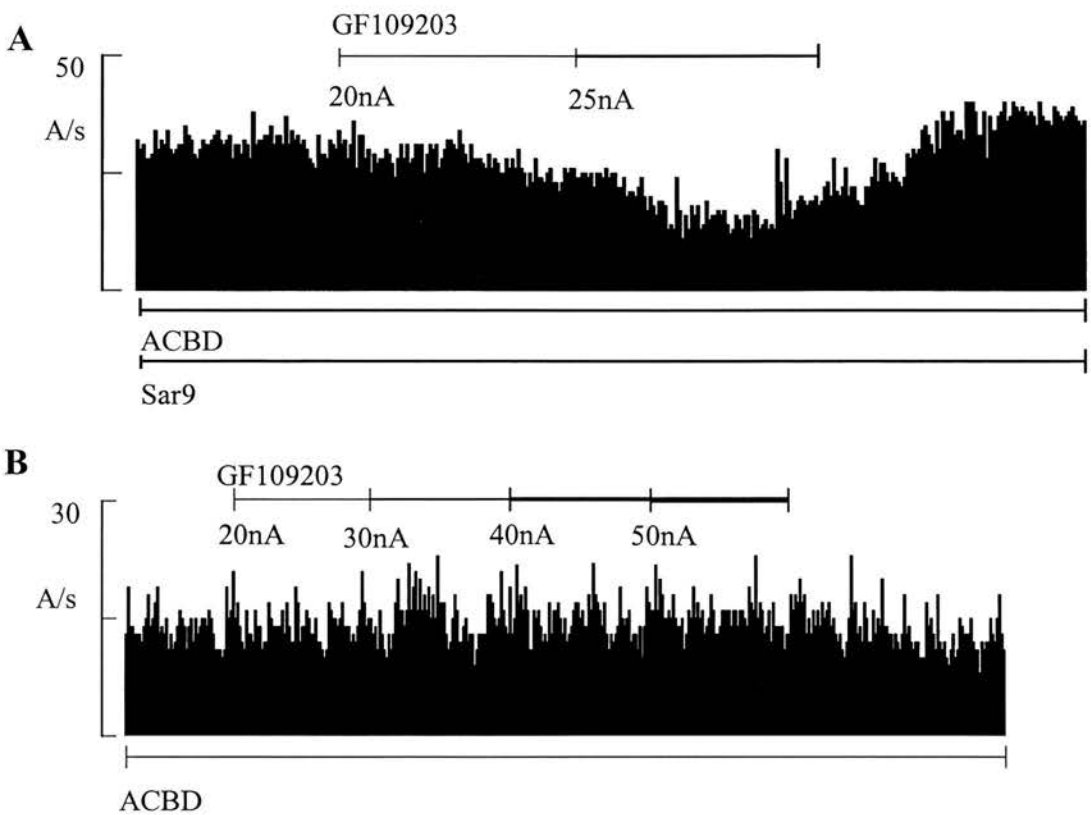
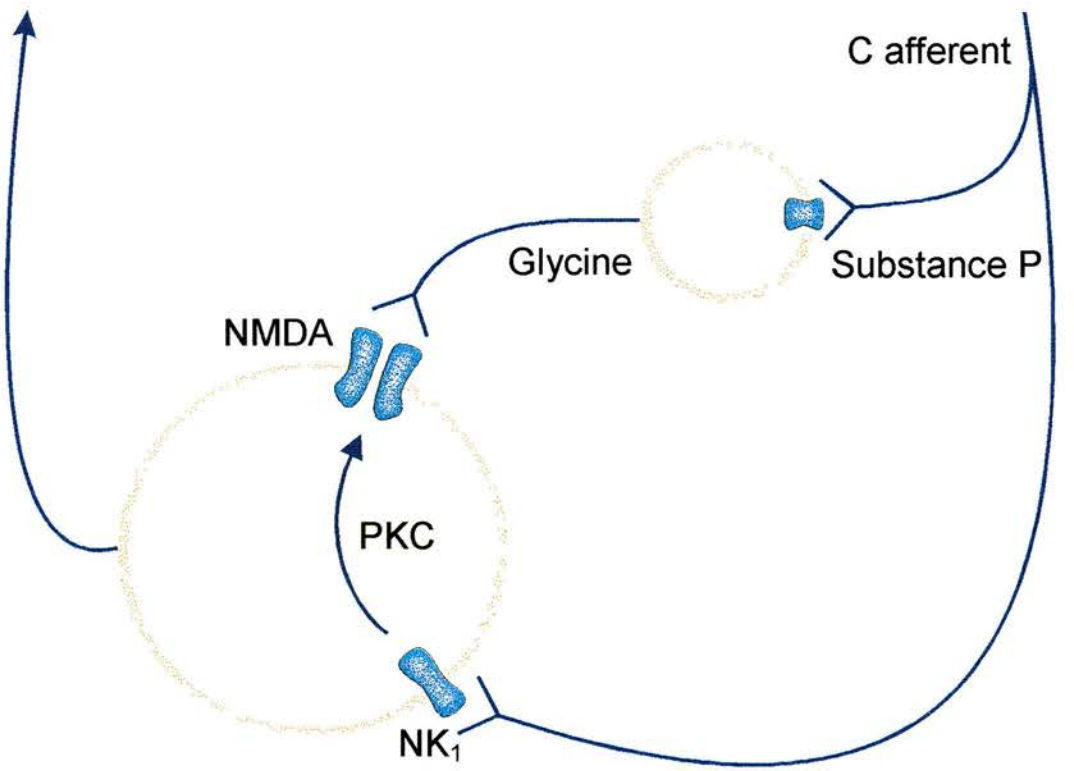

A
GF109203
50
A/s
20nA
25nA
ACBD
Sar9
B
GF109203
30
20nA
30nA
40nA
50nA
A/s
ACBD
1 minute

FIGURE 3.9

A hypothetical model of the actions of SP during prolonged nociception

SP-induced increases in dorsal horn glycine concentrations act at the Gly_{NMDA} site of the NMDA receptor during sustained nociception. Following a severe or prolonged noxious stimulus, PKC mediated phosphorylations of the NMDA receptor increase the sensitivity of the Gly_{NMDA} site. Glycine now acts at this site, unmasks any direct excitatory NK_1 input and contributes to the NMDA/NK_1 interaction. Additional factors may also play a role.


C afferent
Glycine
Substance P
NMDA
PKC
NK1

CHAPTER 4: LIF AS A SPINAL NOCICEPTIVE MEDIATOR

4.1 AIMS

LIF is a neuroactive cytokine that has been extensively characterised in the peripheral nervous system where it acts as an injury-induced neuronal differentiation factor. Recent investigations have explored the role of LIF in the sensory system and there is evidence that it may act on a subset of small diameter neurones suggesting a nociceptive role for this cytokine. This study has addressed the role of LIF as a nociceptive mediator in the spinal cord. Firstly, using *in situ* hybridisation, the distribution of LIF mRNA was examined in the spinal cord of normal animals compared to animals with peripheral inflammation or nerve injury. Secondly the consequences of intrathecally applied LIF to mechanical and thermal behavioural hypersensitivity were determined. Finally, the effects of LIF on the expression of critical components in neuropeptide and excitatory amino acid transmission were assessed. Since changes in behavioural hypersensitivity may reflect altered spinal neurokinin and EAA transmission, expression of NK_1 receptor mRNA (which is upregulated by both nerve and tissue injury) and EAAT2 mRNA (which may regulate synaptic glutamate concentration and hence activation of any of the EAA receptor subtypes) were examined before and after intrathecal LIF infusion. These data are discussed with reference to a nociceptive role for LIF in the spinal cord.

4.2 METHODS

4.2.1 Animals

Experiments were performed on male Wistar rats weighing 200-400g (please see section 2.2.1). LIF expression was assessed in spinal cords taken from normal animals, peripherally inflamed animals and those with a peripheral nerve injury. The effects of LIF on the expression of spinal cord neuropeptide and EAA transmitter components were determined by examining the expression of NK_1 receptor and EAAT2 mRNA before and after intrathecal LIF application.

4.2.1.1 Inflammation and Nerve Ligation

Inflammation (n=3) was induced under halothane anaesthesia with a single unilateral intraplantar injection of 150µl of Complete Freund's Adjuvant (CFA; LCN, Thame, UK) and

animals were left for 52 hours. Increase in paw circumference was measured as a guide to the development of inflammatory oedema.

Peripheral nerve ligation was performed by S.W.N. Thompson: in a group of animals (n=3) under pentobarbitone anaesthesia (40mg/kg i.p.), the L3 spinal nerve was tightly ligated with 5/0 Mersilk for 7 days and a 5mm distal portion of the nerve resected to prevent regeneration.

A third control group (n=3) received no treatment.

Spinal cords comprising segments L2-L4 and injured spinal nerves were then removed from these animals under halothane anaesthesia and snap frozen in isopentane at -40°C to -45°C. Frozen transverse cryostat sections (15μm) were taken at -16°C, thaw mounted onto polysine-coated microscope slides (Merck, Lutterworth, UK) and stored at -70°C.

At all times precautions were taken to avoid the risk of RNase contamination of the tissue. This included wearing gloves, continually cleaning the cryostat blade with ethanol throughout the cutting session and treating all aqueous solutions with the nuclease inhibitor diethyl pyrocarbonate (DEPC).

4.2.1.2 Intrathecal LIF Infusion

Experiments were performed by S.W.N. Thompson on male Wistar rats weighing 200-250g. A laminectomy was performed under pentobarbitone anaesthesia (40mg/kg i.p.) between T4 and T5 vertebrae using sterile precautions. The dura was cut and a silastic tube, external diameter 0.6mm, passed intrathecally so that its tip lay approximately between the L2 and L3 dorsal root ganglia. The silastic tube was connected to a mini-osmotic pump (Alzet type 2001, Alza Corp, Palo Alto,) which delivered at a rate of 1 μl/hr. The pumps were filled with either rat serum albumin (RSA, 5mg/ml) in phosphate buffered saline (PBS) (n=3) (sham controls) or RSA in PBS plus recombinant human LIF (rhLIF, 0.4mg/ml, from J. Heath, University of Birmingham, Birmingham, UK)) (n=3). Pumps were left in place for 7 days.

A control group of animals (n=3) received no treatment.

Sections from spinal cord segments L2-L4 were taken as described above.

4.2.1.3 Behavioural Testing

Behavioural testing was performed by S.W.N. Thompson on male Wistar rats (200-250g) receiving intrathecal applications of LIF or saline and control animals receiving no treatment. The sensitivity to noxious thermal stimuli was assessed by the method of Hargreaves *et al.* {#12705} using a Ugo Basile testing apparatus (Ugo Basile, Comero, Italy). Animals (n=4) were left to acclimatise for 15-20 minutes prior to testing and then the time taken for hindpaw withdrawal was measured automatically. In each testing session the

mean of 4 withdrawal latencies was determined for both hindpaws and these values were averaged.

The hindpaw withdrawal response to a noxious mechanical stimulus was measured using a Randall-Sellito apparatus (Ugo Basile, Comero, Italy). The animals (n=4) were gently restrained and an increasing pressure was applied to the plantar surface of the hindpaw via a pointed rod. The pressure required to elicit withdrawal was determined and the mean of three measurements calculated.

Paws in both thermal and mechanical tests were measured in a pseudo-random order to avoid sensitisation effects and withdrawal thresholds were represented as absolute values.

4.2.2 In situ hybridisation

In situ hybridisation histochemistry was carried out to investigate the spinal distribution of LIF mRNA in normal and injured animals and of NK_1 receptor and EAAT2 mRNA before and after intrathecal LIF infusion.

4.2.2.1 Probe Labelling

Complete gene sequences of the rat LIF, NK_1 receptor and EAAT2 genes were obtained from the EMBL database at http://www.embl-heidelberg.de. Two oligonucleotide probe sequences were chosen from each gene (complementary to bases 86-133 and 434-478 of the rat LIF gene, bases 559-606 and 1075-1105 of the rat NK_1 receptor gene (Elde *et al.* 1990) and bases 300-348 and 851-899 of the rat EAAT2 gene). These were designed to reflect similar characteristics of length (45-50 bases) and CG content (60%) to maintain a comparable affinity for hybridisation within the limits of the *in situ* hybridisation methodology used. The specificity of these sequences was then confirmed using the BLAST search programme at http://www.nih.gov and probes were synthesised by Oswel (Southampton, UK).

Oligonucleotide probes were 3' end-labelled with deoxyadenosine [α-^{35}S]-triphosphate (^{35}S-dATP; specific activity <1250 Ci/mmol; NEN Dupont, Hounslow, UK) using terminal deoxynucleotidyl transferase (TDT; Gibco, Paisley, UK). The reaction was carried out at 37 °C in an autoclaved Eppendorf tube containing the oligonucleotide (1.5μg), TDT buffer (10μl), TDT enzyme (15U/μl), ^{35}S-dATP (4μl) and DEPC distilled water (to give a final volume of 52μl). Following an incubation time of 1 hour a further 2μl of ^{35}S-dATP and 2μl of TDT enzyme were added and the incubation was continued for a further hour. After this time the reaction was stopped on ice for 15 minutes and the labelled probe was separated from unincorporated nucleotide via a Nu-Clean D25 disposable spin column (Scientific Imaging Systems, Cambridge, UK). Percentage incorporation was calculated for each probe and labelling was confirmed by counting the β-emission from liquid scintillation for

duplicate aliquots (1µl) of pre-spun and post-spun mixture. The labelled probe was then stored at -20°C until it was used the following day.

4.2.2.2 Tissue Fixing

Slides were brought to room temperature, immediately fixed in 4% paraformaldehyde/0.1M PBS (0.1M, pH 7.4) for 10 minutes and then rinsed in fresh PBS. Sections were acetylated in ethanolamine (10%)/acetic anhydride (0.25%) for 10 min to reduce non-specific hybridisation of the negatively charged probe to the positively charged glass before being dehydrated in ascending concentrations of aqueous ethanol (50%, 70%, 80%, 90% and 100%) buffered with ammonium acetate (0.3M) followed by 100% ethanol and then air dried.

4.2.2.3 Hybridisation

Labelled oligonucleotide probes were mixed with 40%formamide/60% hybridisation buffer (0.1 % 5x Denhardt' s solution, 10% dextran sulphate, NaCl (600 mM), Tris pH 7.6 (10 mM), EDTA (1 mM), 0.01% salmon sperm DNA, 0.005% yeast tRNA, 0.0005% glycogen and 50% deionised formamide) at a concentration of 750cpm/µl and the solution was heated at 60-70°C for 10 minutes before being cooled on ice for 2-3 minutes. The addition of formamide, which destabilises nucleic acid duplexes, and the heating process ensured that the probe was maintained in a single stranded form and thus achieved a good fidelity of hybridisation. This was further secured by adding dithiothreitol (10mM) to break any random S-S bonds and keep the ^{35}S-dATP in a reduced form following cooling.

The solution was then hybridised with the sections in sealed containers saturated with a solution of 50% de-ionised formamide/50% 4X standard sodium citrate (SSC) solution at 37 °C for 20 hours to give a final count of 1500cpm per slide. These conditions were chosen to optimise the hybridisation reaction and reduce non-specific hybridisation and the ideal temperature was determined from the calculated melting temperature (Tm) for the probes (the temperature at which a hybrid population is half dissociated) to provide a temperature high enough to disrupt random, intrastrand hydrogen bonds within denatured DNA, but not high enough to interfere with intrastrand pairing of complementary bases.

4.2.2.4 Post-Hybridisation Washes

After overnight incubation, post-hybridisation washes were carried out to reduce non-specific binding. Slides were washed over 6 hours at 40°C in decreasing concentration steps of SSC solution (2 x SSC, 1 x SSC and 0.5 x SSC all 2h each). This higher temperature and reduced salt concentration increased the stringency of hybridisation.

4.2.2.5 Emulsion Coating, Developing and Staining

Slides were dehydrated in ascending concentrations of aqueous ethanol (50%, 70%, 80%, 90% and 100%) buffered with ammonium acetate (0.3M) followed by 100% ethanol and then thoroughly air dried overnight. The dried slides were then dipped in photographic emulsion (LM1; Amersham, Chalfont, UK) at 40°C and sealed in light-tight Kartell boxes. After 6-8 weeks exposure at 6°C they were developed at 16-18°C using Kodak D19 developer (4 min) and Ilford Hypam rapid fixer (5 min) and then counterstained in haematoxylin/eosin (Sigma, Poole, UK), mounted and cover-slipped.

4.2.3 Controls

The specificity of the oligonucleotide probe mixture was confirmed by BLAST search, by pretreating sections with RNAse A (Sigma) (1mg/ml in 0.1M PBS) for 1 hour at 37°C prior to hybridisation, by competition for the [^{35}S]-labelled probes in the hybridisation medium with a 100-fold excess of unlabelled probes and by repeating the protocol with corresponding sense probes.

4.2.4 Analysis

Quantitative measurements of hybridisation were made in the lateral part of the superficial dorsal horn and compared with contralateral and control data. Four sections were chosen from each animal and cell counts and silver grain densities measured blind with respect to the treatment groups.

Cell counts were made under the light microscope at X400 magnification over an area of 30625μm^2 and were selected on the criterion that they expressed specific silver grain labelling at five times greater than background levels.

Silver grain densities were measured with an automated image analysis system running "Image v1.49 VDM" software (NIH). The number of silver grains within a standardised circular area of 78.5μm^2 (representing the maximum extent of silver grain clustering over expressing cells) was counted over 4 positively hybridised cells and 1 non-expressing cell (representative of background) for each section and the net density calculated by subtracting background measurements.

Data was pooled for each group and differences between animal means determined using the paired t-test for ipsilateral versus contralateral comparisons and the t-test for treatment versus control groups.

4.3 RESULTS

4.3.1 Spinal expression of LIF mRNA

In situ hybridisation of 2 antisense oligonucleotide probes complementary to the LIF nucleotide sequence revealed that LIF mRNA was expressed in the spinal cord of normal and injured rats. In control animals, expression of LIF mRNA was highest in the superficial laminae I-II of the dorsal horn with only light expression in the deeper laminae III-V. There was also expression around the central canal that extended into the ventral horn where it was observed on motorneurones (Figure 4.1). Peripheral injury profoundly altered levels of LIF mRNA, especially in the lateral superficial dorsal horn.

Spinal nerve ligation increased LIF expression at the injury site and in the ipsilateral spinal cord. In the spinal nerve, a dense band of positively hybridised cells was observed around the ligation and this extended from the injury site by only 100-150μm (Figure 4.2). In the spinal cord, increases in LIF expression were confined to the ipsilateral side and this was most evident in the lateral areas of laminae I and II (Figures 4.3 D-F). Hence in this area, the number of cells positively expressing LIF mRNA increased by 53±8% (mean±SEM, $P<0.05$) compared to control, whereas contralateral to the injury, cell counts were not significantly increased being only 8±12% greater than control ($P=0.61$; Figures 4.3 A-C). Similarly, silver grain densities were also significantly higher in the ipsilateral dorsal horn (64±13%, $P<0.05$) and contralateral levels were unchanged (17±15%, $P=0.23$; Figure 4.4).

To test whether increases in LIF synthesis were associated only with nerve injury, levels of LIF mRNA were also assessed after peripheral inflammation. Intraplantar injection of CFA induced inflammation that was evident as an increase in the circumference of the injured paw by 78±15% compared to contralateral. In the spinal cord, inflammation caused increased synthesis of LIF mRNA throughout the grey matter of the ipsilateral and contralateral sides (Figures 4.3 G-L). However, hybridisation was most dense in the ipsilateral side and was consistently associated with a band of positively hybridised cells across lamina I. Cell counts in this area showed that the number of cells specifically expressing mRNA for this cytokine increased by 27±13% ($P<0.05$) compared to control whereas contralateral counts were unaltered at 104±9% of control ($P=0.63$). Silver grain densities were also significantly higher for ipsilateral (48±22%, $P<0.05$) but not contralateral sides (24±20%, $P=0.17$) although there was no significant difference between these data ($P=0.18$) (Figure 4.4).

4.3.2 Intrathecal LIF infusion

4.3.2.1 Behavioural testing

Intrathecal application of LIF produced both a thermal and mechanical hyperalgesia. However the time course of developing sensitivity in thermal (Hargreaves) and mechanical (Randall-Sellito) tests appeared to differ.

The onset of mechanical hyperalgesia appeared to be rapid and was significantly different from baseline scores three days following initiation of LIF treatment ($P<0.05$). This hyperalgesia persisted for the duration of intrathecal infusion (7 days) and at the end of the infusion period withdrawal thresholds were substantially reduced ($P<0.05$). Furthermore, partial reversal of withdrawal thresholds was evident five days following termination of LIF delivery.

The onset of thermal hypersensitivity was delayed compared to mechanical hypersensitivity. A significant difference in withdrawal thresholds was present five days following LIF infusion. Thermal hypersensitivity was also prolonged with partial recovery five days following cessation of treatment.

Intrathecal saline delivery did not significantly alter mechanical or thermal paw withdrawal thresholds.

4.3.2.2 Expression of NK_1 receptor and EAAT2 mRNA

In the spinal cord, intrathecal application of LIF altered the expression of EAAT2 and NK_1 receptor mRNA.

NK_1 receptor mRNA was expressed diffusely through the superficial dorsal horn, laminae IV and V and on motorneurones in the ventral horn of normal animals (Figures 4.5 A-C). Intrathecal infusion of LIF caused a dramatic increase in NK_1 receptor expression throughout the grey matter (Figures 4.5 D-F). This was quantified in the lateral superficial dorsal horn where the number of hybridised cells rose by 51±15% ($P<0.05$) compared to control and the silver grain density increased by 56±11% ($P<0.05$; Figure 4.6) of control.

EAAT2 expression was normally most strongly associated with cells in laminae I and II, around the central canal and in the ventral horn (Figures 4.5 G-I). In the dorsal horn, intrathecal LIF application decreased the number of cells positively hybridising with the EAAT2 probes by 26±6% ($P<0.05$; Figures 4.5 J-L)) and this was accompanied by a decrease in silver grain density of 21±3% ($P<0.05$; Figure 4.6).

4.3.3 Controls

BLAST searches confirmed the specificity of the probes and sense probe, excess unlabelled probe, or RNAse A-treated sections showed no hybridisation in any of the treatment groups.

4.4 DISCUSSION

4.4.1 LIF Expression in the Spinal Cord

In the peripheral nervous system, LIF has been characterised as a lesion-induced injury factor that promotes the differentiation and regeneration of injured neurones (Zigmond, 1997). However, peripheral injury also alters the characteristics of spinal neurones (Cook *et al.* 1987) and the hypothesis that peripheral challenges and trauma associated with nociception may increase LIF synthesis in this area has not been previously considered. We therefore examined the distribution of LIF mRNA in the dorsal horn of the spinal cord and show for the first time that injury induces significant increases in the spinal expression of LIF. Furthermore, this increase occurs after injury to both peripheral nerve and tissue, indicating that LIF might be an important factor in the induction of central neuronal sensitisation in both of these experimental models.

In normal animals, LIF was distributed at low levels in laminae I, II, IV and V of the dorsal horn, around the central canal and on motorneurones in the ventral horn. This basal expression of LIF appears to differ from the expression pattern seen in the peripheral nervous system of uninjured animals. In the periphery, evidence suggests that LIF is not expressed until the nerve is injured (Banner and Patterson, 1994). Kurek *et al.* (1996) have demonstrated that expression occurs in Schwann cells and is confined to a proximal and distal area close to the site of injury. In agreement with this data, we have reported that LIF mRNA is expressed in ligated spinal nerve, only extends by approximately 100-150μm from the injury site, and the remaining portion of nerve shows no detectable hybridisation. This serves as an important control and implies that the oligonucleotide probes used were most likely hybridising to LIF mRNA. However, these probes detected a basal expression of LIF in the spinal cord of uninjured animals and this suggests that LIF expression may be differentially regulated in peripheral versus central nervous tissue.

The expression of LIF was most prominent in the superficial dorsal horn and in lamina V. This study did not address the types of cell that were producing LIF mRNA, but this area is a major termination zone for small diameter Aδ and C-fibres transmitting nociceptive information (Willis and Coggeshall, 1991). Furthermore, inflammation and nerve injury increased LIF mRNA expression throughout the spinal cord, especially in the superficial dorsal horn where a dense band of hybridisation extended across lamina I. The data therefore indicate that LIF might have important actions on the regulation of nociceptive transmission and this is supported by observations that the neuropoietic cytokine IL6 has also been shown to be upregulated in the spinal cord in response to a peripheral nerve injury (DeLeo *et al.* 1996).

If LIF does have a pro-nociceptive role in the central nervous system, it accords well with aspects of its putative function in the periphery. Thompson *et al.* (1996; 1997) have shown that both systemic and local administration of LIF increased nociceptive reflexes to mechanical stimuli and that a subset of nociceptive sensory neurones retrogradely transport LIF.

These observations imply that LIF may have important actions on central as well as peripheral sensory nervous tissue and might be an essential regulator of neuronal responsiveness to inflammation or nerve injury.

4.4.2 Intrathecal LIF infusion and NK_1 and EAAT2 expression

Injury-induced increases in the expression of LIF in the spinal cord might parallel the neuronal hypersensitivity that occurs in the dorsal horn following sustained nociceptive stimulation. This central sensitisation contributes to behavioural hyperalgesia and is partly mediated by increased efficacy of neuropeptide and EAA transmission in the dorsal horn (Urban *et al.* 1994b). The present study has investigated the role of intrathecally-applied LIF in this process and addressed its function as a mediator of behavioural hypersensitivity and as an induction factor in the regulation of NK_1 receptor and EAAT2 gene expression.

Intrathecal administration of LIF altered both thermal and mechanical behavioural sensitivities without affecting motor responses. LIF was applied for 7 days and mechanical thresholds were significantly reduced after 3 days with thermal paw withdrawal latencies dropping after 5 days. Behavioural responses returned to normal after 12 days. This data is in broad agreement with the effects of systemic application of LIF on behavioural reflexes (Thompson *et al.* 1996). However, in contrast to that study, here LIF evoked changes in thermal nociception. This could be due to differences in both the dosing regime and route of administration. In the present study, LIF was applied over a chronic 7-day period compared to a single acute injection. From the data, it is apparent that thermal hypersensitivity develops later than mechanical changes indicating that a more prolonged exposure to LIF is required for thermal sensitisation to develop. LIF was also applied intrathecally where it had direct access to spinal cord tissue. Kerekes *et al.* (1997) have recently considered the effects of different routes of application on drug efficacy in related models. They reported that axotomy induced increases in DRG galanin expression were more sensitive to blockade by NGF when this neurotrophin was applied via capsule form to the injured nerve, than *in vitro* bath application or *in vivo* intrathecal administration. Although the effects of alternative routes of application of LIF have not been considered, Thompson (S.W.N. Thompson unpublished observations) has investigated the spreading of brain derived neurotrophic factor-like immunoreactivity (BDNF-LI) after a chronic intrathecal application of BDNF. The distribution of BDNF-LI extended around the spinal cord where it penetrated into the

dorsal horn and importantly it was found around and within the DRG. Furthermore, induction of DRG c-fos expression after intrathecal infusion of BDNF was too rapid to be explained solely by retrograde transport from central terminals, indicating a direct effect on DRG cells. If a similar spreading of peptide occurs following intrathecal administrating of LIF, it is possible that the behavioural changes result from effects in only the DRG. However, data from this study indicates that LIF mRNA is upregulated in the spinal cord which makes it attractive to propose that LIF is have having at least some effect centrally.

Increased neuronal hyperexcitability in the spinal cord is known to contribute to behavioural hyperalgesia and allodynia (Treede *et al.* 1992). This process may be dependent upon both increased release of transmitter from primary afferent central terminals and increased efficacy of postsynaptic receptor responses. A peripheral action of LIF may raise neuropeptide synthesis in the DRG and enhance the release of these substances. However, central actions of LIF could also alter postsynaptic elements in the spinal cord. Indeed pretreatment of cerebellar granule neurones with IL-6 (a hematopoietic cytokine that shares a similar signal transduction mechanism to LIF) has been demonstrated to selectively enhance the intracellular calcium response of NMDA receptors (Qiu *et al.* 1995) and it is possible that injury induced increases in LIF mRNA might have similar effects in the spinal cord. Changes in behavioural sensitivity evoked by intrathecal application of LIF could therefore be caused by a combination of peripheral and central effects where LIF induces changes in the chemical phenotype of nociceptive primary afferents and alters gene expression and neuronal excitability in the spinal cord.

In the periphery, LIF has been demonstrated to induce the synthesis of a number of peptides involved in the cell body reaction to axotomy which are upregulated in the DRG in response to nerve injury. These include SP, VIP, galanin and NK_1 receptor (Rao *et al.* 1993; Shadiack *et al.* 1993; Fann and Patterson, 1994; Sun *et al.* 1994; Ludlam *et al.* 1995; Corness *et al.* 1996; Sun and Zigmond, 1996). In a recent study by Livesey *et al.* (1997), Reg-2 a Schwann cell mitogen that is essential for neuronal regeneration, was also shown to be regulated by LIF. Indeed DRG cells undergoing regeneration are sensitive to exogenous application of this cytokine, such that the switch from a synaptically transmitting neurone to a regenerating cell may be regulated by LIF in response to neuronal damage (Zigmond *et al.* 1996). Furthermore, the induction of many of these factors has been demonstrated to be dependent upon the existence of a 180 base pair cytokine response element on the promoter region of the gene that can be activated by the LIF/JAK/STAT pathway and can initiate increased transcription (Symes *et al.* 1994; Rajan *et al.* 1995; Frank and Greenberg, 1996).

This study has investigated the effects of LIF on gene expression in the central nervous system. We report that LIF is upregulated in the spinal cord in response to peripheral injury and may therefore induce alterations in neuronal chemical phenotype and indirectly increase

spinal cord excitability. As markers for this process, NK_1 receptor and EAAT2 gene expression were examined. Central sensitisation is believed to involve the synergism of a number of factors, especially a positive co-operation between NK_1 and EAA transmission (Urban *et al.* 1994b), therefore LIF induced changes in NK_1 receptor and EAAT2 expression might contribute to this neuronal hyperexcitability. NK_1 receptor expression is known to be upregulated by nerve and tissue injury (Schäfer *et al.* 1993; Abbadie *et al.* 1996) and these receptors may have crucial actions in the induction and maintenance of central sensitisation. Furthermore, alterations in the expression of EAA transporters could have significant effects on EAA transmission via changes in extracellular glutamate concentration and modified activity at any of the EAA subtypes. There are currently four known EAA transporters and all of these could contribute to the regulation of glutamate uptake in the nociceptive system. However, EAAT2 has been located in the spinal cord and is extensively characterised in this area (Rothstein *et al.* 1994). Therefore both NK_1 receptors and EAAT2 could represent potential targets for intrathecally applied LIF and reflect important factors to study.

In untreated control animals NK_1 receptor mRNA was distributed in the superficial dorsal horn and in laminae IV with a more diffuse hybridisation through the deeper dorsal horn. Application of LIF brought about a marked increased in the number of positive neurones throughout this area. This is in agreement with previous studies that have examined the distribution of NK_1 mRNA in the spinal cord before and after peripheral injury. It has been reported that NK_1 mRNA is upregulated following peripheral inflammation (Schäfer *et al.* 1993) and NK_1 receptor-like immunoreactivity increases after inflammation and nerve injury (Abbadie *et al.* 1996). Interestingly, these increases do not correlate with injury-induced changes in the levels of substance P, the endogenous ligand for NK_1 receptors. Spinal substance P gene expression has been demonstrated to increase after inflammation (Minami *et al.* 1989) whilst substance P immunoreactivity and expression decreases after nerve injury (Hokfelt *et al.* 1994; Sommer and Myers, 1995) indicating that ligand and receptor expression is differentially regulated in these situations. However, injury-induced increases in NK_1 receptor mRNA correspond to LIF-induced increases in NK_1 receptor expression and represent evidence consistent with the hypothesis of a pro-nociceptive role for LIF.

EAAT2 is a member of a family of sodium-dependant glutamate transporters that rapidly terminate glutamatergic synaptic transmission via high affinity uptake from the synaptic cleft. There are 3 additional glutamate transporters termed EAAT1, EAAT3 and EAAT4 (Rothstein *et al.* 1994) but EAAT2 has been most extensively characterised in the spinal cord. EAAT2-like immunoreactivity is distributed throughout the central nervous system and is localised exclusively on astroglial cells (Milton *et al.* 1997). In the spinal cord this is enriched in the grey matter and is particularly intense in laminae I-III (Rothstein *et al.* 1994).

In agreement, we have shown that EAAT2 mRNA is expressed in laminae I-II of the dorsal horn and furthermore, intrathecal LIF infusion decreases levels of its expression.

The functional consequences of a decrease in EAAT2 synthesis are unknown. The selective loss of EAAT2-like immunoreactivity has been observed in the motor cortex and spinal cord of human patients with ALS and has been shown to contribute to glutamate excitotoxicity and the death of motorneurones (Rothstein *et al.* 1995). However, in axotomised hypoglossal rat motorneurones, Kiryu *et al.* (1995) reported that the glutamate transporter EAAT3 was upregulated compared to control. This transporter is located on glutamatergic nerve endings and it was suggested that upregulation allowed these motorneurones to resist neurotoxic glutamate accumulation during regeneration.

In the dorsal horn, a LIF-induced decrease in EAAT2 expression could regulate the neuronal hypersensitivity associated with inflammation and nerve injury. Increased synaptic concentrations of glutamate might raise the excitability of postsynaptic neurones and contribute to this process. Furthermore, we have demonstrated that NK_1 receptors are upregulated by intrathecal LIF infusion and interactions between these and glutamate receptors have been shown to sensitise dorsal horn neurones (Dougherty *et al.* 1993b; Urban *et al.* 1994b).

When considering the effects of LIF on gene expression in central compared to nervous tissue, it is important to bear in mind the differences between the two situations. In the peripheral nervous system, LIF expression is induced at the site of injury and in direct response to the neuronal damage. It then induces changes in the chemical phenotype of these neurones to promote cell survival and regeneration. However, evidence from the present study suggests that in the central nervous system, LIF may act differently. It appears that LIF is present at basal levels in the spinal cord of uninjured animals. Furthermore, peripheral inflammation and injury of sensory neurones "upstream" of the spinal cord evoke changes in LIF expression. Thus, whilst the spinal neurones are not injured per se, central tissue still responds to peripheral injury by increasing LIF mRNA. This would suggest that LIF is not acting as a regeneration factor for these neurones, but may be redefining their properties to change the way in which they respond to subsequent inputs. However, the current experiments do not exclude the possibility of indirect effects of LIF on NK_1 and EAAT2 expression. It is possible that intrathecally applied LIF may access peripheral sites such as the nerve trunk and ganglion (sympathetic and sensory) as well as central sites in the dorsal and ventral spinal cord. This could result in structural changes such as in the sympathetic innervation of sensory ganglia (Thompson and Majithia, 1998) and the induction of a number of factors both peripherally and centrally that could elevate dorsal horn excitability and then contribute to secondary changes in NK_1 and EAAT2 gene expression. However, the

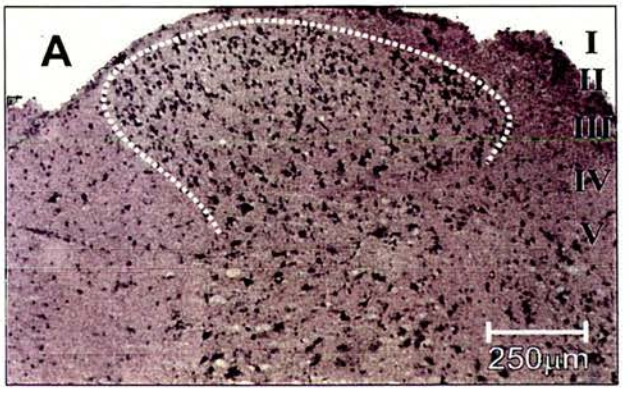
upregulation of LIF in specific regions of the spinal cord suggests that it may have a defined role in these areas.

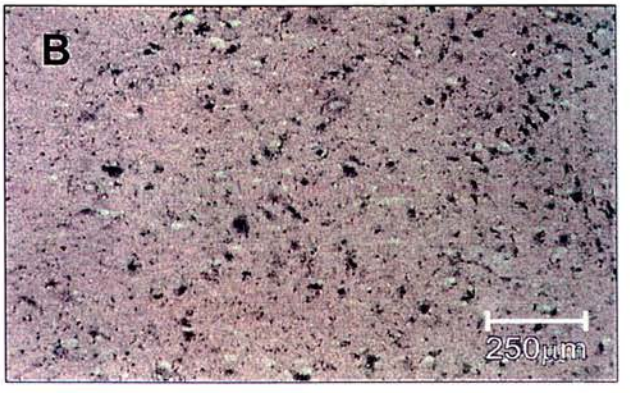
LIF-induced regulation of neuropeptide and EAA systems in the spinal cord will presumably have significant effects on sensory transmission. Correspondingly, we have demonstrated that the same LIF treatment causes behavioural hypersensitivity and that this may be due to the altered chemical phenotype of spinal neurones. Therefore the actions of LIF may indirectly affect the excitability of central neurones and possibly contribute both to the sensitisation of central neurones and to the development of inflammatory and neuropathic pain states.

FIGURE 4.1

Photomicrographs showing expression of LIF mRNA in the spinal cord

LIF mRNA is normally expressed in the superficial laminae of the dorsal horn (A), around the central canal and on ventral horn motor neurones (B). Part C shows a high power photomicrograph of LIF distribution in the superficial dorsal horn. Roman numerals indicate Rexed's laminae.


A
I
II
III
IV
V
250µm


B
250µm

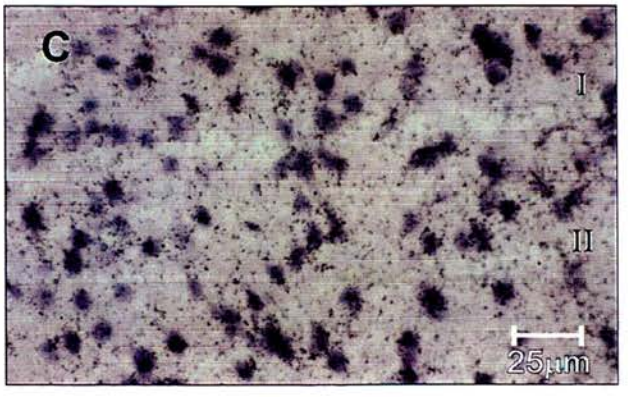
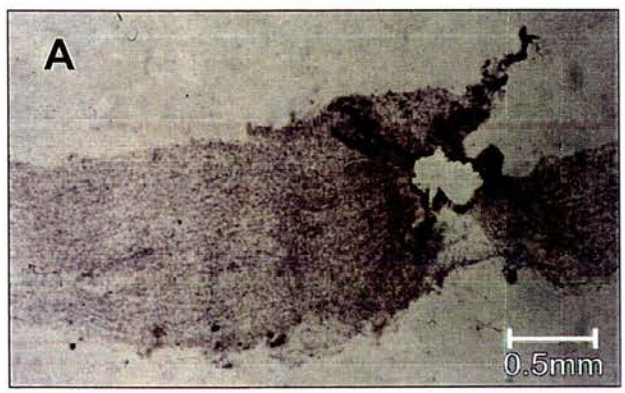

C
I
II
25µm

FIGURE 4.2

Photomicrographs showing expression of LIF mRNA in a ligated spinal nerve

LIF mRNA is expressed solely at the ligation site of the injured nerve (A). Part B shows a high power photomicrograph of LIF distribution at the ligation site.


A
0.5mm

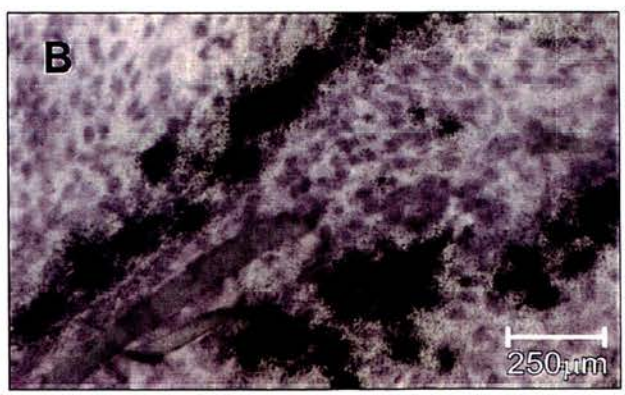

B
250μm

FIGURE 4.3

Photomicrographs showing expression of LIF mRNA in the spinal cord following peripheral inflammation and nerve injury

Peripheral inflammation and nerve injury induce upregulation of LIF mRNA in the spinal cord. Parts A-C indicate the expression of LIF in spinal cord contralateral to a peripheral nerve injury and this is upregulated on the ipsilateral side (D-F). Parts G-I show the expression of LIF in spinal cord contralateral to peripheral inflammation and this is also upregulated on the ipsilateral side (J-L). (A, D, G and J represent dorsal horn, B, E, H and K show ventral horn and C, F, I and L are high power photomicrographs of the superficial dorsal horn)

A
B
C
D
E
F
G
H
I
J
K
L
100µm
200µm
25µm
I
II

FIGURE 4.4

Quantification of silver grain density and number of cells expressing LIF in the superficial dorsal horn

Histogram of the number of silver grains (representing hybridised probe) per expressing cell (calculated as the mean number of silver grains per expressing cell (a standardised area of 78.5μm^2) minus the background density) against treatment groups of control animals ( ) (n=3), contralateral ( ) and ipsilateral ( ) nerve injury (n=3) and contralateral ( ) and ipsilateral ( ) hindpaw inflammation (n=3). The inset shows the number of positively hybridised cells (expression greater than 5 times background) per 30625μm^2 for each treatment. All measurements were made from the lateral superficial dorsal horn in laminae I-II. ★ Represents a significant difference from control ($P<0.05$).

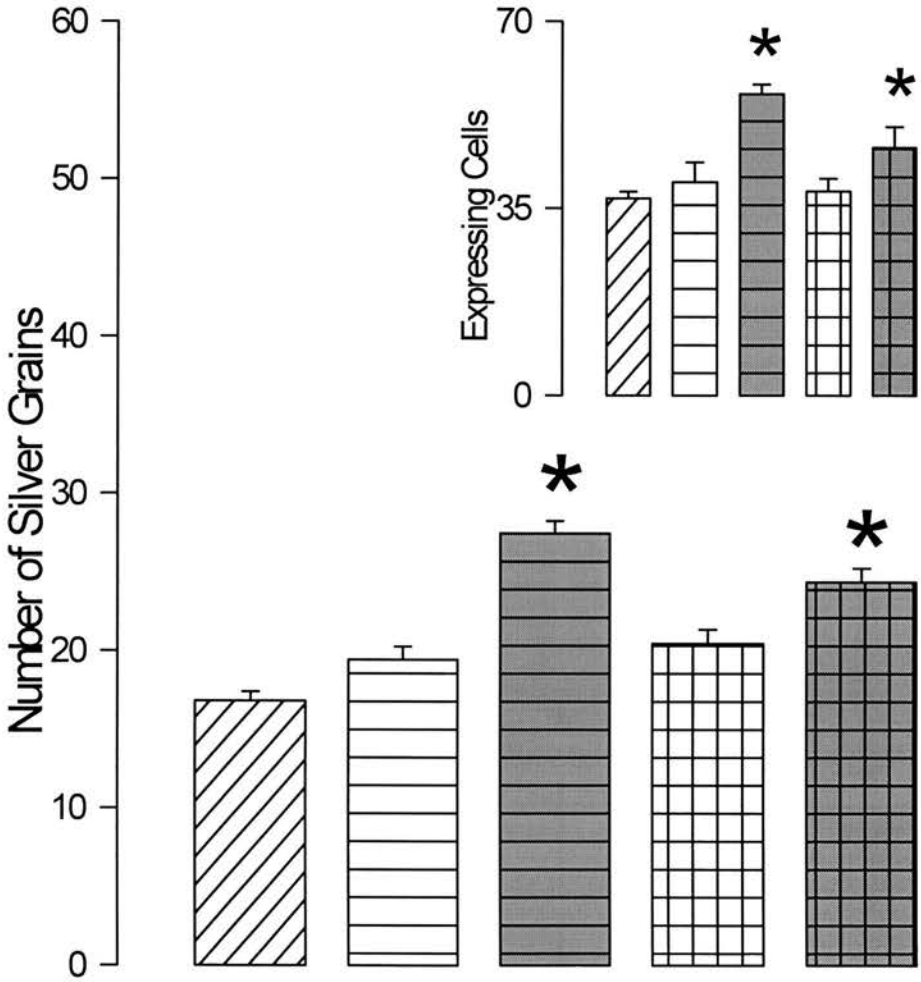

Number of Silver Grains
60
50
40
30
20
10
0
Expressing Cells
70
35
0
*
*
*
*

FIGURE 4.5

Photomicrographs showing expression of NK_1 receptor and EAAT2 mRNA in the spinal cord

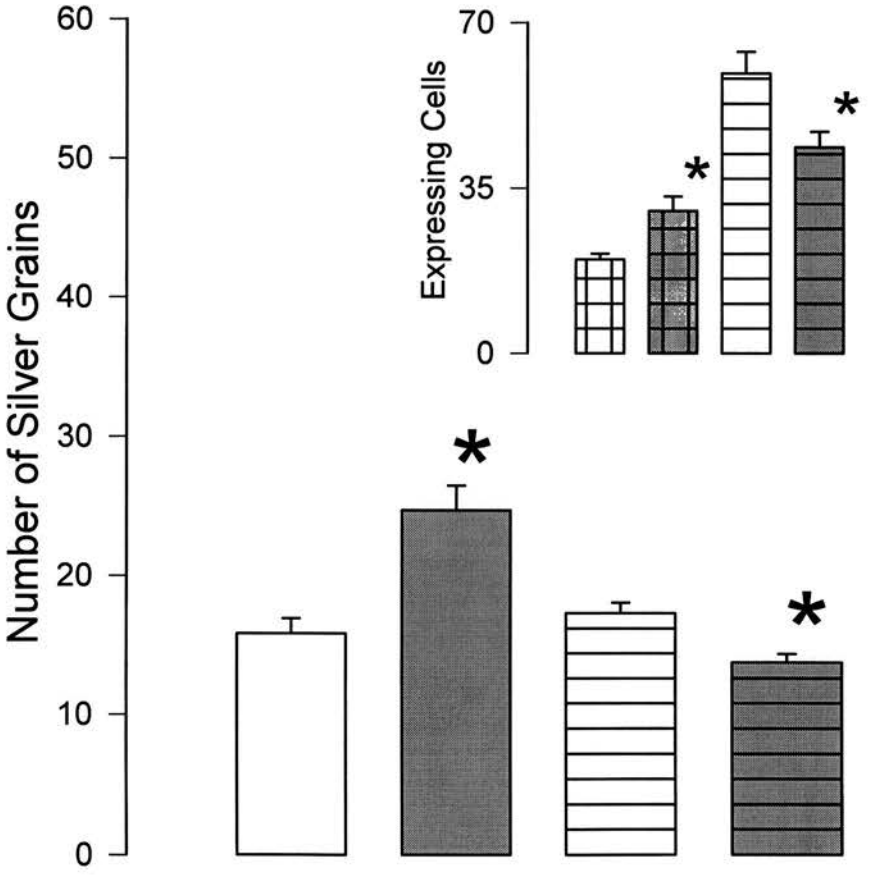
Intrathecal LIF application induces alterations in NK_1 receptor and EAAT2 gene expression. Parts A-C show the normal distribution of NK_1 receptor and this is increased by LIF administration (D-F). Parts G-I show the normal distribution of EAAT2 and this is decreased by LIF administration (J-L).

A
B
C
D
E
F
G
H
I
J
K
L
I
II
III
100μm
200μm
25μm

FIGURE 4.6

Quantification of silver grain density and the number of cells expressing NK_1 receptor or EAAT2 mRNA

Histogram of the number of silver grains (representing hybridised probe) per expressing cell (calculated as the mean number of silver grains per expressing cell (a standardised area of $78.5\mu m^2$) minus the background density) against treatment groups of normal expression of NK_1 receptor (▦) and EAAT2 (▤) compared to expression after intrathecal LIF infusion, (NK_1 receptor =▦ , EAAT2 =▤). The inset shows the number of positively hybridised cells (expression greater that 5 times background) per $30625\mu m^2$ for each treatment. All measurements were made from the lateral superficial dorsal horn (laminae I-II). ★ Represents a significant difference from control ($P<0.05$).


Number of Silver Grains
60
50
40
30
20
10
0
Expressing Cells
70
35
0
*
*
*
*

CHAPTER 5: GENERAL DISCUSSION

The transmission of sensory inputs in the spinal cord relies upon a complex series of interactions involving several classes of chemical mediator. These include classical neurotransmitters such as EAAs and neuropeptides, neuromodulators such as growth factors and compounds traditionally associated with the immune system such as inflammatory cytokines. Recently it has become apparent that these systems do not act in isolation, but co-operate with varying degrees of interaction to modify spinal sensory processing.

This study has addressed the role of NK_1 receptors as spinal nociceptive mediators. There is evidence that the involvement of these receptors in nociceptive transmission may be dependent upon the duration and severity of the stimulus. Using an *in vivo* electrophysiology protocol utilising ionophoresis and extracellular recording, a series of interactions involving NK_1 receptors, glycine and NMDA receptors were investigated to determine a mechanism for NK_1 receptor influence in sensory processing. The functions of the inflammatory cytokine LIF were also considered. Using *in situ* hybridisation, the distribution of this factor in the spinal cord and its effects on the expression of critical components in neuropeptide and excitatory amino acid transmission were assessed. These data are discussed with reference to injury induced alterations in dorsal horn processing of nociceptive information.

5.1 NK_1 Receptor Interactions With Other Mediators/Modulators of Nociceptive Transmission

SP and NK_1 receptors are located in the superficial spinal cord where they are likely to play a pro-nociceptive role. SP-LI boutons have been demonstrated to make appositions with high threshold and WDR neurones (Weiya Ma *et al.* 1996) and many NK_1 receptor immunoreactive dorsal horn neurones project via the STT to the thalamus and may be nociceptive in nature (Marshall *et al.* 1996). Liu *et al.* (1994a) reported that few (15%) of these neurones make direct synaptic contact with SP terminals indicating that much of the transmission in this area could be due to diffusion of the ligand. Studies addressing the release of SP are in agreement with this. Using the antibody microprobe technique it has become apparent that a significant SP release is dependent upon a sustained and severe stimulus such as tissue damage and this form of injury will produce a massive release of SP over a wide area (Schaible *et al.* 1990). Diffusion of the ligand would therefore access additional receptor sites raising the gain of the neurones and perhaps contribute to secondary hyperalgesia by activating neurone populations that innervate tissue adjacent to the injury site.

Behavioural and electrophysiological investigations also support a role for NK_1 receptors in spinal nociception. However their relative involvement may be dependent upon the duration and severity of the stimulus. NK_1 receptors do not appear to mediate brief nociceptive inputs: NK_1 antagonists don't reduce behavioural reflexes evoked by brief noxious pinch or acute noxious thermal stimulation of the tail or paw (Malmberg and Yaksh, 1992; Yamamoto and Yaksh, 1992; Garces *et al.* 1993) and in electrophysiological experiments they do not alter baseline nociceptive reflex activity (Laird *et al.* 1993), single C-fibre volleys (De Koninck and Henry, 1991; Chapman and Dickenson, 1993) or sustained C-fibre stimulation in normal animals (Thompson *et al.* 1994). More evidence implies that NK_1 receptors are mediators of sustained nociceptive inputs. For example, NK_1 antagonists reduce behavioural and dorsal horn neuronal responses to severe stimulation such as intraplantar injection of capsaicin (Sakurada *et al.* 1993; Dougherty *et al.* 1994), carrageenan (Yamamoto *et al.* 1993b) or formalin (Charriaut-Marlangue *et al.* 1991; Chapman and Dickenson, 1993). Indeed, much of this evidence suggests that SP does not function as a pain transmitter per se, but may be more important in sensitising spinal neurones (Dirig and Yaksh, 1996).

The main role of SP and NK_1 receptors in sensory processing therefore appears to be as a positive modulator of nociceptive transmission. This is supported by anatomical, behavioural and electrophysiological studies with little evidence for a direct role of SP in the transmission of brief nociceptive inputs. There are several feasible mechanisms for this delayed mode of action of NK_1 receptors. One possibility is that the release of SP has to accumulate enough to spread to pertinent receptor sites some way from the actual release site. However, even when NK_1 agonists are applied directly onto dorsal horn cells, neuronal responses to brief inputs are often not enhanced (Fleetwood-Walker *et al.* 1987; De Koninck and Henry, 1991; Malmberg and Yaksh, 1992; Yamamoto and Yaksh, 1992; Couture *et al.* 1993) and in some cases are even inhibited (Davies and Dray, 1980; Murase and Randic, 1984; Willcockson *et al.* 1984; Ryall and Pini, 1987; Fleetwood-Walker *et al.* 1988; Fleetwood-Walker *et al.* 1990; Masuyama *et al.* 1996). This would suggest that there might be an endogenous regulatory mechanism that precludes NK_1 receptor involvement in acute nociception, perhaps via the co-activation of an inhibitory mechanism.

In contrast, sustained nociceptive stimuli are believed to activate an overt NK_1 receptor-mediated component and there is a large body of evidence to demonstrate that this occurs in co-operation with NMDA receptors. This hypothesis stems from observations that glutamate and SP coexist in primary afferent terminals in the superficial laminae (De Biasi and Rustioni, 1988) and may be co-released following noxious stimulation. Furthermore, a synergistic interaction between NK_1 and NMDA receptors has been demonstrated in a variety of experimental models. For example, SP is known to potentiate NMDA evoked neuronal responses *in vivo* (Dougherty and Willis, 1991; Dougherty *et al.* 1995) and *in vitro*

(Randic *et al.* 1990), and combined applications of NMDA and NK_1 antagonists reduce wind-up (Chapman *et al.* 1994) and block behavioural responses to inflammatory stimuli more significantly than application of each type of antagonist alone (Murray *et al.* 1991; Mjellem-Joly *et al.* 1992; Okano *et al.* 1994; Okano *et al.* 1995). This data emphasises the importance of NK_1 receptors in the transmission of prolonged nociceptive inputs and also gives an insight into the mechanistics of the central sensitisation phenomenon. If a situation arises where co-release of SP and glutamate activates NMDA and NK_1 receptors, then co-operation between these receptors should increase the gain of the system and sensitise dorsal horn neurones to subsequent inputs.

This study has investigated the role of NK_1 receptor activation in spinal nociceptive processing. On the basis of a relationship between stimulus severity and NK_1 receptor involvement, a hypothesis has been proposed that links preclusion of the NK_1 receptor component during acute nociception with NMDA/NK_1 co-operation during sustained stimulation. Since SP is known to increase levels of glycine in the dorsal horn (Kangrga and Randic, 1990b; Smullin *et al.* 1990b; Maehara *et al.* 1995b), the dual actions of this amino acid at either its inhibitory strychnine-sensitive receptor or its excitatory site on the NMDA receptor have been investigated. The model (Figure 5.1) predicts that during brief noxious stimuli the NK_1 receptor component is masked by SP-evoked glycine activity at its inhibitory receptor. However, sustained nociception leads to increased SP release and NMDA receptor activation. SP-evoked glycine release can now act at the allosteric Gly_{NMDA} site, unmasking the covert action of NK_1 receptors and further potentiating NMDA receptor activity.

To investigate the role of NK_1 receptors in brief nociception, the effects of NK_1 antagonists on dorsal horn neuronal responses to the algogen mustard oil were investigated. This model reflects a fairly brief nociceptive stimulus that is not affected by NK_1 antagonists (Munro *et al.* 1993). However, in the present study, co-application of glycine receptor antagonists such as strychnine, with NK_1 antagonist brought about a dose-dependent inhibition of the neuronal activity. This could be interpreted as a lifting of an inhibitory control. If NK_1 receptor influences are regulated via an inhibitory action of SP-evoked increases in glycine concentrations, by blocking the negative modulation (with glycine receptor antagonists), the regulatory aspect will be removed and an effect of the NK_1 antagonist will become apparent.

Longer duration nociceptive stimuli evoke NMDA receptor involvement in neuronal responses and NK_1 receptors now exert a positive modulation (Urban *et al.* 1994b). The effects on the this interaction of an NK_1-evoked increase in glycine levels on this interaction were investigated by testing Gly_{NMDA} antagonists on dorsal horn neuronal activity evoked by NMDA and NK_1 receptor agonists. Data from this study demonstrate that the interaction is highly sensitive to blockade of the Gly_{NMDA} site. Furthermore, it is accompanied by an increase in the sensitivity of the Gly_{NMDA} site that is not apparent at other sites on the NMDA

receptor complex or when NMDA agonist is applied alone. Similarly, PKC inhibitors at low ejection currents also block the interaction. This raises the possibility that site specific phosphorylations within the NMDA receptor complex (as a consequence of NK_1 receptor activation) may alter the sensitivity of the Gly_{NMDA} site and when coupled with increased activity at this site, contribute to the NK_1/NMDA interaction.

The central concept of both of these series of experiments is the functional change of NK_1 receptors from a covert activity during brief stimulation to a potentiating role during severe or sustained nociception and the dual actions of glycine might account for this change. Such arrangements of inhibitory and excitatory actions of glycine receptors have previously been described. Budai *et al.* (1992) reported that ionophoretic application of high doses of glycine inhibited the NMDA-evoked firing of nociceptive neurones in rat dorsal horn whereas low doses increased activity. Furthermore Beyer *et al.* (1992) demonstrated that in behavioural tests, combined intrathecal injections of glycine and the glutamate antagonist D-AP5 produced marked and prolonged analgesia compared to individual applications of these compounds suggesting that activation of the strychnine-sensitive receptor produces analgesia provided that the NMDA receptor is also blocked. Therefore, the actions of this amino acid, either at its inhibitory strychnine-sensitive receptor or its positive modulatory site on the NMDA receptor would determine the role of the NK_1 receptor.

When discussing such a mechanism it is important to consider what element switches the negative actions of glycine to a positive effect and how this relates to the severity and duration of the stimulus. Data from the present and other studies suggests that this might be due to the accumulation of intracellular phosphorylations perhaps mediated via PKC. We have demonstrated that the NK_1/NMDA interaction is highly sensitive to inhibition by PKC blockers and this accords well with other evidence. PKC inhibitors block the SP-mediated facilitation of NMDA responses in electrophysiological preparations (Rusin *et al.* 1993a; Urban *et al.* 1994a) and inhibit enhanced behavioural responses to subcutaneous formalin injection induced by co-application of SP and NMDA (Coderre, 1992; Coderre and Yashpal, 1994). Furthermore PKC has been implicated as a sensitiser of dorsal horn neuronal responsiveness (Munro *et al.* 1994; Yashpal *et al.* 1995; Peng *et al.* 1997) and Lin *et al.* (1997) have demonstrated that this may be partly due to an attenuation of the inhibition of (spinothalamic tract) neurones by glycine. They reported that during central sensitisation, PKC desensitised glycine receptors and removed the inhibitory modulation. It is therefore possible that a combination of glycine receptor desensitisation and phosphorylation of the NMDA receptor complex diverts the action of glycine from an inhibitory function to an excitatory role and helps initiate the process of central sensitisation.

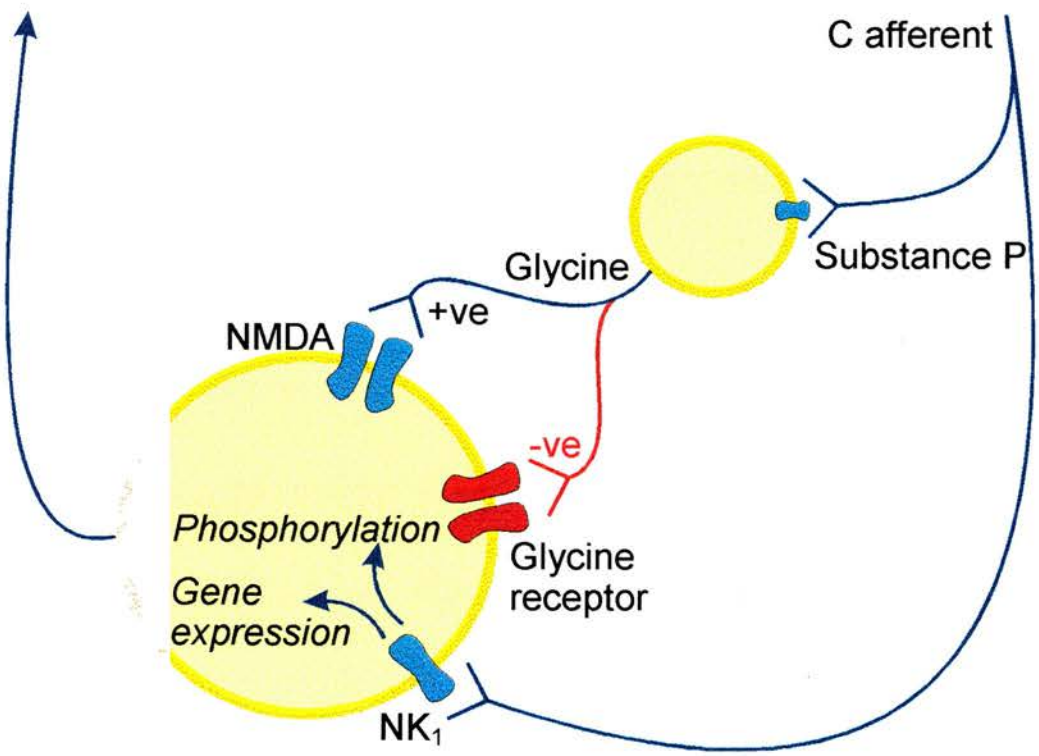
This study shows that the effects of NK_1 receptors in spinal nociception may occur via a series of time-dependent interactions. During acute situations the inhibitory glycine receptor

reduces the role played by NK_1 receptors in pain transmission, presumably via an NK_1 induced increase in glycine levels. However in more prolonged or severe models of pain other factors may overcome this influence. Increased release of substance P coupled with the accumulation of intracellular phosphorylations and changes in the sensitivity of the Gly_{NMDA} site (and perhaps also the strychnine-sensitive glycine receptor) reduce the inhibitory modulation and NK_1 receptors now result in an overall potentiation of NMDA receptor-mediated activity.

FIGURE 5.1

A hypothetical model of the effects of SP-evoked increases in glycine concentrations in the dorsal horn.

During brief noxious stimuli SP-evoked glycine acts at its inhibitory receptor to mask any direct effects of NK_1 receptors. Following a more severe stimulus, PKC-mediated phosphorylation of the NMDA receptor leads to changes in the sensitivity of the Gly_{NMDA} site. Glycine now exerts a greater action at this site and contributes positively to the NMDA/NK_1 interaction while this cumulative potentiation of NMDA receptor effect outweighs the acute inhibitory effect of strychnine-sensitive glycine receptors. Additional factors may well also play a role.


C afferent
Substance P
Glycine
+ve
NMDA
-ve
Phosphorylation
Gene
expression
Glycine
receptor
NK1

5.2 LIF AS A REGULATOR OF NOCICEPTIVE TRANSMISSION

The first part of this study addressed short-term changes in dorsal horn processing of nociceptive information. This form of plasticity may be important in initiating alterations in neuronal responsiveness. However long term modifications of neuronal phenotype are also known to occur via alterations in gene expression. These changes can lead to pathological conditions where the dorsal horn remains in a sensitised state in the absence of peripheral stimulation (Treede *et al.* 1992). Understanding the mechanisms of longer term plasticity in the dorsal horn may therefore lead to the development of new pharmaceutical strategies for the treatment of pain and sensory neuropathies.

LIF is a neuropoietic cytokine that has been demonstrated to have a function in the injured sympathetic nervous system. LIF synthesis is upregulated at the site of injury and in the SCG by Schwann cells (Banner and Patterson, 1994; Curtis *et al.* 1994; Kurek *et al.* 1996; Sun *et al.* 1996) and participates in the cell body reaction to axotomy (Zigmond *et al.* 1996). This may be considered as essentially a protective process reflecting a change in neuronal function from synaptic transmission to regeneration (Grafstein and McQuarrie, 1978) and is accompanied by increased production of a number of structural proteins and neuropeptides (Koo *et al.* 1988; Hoffman, 1989; Hyatt-Sachs *et al.* 1993; Schreiber *et al.* 1994). LIF induces the synthesis of many of these factors including SP, VIP, galanin and NK_1 receptor (Rao *et al.* 1993; Shadiack *et al.* 1993; Fann and Patterson, 1994; Sun *et al.* 1994; Ludlam *et al.* 1995) and may be a crucial component in the cellular response to injury.

Recent studies have also implicated LIF as a neuronal modulator in the sensory nervous system. In this environment LIF synthesis is restricted to Schwann cells at the injury site and secreted LIF is retrogradely transported to the DRG. Thompson *et al.* (1997) have examined this process in detail and report that the transport is undertaken solely by a population of fine diameter nociceptive neurones. Furthermore, systemically administered LIF evokes mechanical hypersensitivity in concious rats which suggests that LIF may have a function as a nociceptive agent (Thompson *et al.* 1996). As in the sympathetic system, retrogradely transported LIF induces changes in the chemical phenotype of sensory nerves, (Corness *et al.* 1996; Sun and Zigmond, 1996) and the increased synthesis and release of neuropeptides from central terminals may partly account for this pro-nociceptive role of LIF.

The functional diversity of LIF's actions in the peripheral nervous system makes it an interesting candidate for study in the spinal cord. Very few studies have addressed the role or distribution of LIF in the central nervous system, although the related cytokine IL-6 has recently attracted some attention with the demonstration that it may be involved in the regulation of spinal neuronal responses to peripheral nerve injury (DeLeo *et al.* 1996). We

have examined the action of LIF in the spinal cord. Initially, its spinal distribution was investigated and this was compared to expression after peripheral inflammation and nerve injury. We report that LIF mRNA is located in the spinal cord and is upregulated in response to peripheral injury. Therefore LIF may have a similar function in the central nervous system to its regulatory action on gene expression in peripheral ganglia. This was tested by assessing the effects of chronic intrathecal LIF application on the expression of NK_1 receptor and EAAT2 mRNA and measuring subsequent behavioural responses to intrathecally infused LIF. LIF increased the expression of NK_1 receptors and decreased EAAT2 mRNA in dorsal horn. Furthermore rats developed behavioural hypersensitivity to mechanical and thermal stimuli.

This is the first report of a nociceptive role for LIF in the spinal cord. In agreement with data on its role in the peripheral nervous system, LIF appears to be evoking behavioural hypersensitivity via increased expression of key components of nociceptive transmission. However, there is a basal expression of LIF in the spinal cord, and whilst this is increased by peripheral injury, these changes occur at sites removed from the actual injury zone. This would suggest that in this situation, spinal LIF does not have a function as a regeneration factor but may be more important in regulating neuronal hyperexcitability via changes in gene expression. It is also possible that LIF might have fast actions in the central nervous system. Recently, factors associated with trophic functions such as NGF and other members of the neurotrophin family, have been demonstrated to acutely alter synaptic efficacy and contribute to behavioural hyperalgesia (Lewin *et al.* 1993; Lewin and Barde, 1996). It is therefore possible that LIF might also evoke rapid changes in dorsal horn neuronal responses by directly modifying the strength of synaptic connections. This has not yet been investigated.

LIF is an injury-induced regeneration factor in the peripheral nervous system with important functions in sympathetic and sensory tissue. This study provides new evidence for a role in the central nervous system. LIF mRNA expression is upregulated in the spinal cord in response to a peripheral injury and it induces behavioural hypersensitivity when applied intrathecally. We report that this may be due to increased synthesis of a number of pro-nociceptive components such as NK_1 receptors and EAAT2 glutamate transporters. LIF might therefore be a critical regulator of the central changes that occur following peripheral injury and could contribute to the development of inflammatory and neuropathic pain states associated with these changes.

BIBLIOGRAPHY

Aanonsen, L.M., Lei, S. and Wilcox, G.L. (1990) Excitatory amino acid receptors and nociceptive neurotransmission in rat spinal cord. *Pain*, **41**: 309-321.

Aanonsen, L.M., Kajander, K.C., Bennett, G.J. and Seybold, V.S. (1992) Autoradiographic analysis of ^{125}I-substance P binding in rat spinal cord following chronic constriction injury of the sciatic nerve. *Brain Res*, **596**: 259-268.

Aanonsen, L.M. and Wilcox, G.L. (1987) Nociceptive action of excitatory amino acids in the mouse: effects of spinally administered opioids, phencyclidine and sigma agonists. *J Pharmacol Exp Ther*, **243**: 9-19.

Abbadie, C., Brown, J.L., Mantyh, P.W. and Basbaum, A.I. (1996) Spinal cord substance P receptor immunoreactivity increases in both inflammatory and nerve injury models of persistent pain. *Neuroscience*, **70**: 201-209.

Abbadie, C., Trafton, J., Liu, H., Mantyh, P.W. and Basbaum, A.I. (1997) Inflammation increases the distribution of dorsal horn neurons that internalize the neurokinin-1 receptor in response to noxious and non-noxious stimulation. *Journal of Neuroscience*, **17**: 8049-8060.

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N. and Nakanishi, S. (1992) Molecular characterisation of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phophate/Calcium signal transduction. *J Biol Chem*, **267**: 13361-13368.

Aicher, S.A., Sharma, S., Cheng, P.Y. and Pickel, V.M. (1997) The NMDA receptor is postsynaptic to substance P-containg axon terminals in the rat superficial dorsal horn. *Brain Research*, **772**: 71-81.

Aizenman, E., Lipton, S.A. and Loring, R.H. (1989) Selective modulation of NMDA responses by reduction and oxidation. *Neuron*, **2**: 1257-1263.

Alford, S. and Grillner, S. (1990) CNQX and DNQX block non-NMDA synaptic transmission but not NMDA-evoked locomotion in lamprey spinal cord. *Brain Res*, **506**: 297-302.

Allan, R.D., Hanrahan, J.R., Hambley, T.W., Johnston, G.A., Mewett, K.N. and Mitrovic, A.D. (1990) Synthesis and activity of a potent NMDA agonist, trans-1,3-dicarboxylic acid and related phosphonic and carboxylic acids. *J Med Chem*, **33**: 2905-2915.

Angaut-Petit, D. (1975) The dorsal column system: I. Existence of long ascending postsynaptic fibres in the cats fasciculus gracilis. *Exp Brain Res*, **22**: 457-470.

Anis, N.A., Berry, S.C., Burton, N.R. and Lodge, D. (1983) The dissociative anaesthetics ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl -D-aspartate. *Brit J Pharm*, **79**: 471-493.

Apkarian, A.V. and Shi, T. (1994) Squirrel monkey lateral thalamus. I. Somatic nociresponsive neurons and their relation to spinothalamic terminals. *J Neurosci*, **14**: 6779-6795.

Applebaum, A.E., Beall, J.E., Foreman, R.D. and Willis, W.D. (1975) Organisation and receptive fields of primate spinothalamic tract neurones. *J Neurophysiol*, **38**: 572-586.

Aragon, M.C., Gimenez, C. and Mayor, F. (1987) Stoichiometry of sodium- and chloride-coupled glycine transport in synaptic plasma membrane vesicles derived from rat brain. *FEBS Letters*, **212**: 87-90.

Araki, T., Yamano, M., Murakami, T., Wanaka, A., Betz, H. and Toyhama, M. (1988) Localisation of glycine receptors in the rat central nervous system: an immunocytochemical analysis using monoclonal antibody. *Neurosci*, **25**: 613-624.

Aramori, I. and Nakanishi, S. (1992) Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected chinese hamster ovary cells. *Neuron*, **8**: 757-765.

Artola, A. and Singer, W. (1987) Long-term potentiation and NMDA receptors in rat visual cortex. *Nature*, **330**: 649-652.

Attwell, D., Szatkowski, M. and Barbour, B. (1993) Non-vesicular release of neurotransmitter. *Neuron*, **11**: 401-407.

Bakhle, Y.S. and Bell, C. (1995) Neurokinin A and substance P vary independently in different regions of rat sensory neurons. *Neuropeptides*, **28**: 237-241.

Banner, L.R. and Patterson, P.H. (1994) Major changes in the expression of the mRNAs for cholinergic differentiation factor/leukemia inhibitory factor and its receptor after injury to adult peripheral nerves and ganglia. *Proceedings of the National Academy of Sciences of the United States of America*, **91**: 7109-7113.

Baranauskas, G., Traversa, U., Rosati, A.M. and Nistri, A. (1995) An NK1 receptor-dependent component of the slow excitation recorded intracellularly from rat motoneurons following dorsal root stimulation. *European Journal of Neuroscience*, **7**: 2409-2417.

Barker, J.L. and Nicoll, R.A. (1973) The pharmacology and ionic dependency of amino acid responses in the frog spinal cord. *Journal of Physiology*, **228**: 259-277.

Bars, S.J.P.T. and Hanna, M.H. (1987) A double blind comparison of epidural ketamine and diamorphine for postoperative anaesthesia. *Anaesthesia*, **44**: 555-558.

Basbaum, A.I. (1988) Distribution of glycine receptor immunoreactiviy in the spinal cord of the rat: cytochemical evidence for a differential glycinergic control of laminae I and V nociceptive neurons. *J Comp Neurol*, **278**: 1168-1169.

Battaglia, G. and Rustioni, A. (1988a) Coexistence of glutamate and substance P in dorsal root ganglion neurons of the rat and monkey. *J Comp Neurol*, **277**: 302-312.

Battaglia, G. and Rustioni, A. (1988b) Coexistence of glutamate and substance P in dorsal root ganglion neurons of the rat and monkey. *Journal of Comparative Neurology*, **277**: 302-312.

Bazan, J.F. (1991) Neuropoietic cytokines in the hematopoietic fold. *Neuron*, **7**: 197-208.

Becker, C.M. (1990) Disorders of the inhibitory glycine receptor: the spastic mouse. *FASEB Journal*, **4**: 2767-2774.

Bennett, G.J., Hayashi, H., Abdelmoumene, M. and Dubner, R. (1979) Physiology properties of stalked cells of the substantia gelatinosa intracellulary stained with horseradish peroxidase. *Brain Res*, **164**: 285-289.

Berthele, A., Neudart, C., Castro-Lopes, J.M., Wisden, W., Thomas, K.L., Hunt, S.P., Zieglgansberger, W. and Tolle, T.R. (1996) Protein Kinase C isoform, but not glutamate receptor subunit gene expression is differentially regulated in the spinal cord of the rat following induction of monoarthritis. *8th World Congress on Pain*, 241

Besson, J.M., Guilbaud, G. and Le Bars, D. (1975) Descending inhibitory influences exerted by the brainstem upon the activities of dorsal horn laminae V cells induced by intraarterial injection of bradykinin into the limbs. *J Physiol*, **248**: 725-739.

Besson, J.M. and Chaouch, A. (1987) Peripheral and spinal mechanisms of nociception. *Physiol Rev*, **67**: 67-186.

Bessou, P. and Perl, E.R. (1969) Response of cutaneous sensory units with unmyelinated fibres to noxious stimuli. *J Neurophysiol*, **32**: 1025-1043.

Bettler, B., Boutler, J., Hermans-Borgmeyer, I., O'SheaGreen-field, A., Deneris, E.S., Moll, C., Borgmeyer, U., Hollmann, M. and Heinemann, S. (1990) Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. *Neuron*, **5**: 583-595.

Bettler, B., Egebjerg, J., Sharma, G., Pecht, G., Hermans-Borgmeyer, I., Moll, C., Stevens, C.F. and Heinemann, S. (1992) Cloning of a putative glutamate receptor: a low affinity kainate-binding subunit. *Neuron*, **8**: 257-265.

Bettler, B. and Mulle, C. (1995) Review: neurotransmitter receptors. II. AMPA and kainate receptors. *Neuropharmacology*, **34**: 123-139.

Betz, H., Kuhse, J., Fischer, M., Schmieden, V., Laube, B., Kuryatov, A., Langosch, D., Meyer, G., Bormann, J., Rundstrom, N. and et al. (1994) Structure, diversity and synaptic localization of inhibitory glycine receptors. *Journal of Physiology,Paris*, **88**: 243-248.

Beyer, C., Roberts, L.A. and Komisaruk, B.R. (1985) Hyperalgesia induced by altered glycinergic activity at the spinal cord. *Life Sciences*, **37**: 875-882.

Beyer, C., Banas, C., Gonzalez-Flores, O. and Komisaruk, B.R. (1989) Blockage of substance P-induced scratching behavior in rats by the intrathecal administration of inhibitory amino acid agonists. *Pharmacology, Biochemistry and Behavior*, **34**: 491-495.

Beyer, C., Komisaruk, B.R., Lopez-Colome, A.M. and Caba, M. (1992) Administration of AP5, a glutamate antagonist, unmasks glycine analgesic actions in the rat. *Pharmacology,Biochemistry and Behavior*, **42**: 229-232.

Billups, B. and Attwell, D. (1996) Modulation of non-vesicular glutamate release by pH. *Nature*, **379**: 171-174.

Bjorkman, R., Hallman, K.M., Hedner, J., Hedner, T. and Henning, M. (1994) Acetaminophen blocks spinal hyperalgesia induced by NMDA and substance P. *Pain*, **57**: 259-264.

Bleakman, D., Rusin, K.I., Chard, P.S., Glaum, S.R. and Miller, R.J. (1992) Metabotropic glutamate receptors potentiate ionotropic glutamate responses in the rat dorsal horn. *Molecular Pharmacology*, **42**: 192-196.

Bleazard, L., Hill, R.G. and Morris, R. (1994) The correlation between the distribution of the NK_1 receptor and the actions of tachykinin agonists in the dorsal horn of the rat indicates that substance P does not have a functional role on substantia gelatinosa (lamina II) neurons. *J Neurosci*, **14**: 7655-7664.

Bloomenthal, A.B., Goldwater, E., Pritchett, D.B. and Harrison, N.L. (1994) Biphasic modulation of the strychnine-sensitive glycine receptor by Zn2+. *Molecular Pharmacology*, **46**: 1156-1159.

Bond, A. and Lodge, D. (1995) Pharmacology of metabotropic glutamate receptor-mediated enhancement of responses to excitatory and inhibitory amino acids on rat spinal neurones in vivo. *Neuropharmacology*, **34**: 1015-1023.

Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. and Heinemann, S. (1990) Molecular cloning and functional expression of glutamate receptor subunit genes. *Science*, **249**: 1033-1037.

bovetto, S., Boyer, B.-A., Skolnick, P. and Fossom, L.H. (1997) Chronic administration of a glycine partial agonist alters the expression of N-Methyl-D-aspartate receptor subunit mRNAs. *Journal of Pharmacology and Experimental Therepeutics*, **283**: 1503-1508.

Boxall, S.J., Thompson, S.W., Dray, A., Dickenson, A.H. and Urban, L. (1996) Metabotropic glutamate receptor activation contributes to nociceptive reflex activity in the rat spinal cord in vitro. *Neuroscience*, **74**: 13-20.

Broman, J., Anderson, S. and Ottersen, O.P. (1993) Enrichment of glutamate-like immunoreactivity in primary afferent terminals throughout the spinal cord dorsal horn. *European Journal of Neuroscience*, **5**: 1050-1061.

Brown, A.G. (1971) Effects of descending impulses on transmission through the spinocervical tract. *J Physiol (Lond)*, **219**: 103-125.

Brown, A.G., Fyffe, R.E.W., Noble, R., Rose, P.K. and Snow, P.J. (1980) The density, distribution and topographical organisation of spinocervical tract neurones in the cat. *J Physiol (Lond)*, **300**: 409-428.

Brown, A.G. (1981) *Organization in the Spinal Cord: the Anatomy and Physiology of Identified Neurones.* Springer-Verlag, Berlin.

Brown, A.G., Brown, P.B., Fyffe, R.E. and Pubols, L.M. (1983) Receptive field organisation and response properties of spinal neurones with axons ascending the dorsal columns in the cat. *J Physiol*, **337**: 575-588.

Brown, A.G. and Franz, D.N. (1969) Responses of spinocervical tract neurones to natural stimulation of identified cutaneous receptors. *Exp Brain Res*, **7**: 231-249.

Brown, J.L., Liu, H., Maggio, J.E., Vigna, S.R., Mantyh, P.W. and Basbaum, A.I. (1995) Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis. *Journal of Comparative Neurology*, **356**: 327-344.

Budai, D., Wilcox, G.L. and Larson, A.A. (1992) Enhancement of NMDA-evoked neuronal activity by glycine in the rat spinal cord in vivo. *Neuroscience Letters*, **135**: 265-268.

Burgess, P.R. and Perl, E.R. (1967) Myelinated afferent fibers responding specifically to noxious stimulation of skin. *J Physiol (Lond)*, **190**: 541-562.

Burgess, P.R. and Perl, E.R. (1973) Cutaneous mechanoreceptors and nociceptors. *Handbook of sensory physiology* (ed. by A. Iggo), p.29. Springer, New York.

Cahusac, P.M.B., Evans, R.H., Hill, R.G., Rodriquez, R.E. and Smith, D.A.S. (1984) The behavioural effects of an N-methylaspartate receptor antagonist following application to the lumbar spinal cord of conscious rats. *Neuropharmac*, **23**: 719-724.

Campbell, J.N., Meyer, R.A. and LaMotte, R.H. (1979) Sensitization of myelinated nociceptive afferents that innervate the monkey hand. *J Neurophysiol*, **42**: 1669-1679.

Cangro, C.B., Sweetnam, P.M., Wrathall, J.R., Haser, W.B., Curthoys, N.P. and Neale, J.H. (1985) Localization of elevated glutaminase immunoreactivity in small DRG neurons. *Brain Research*, **336**: 158-161.

Carlton, S.M., Hargett, G.L. and Coggeshall, R.E. (1996a) Distribution of glycine-immunoreactive profiles in the monkey spinal cord: a light microscopic and ultrastructural study. *Journal of Comparative Neurology*, **371**: 589-602.

Carlton, S.M., Zhou, S. and Coggeshall, R.E. (1996b) Localization and activation of substance P receptors in unmyelinated axons of rat glabrous skin. *Brain Research*, **734**: 103-108.

Cerne, R., Jiang, M. and Randic, M. (1992) Cyclic adenosine 3'5'-monophosphate potentiates excitatory amino acid and synaptic responses of rat spinal dorsal horn neurons. *Brain Research*, **596**: 111-123.

Cervero, F., Iggo, A. and Ogawa, H. (1976) Nociceptor-driven dorsal horn neurons in the lumbar spinal cord of the rat. *Pain*, **2**: 5-14.

Cervero, F., Molony, V. and Iggo, A. (1979) Supraspinal linkage of substantia gelatinosa neurons: effects of descending inputs. *Brain Res*, **175**: 351-355.

Cervero, F. and Iggo, A. (1980) The substantia gelatinosa of the spinal cord. A critical review. *Brain*, **103**: 717-772.

Chambers, M.R., Andres, K.H., Von During, M. and Iggo, A. (1972) The structure and function of the slowly adapting type II mechanoreceptor in hairy skin. *Quart J Exp Physiol*, **57**: 417-445.

Chapman, L.F., Ramos, A.O., Goodell, H. and Wolff, H.G. (1961) Neurohumoral features of afferent fibres in man. *A M A Arch Neurology*, **4**: 617-650.

Chapman, V., Dickenson, A.H. and Tjolsen, A. (1994) Bi-directional effects of intrathecal NMDA and substance P on rat dorsal horn neuronal responses. *Neuroscience Letters*, **178**: 90-94.

Chapman, V., Buritova, J., Honore, P. and Besson, J.M. (1996) Physiological contributions of neurokinin 1 receptor activation, and interactions with NMDA receptors, to inflammatory-evoked spinal c-Fos expression. *Journal of Neurophysiology*, **76**: 1817-1827.

Chapman, V. and Dickenson, A.H. (1993) The effect of intrathecal administration of RP67580, a potent neurokinin 1 antagonist on nociceptive transmission in the rat spinal cord. *Neurosci Lett*, **157**: 149-152.

Chapman, V. and Dickenson, A.H. (1994) Enhanced responses of rat dorsal horn neurones after UV irradiation of the hindpaw; Roles of the NMDA receptor. *Neurosci Lett*, **176**: 41-44.

Charriaut-Marlangue, C., Otani, S., Creuzet, C., Ben-Ari, Y. and Loeb, J. (1991) Rapid activation of hippocampal casein kinase II during long-term potentiation. *Proc Natl Acad Sci USA*, **88**: 10232-10236.

Cheema, S.S., Richards, L., Murphy, M. and Bartlett, P.F. (1994a) Leukemia inhibitory factor prevents the death of axotomised sensory neurons in the dorsal root ganglia of the neonatal rat. *Journal of Neuroscience Research*, **37**: 213-218.

Cheema, S.S., Richards, L.J., Murphy, M. and Bartlett, P.F. (1994b) Leukaemia inhibitory factor rescues motoneurones from axotomy-induced cell death. *Neuroreport*, **5**: 989-992.

Chen, L. and Huang, L.Y. (1992) Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation. *Nature*, **356**: 521-523.

Chizh, B.A., Cumberbatch, M.J., Birch, P.J. and Headley, P.M. (1995) Endogenous modulation of excitatory amino acid responsiveness by tachykinin NK1 and NK2 receptors in the rat spinal cord. *British Journal of Pharmacology*, **115**: 1013-1019.

Christensen, B.N. and Perl, E.R. (1970) Spinal neurones specifically excited noxious or thermal stimuli: marginal zone of the dorsal horn. *J Neurophysiol*, **33**: 294-307.

Chung, J.M., Kenshalo, D.R., Gerhart, K.D. and Willis, W.D. (1979) Excitation of primate spinothalamic neurons by cutaneous C-fibre volleys. *J Neurophysiol*, **42**: 1354

Chung, S.K., Chan, K.H., Lee, T.Y., Lui, P.W., Yang, M.W., Huang, Y.L., Liu, K., Wu, C.L. and Fu, Y.P. (1986) Epidural ketamine for postoperative pain relief. *Acta Anaesthesiol Sin*, **24**: 251-257.

Ciabarra, A.M., Sullivan, J.M., Gahn, L.G., Pecht, G., Heinemann, S. and Sevarino, K.A. (1995) Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *Journal of Neuroscience*, **15**: 6498-6508.

Coan, E.J. and Collingridge, G.L. (1984) Magnesium ions affect synaptic transmission in rat hippocampal slices via an action at N-methyl-D-aspartate receptors. *J Physiol*, **357**: 121P

Coderre, T.J. (1992) Contribution of protein kinase C to central sensitization and persistent pain following tissue injury. *Neuroscience Letters*, **140**: 181-184.

Coderre, T.J. and Melzack, R. (1991a) Central neural mediators of secondary hyperalgesia following heat injury in rats: neuropeptides and excitatory amino acids. *Neuroscience Letters*, **131**: 71-74.

Coderre, T.J. and Melzack, R. (1991b) Central neural mediators of secondary hyperalgesia following heat injury in rats: Neuropeptides and excitatory amino acids. *Neurosci Lett*, **131**: 71-74.

Coderre, T.J. and Melzack, R. (1992) The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. *J Neurosci*, **12**: 3665-3670.

Coderre, T.J. and Van Empel, I. (1994a) The utility of excitatory amino acid (EAA) antagonists as analgesic agents I. Comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests. *Pain*, **94**: 345-352.

Coderre, T.J. and Van Empel, I. (1994b) The utility of excitatory amino acid (EAA) antagonists as analgesic agents. II. Assessment of the antinociceptive activity of combinations of competitive and non-competitive NMDA antagonists with agents acting at allosteric-glycine and polyamine receptor sites. *Pain*, **59**: 353-359.

Coderre, T.J. and Yashpal, K. (1994a) Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model. *Eur J Neurosci*, **6**: 1328-1334.

Coderre, T.J. and Yashpal, K. (1994b) Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model. *European Journal of Neuroscience*, **6**: 1328-1334.

Coggeshall, R.E. and Carlton, S.M. (1997) Receptor localisation in the mammalian dorsal horn and primary afferent neurons. *Brain Res Rev*, **24**: 28-66.

Collingridge, G.L., Kehl, S.J. and McLennan, H. (1983) Excitatory amino acids in synaptic transmission in the Schaffer-commissural pathway of the rat hippocampus. *J Physiol (Lond)*, **334**: 35-46.

Cook, A.J., Woolf, C.J., Wall, P.D. and McMahon, S.B. (1987) Dynamic receptive field plasticity in rat spinal cord dorsal horn following C-primary afferent input. *Nature Lond*, **325**: 151-153.

Corness, J., Shi, T.J., Xu, Z.Q., Brulet, P. and Hokfelt, T. (1996) Influence of leukemia inhibitory factor on galanin/GMAP and neuropeptide Y expression in mouse primary sensory neurons after axotomy. *Experimental Brain Research*, **112**: 79-88.

Corsi, M., Fina, P. and Trist, D.G. (1996) Co-agonism in drug-receptor interaction: illustrated by the NMDA receptors. *Trends in Pharmacological Sciences*, **17**: 220-222.

Couture, R., Boucher, S., Picard, P. and Regoli, D. (1993) Receptor characterization of the spinal action of neurokinins on nociception: A three receptor hypothesis. *Regul Pept*, **46**: 426-429.

Cumberbatch, M.J., Chizh, B.A. and Headley, P.M. (1995a) Modulation of excitatory amino acid responses by tachykinins and selective tachykinin receptor agonists in the rat spinal cord. *British Journal of Pharmacology*, **115**: 1005-1012.

Cumberbatch, M.J., Chizh, B.A. and Headley, P.M. (1995b) Modulation of excitatory amino acid responses by tachykinins and selective tachykinin receptor agonists in the rat spinal cord. *Br J Pharmacol*, **115**: 1005-1012.

Curtis, D.R., Phillis, J.W. and Watkins, J.C. (1960) The chemical excitation of spinal neurones by certain acidic amino acids. *J Physiol (Lond)*, **150**: 656-682.

Curtis, D.R., Hosli, L. and Johnston, G.A. (1967) Inhibition of spinal neurons by glycine. *Nature*, **215**: 759-762.

Curtis, D.R., Duggan, A.W. and Johnston, G.A. (1969) Glycine, strychnine, picrotoxin and spinal inhibition. *Brain Res*, **14**: 759-762.

Curtis, R., Scherer, S.S., Somogyi, R., Adryan, K.M., Ip, N.Y., Zhu, Y., Lindsay, R.M. and DiStefano, P.S. (1994) Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. *Neuron*, **12**: 191-204.

D'Angelo, E., Rossi, P. and Garthwaite, J. (1990) Dual component NMDA receptor currents at a single central synapse. *Nature*, **346**: 467-470.

Dabgaard, Hokfelt, T. and et al. (1985) Neurokinin A-like immunoreactivity in rat primary sensory neurons, coexistence with substance P. *Histochemistry*, **83**: 37-40.

Davar, G., Hama, A., Deykin, A., Vos, B. and Maciewicz, R. (1991) MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. *Brain Research*, **553**: 327-330.

Davidoff, R.A., Aprison, M.H. and Werner, R. (1969) The effects of strychnine on the inhibition of interneurons by glycine and GABA. *J Neuropharmacol*, **8**: 191-194.

Davidoff, R.A. and Aprison, M.H. (1969) Picrotoxin antagonism of the inhibition of interneurons by glycine. *Life Sci*, **8**: 107-112.

Davies, J. and Dray, A. (1980) Depression and facilitation of synaptic responses in cat dorsal horn by substance P administered into substantia gelatinosa. *Life Sciences*, **27**: 2037-2042.

Davies, J. and Watkins, J.C. (1983) Role of excitatory amino acid receptors in mono-and polysynaptic excitation in the cat spinal cord. *Exp Brain Res*, **49**: 280-290.

Davies, S.N. and Lodge, D. (1987) Evidence for involvement of N-methylaspartate receptors in "wind-up" of class 2 neurons in the dorsal horn of the rat. *Brain Res*, **424**: 402-406.

Davis, K.D., Meyer, R.A., Treede, R.-D., Cohen, R.H. and Campbell, J.N. (1990) Chemosensitivty and sensitisation of mechanically insensitive afferents in the primate. *Soc Neurosci Abstr*, **16**, 415

De Biasi, S. and Rustioni, A. (1988) Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord. *Proceedings of the National Academy of Sciences of the United States of America*, **85**: 7820-7824.

De Koninck, Y., Ribeiro-da-Silva, A., Henry, J.L. and Cuello, A.C. (1992) Spinal neurons exhibiting a specific nociceptive response receive abundant substance P-containing synaptic contacts. *Proc Natl Acad Sci USA*, **89**: 5073-5077.

De Koninck, Y. and Henry, J.L. (1991) Substance P-mediated slow excitatory postsynaptic potential elicited in dorsal horn neurons *in vivo* by noxious stimulation. *Proc Natl Acad Sci USA*, **88**: 11344-11348.

DeLeo, J.A., Colburn, R.W., Nichols, M. and Malhotra, A. (1996) Interleukin-6-mediated hyperalgesia/allodynia and increased spinal il-6 expression in a rat mononeuropathy model. *Journal Of Interferon And Cytokine Research*, **16**: 695-700.

Dickenson, A.H. and Aydar, E. (1991) Antagonism at the glycine site on the NMDA receptor reduces spinal nociception in the rat. *Neuroscience Letters*, **121**: 263-266.

Dickenson, A.H. and Sullivan, A.F. (1990) Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurones in the rat. *Brain Research*, **506**: 31-39.

Ding, M., Hart, R.P. and Jonakait, G.M. (1995) Tumor necrosis factor-alpha induces substance p in sympathetic ganglia through sequential induction of interleukin-1 and leukemia inhibitory factor. *Journal of Neurobiology*, **28**: 445-454.

Dirig, D.M. and Yaksh, T.L. (1996) Thermal hyperalgesia in rat evoked by intrathecal substance P at multiple stimulus intensities reflects an increase in the gain of nociceptive processing. *Neuroscience Letters*, **220**: 93-96.

Dougherty, P. and Willis, W. (1991) Modification of the response of private STT neurones to mechanical stimulation by excitatory acids and an NMDA antagonist. *Brain Res*, **542(1)**: 15-22.

Dougherty, P.M., Palecek, J., Paleckova, V., Sorkin, L.S. and Willis, W.D. (1992) The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, thermal and electrical stimuli. *J Neurosci*, **12**: 3025-3041.

Dougherty, P.M., Palecek, J., Zorn, S. and Willis, W.D. (1993a) Combined application of excitatory amino acids and substance P produces long-lasting changes in responses of primate spinothalamic tract neurons. *Brain Research - Brain Research Reviews*, **18**: 227-246.

Dougherty, P.M., Palecek, J., Zorn, S. and Willis, W.D. (1993b) Combined application of excitatory amino acids and substance P produces long-lasting changes in responses of primate spinothalamic tract neurons. *Brain Res Rev*, **18**: 227-246.

Dougherty, P.M., Palecek, J., Palecková, V. and Willis, W.D. (1994) Neurokinin 1 and 2 antagonists attenuate the responses and NK1 antagonists prevent the sensitization of primate spinothalamic tract neurons after intradermal capsaicin. *J Neurophysiol*, **72**: 1464-1475.

Dougherty, P.M., Palecek, J., Paleckova, V. and Willis, W.D. (1995) Infusion of substance P or neurokinin A by microdialysis alters responses of primate spinothalamic tract neurons to cutaneous stimuli and to iontophoretically released excitatory amino acids. *Pain*, **61**: 411-425.

Dougherty, P.M. and Willis, W.D. (1990) Long-lasting enhancement of excitability of spinothalamic neurons following combined application of excitatory amino acid and substance P in anaesthetized macaques. *J Physiol*, **425**: 38P

Dougherty, P.M. and Willis, W.D. (1991a) Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of N-methyl-D-aspartic acid and substance P. *Pain*, **47**: 85-93.

Dougherty, P.M. and Willis, W.D. (1991b) Modification of the responses of primate spinothalamic neurons to mechanical stimulation by excitatory amino acids and an N-methyl-D-aspartate antagonist. *Brain Res*, **542**: 15-22.

Dougherty, P.M. and Willis, W.D. (1992) Enhanced responses of spinothalamic tract neurons to excitatory amino acids accompany capsaicin-induced sensitization in the monkey. *J Neurosci*, **12**: 883-894.

Duce, I.R. and Keen, P. (1983) Selective uptake of [3H]glutamine and [3H]glutamate into neurons and satellite cells of dorsal root ganglia in vitro. *Neuroscience*, **8**: 861-866.

Duggan, A.W., Griersmith, B.T. and Johnson, S.M. (1981) Supraspinal inhibition of the excitation of dorsal horn neurones by impulses in unmyelinated primary afferents: lack of effect of strychnine and bicuculline. *Brain Res*, **210**: 231-241.

Duggan, A.W., Hendry, I.A., Morton, C.R., Hutchison, W.D. and Zhao, Z.Q. (1988) Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Research*, **451**: 261-273.

Duggan, A.W., Hope, P.J., Jarrott, B., Schiable, H.-D. and Fleetwood-Walker, S.M. (1990) Release,spread and persistence of immunoreactive Neurokinin A in the dorsal horn of the cat following noxious cutaneous stimulation. Studies with antibody microprobes. *Neuroscience*, **35**: 195-202.

Duggan, A.W., Schaible, H.-G., Hope, P.J. and Lang, C.W. (1992) Effect of peptidase inhibition on the pattern of intraspinally released immunoreactive substance P detected with antibody microprobes. *Brain Res*, **579**: 261-269.

Eccleston, C. (1995) Chronic pain and distraction: an experimental investigation into the role of sustained and shifting attention in the processing of chronic persistent pain. *Behaviour Research and Therapy*, **33**: 391-405.

Egebjerg, J., Bettler, B., Hermans-Borgmeyer, I. and Heinemann, S. (1991) Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA, *Nature*, **351**: 745-748.

Elde, R., Schalling, M., Ceccatelli, S., Nakanishi, S. and Hokfelt, T. (1990) Localization of neuropeptide receptor mRNA in rat brain: initial observations using probes for neurotensin and substance P receptors. *Neurosci Lett*, **120**: 134-138.

Fairman, W.A., Vandenberg, R.A., Arriza, J.L., Kavanaugh, M.P. and Amara, S.G. (1995) An excitatory amino acid transporter with properties of a ligand gated chloride channel. *Nature*, **375**: 599-603.

Fann, M.J. and Patterson, P.H. (1994) Neuropoietic cytokines and activin A differentially regulate the phenotype of cultured sympathetic neurons. *Proceedings of the National Academy of Sciences of the United States of America*, **91**: 43-47.

Felsby, S., Nielsen, J., Arendt-Nielsen, L. and Jensen, T.S. (1996) NMDA receptor blockade in chronic neuropathic pain: a comparison of ketamine and magnesium chloride. *Pain*, **64**: 283-291.

Fields, H.L., Clawton, C.H. and Anderson, S.D. (1977) Somatosensory properties of spinoreticular neurons in the cat. *Brain Res*, **120**: 49-66.

Fisher, K. and Coderre, T.J. (1996) The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. *Pain*, **68**: 255-263.

Fitzgerald, M. (1981) A study of the cutaneous afferent input to substantia gelatinosa. *Neuroscience*, **6**: 2229-2238.

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., El-Yassir, N. and Molony, V.M. (1987) The roles of tachykinin and opioid receptor types in nociceptive and non-nociceptive processing in superficial dorsal horn. *Fine Afferent Nerve Fibres and Pain* (ed. by R.F.

Schmidt, H.G. Schaible and C. Vahle-Hinz), p.239. VCH Verlagsgesellschaft mbH, Weinheim.

Fleetwood-Walker, S.M., Hope, P.J., Mitchell, R. and El Yassir, N. (1988) Effects of agonists selective for NK1, NK2 and NK3 receptors on somatosensory transmission in the superficial dorsal horn. *Regul Peptides*, **22**: 67

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., El-Yassir, N., Molony, V. and Bladon, C.M. (1990a) The involvement of neurokinin receptor subtypes in somatosensory processing in the superficial dorsal horn of the cat [published erratum appears in Brain Res 1992 May 8;579(2):357]. *Brain Research*, **519**: 169-182.

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., El-Yassir, N., Molony, V.M. and Bladon, C.M. (1990b) The involvement of neurokinin receptor subtypes in somatosensory processing in the superficial dorsal horn of the cat. *Brain Res*, **519**: 169-182.

Fleetwood-Walker, S.M., Parker, R.M., Munro, F.E., Young, M.R., Hope, P.J. and Mitchell, R. (1993) Evidence for a role of tachykinin NK2 receptors in mediating brief nociceptive inputs to rat dorsal horn (laminae III-V) neurons. *European Journal of Pharmacology*, **242**: 173-181.

Fletcher, E.J. and Lodge, D. (1988) Glycine reverses antagonism of NMDA by 1-hydroxy-3-aminopyrrolidine-2-one (HA-966), but not by D-2-Amino-5-phophonovalerate (D-AP5) on rat cortical slices. *Eur J Pharmacol*, **151**: 161-162.

Foong, F.W. and Duggan, A.W. (1986) Brainstem areas tonically inhibiting dorsal horn neurons: studies with microinjection of the GABA analogue piperidine-4-sulphonic acid. *Pain*, **27**: 361-372.

Foreman, R.D., Applebaum, A.E., Beall, J.E., Trevino, D.L. and Willis, W.D. (1975) Responses of primate spinothalamic tract neurons to electrical stimulation of hindlimb peripheral nerves. *J Neurophysiol*, **38**: 132-145.

Fotuhi, M., Sharp, A.H., Glatt, C.E., Hwang, P.M., von Krosigk, M., Snyder, S.H. and Dawson, T.M. (1993) Differential localization of phosphoinositide-linked metabotropic glutamate receptor (mGluR1) and the inositol 1,4,5-trisphosphate receptor in rat brain. *Journal of Neuroscience*, **13**: 2001-2012.

Frank, D.A. and Greenberg, M.E. (1996) Signal transduction pathways activated by ciliary neurotrophic factor and related cytokines. *Perspectives On Developmental Neurobiology*, **4**: 3-18.

Fujita, M., Sato, K., Sato, M., Inoue, T., Kozuka, T. and Tohyama, M. (1991) Regional distribution of the cells expressing glycine receptor beta subunit mRNA in the rat brain. *Brain Research*, **560**: 23-37.

Furuyama, T., Kiyama, H., Sato, K., Park, H.T., Maeno, H., Takagi, H. and Tohyama, M. (1993) Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. *Brain Res Mol Brain Res*, **18**: 141-151.

Furuyama, T., Sato, M., Sato, K., Araki, T., Inagaki, S., Takagi, H. and Tohyama, M. (1994) Co-expression of glycine receptor beta subunit and GABAA receptor gamma subunit mRNA in the rat dorsal root ganglion cells. *Brain Research*, **Molecular**: 335-338.

Garces, Y.I., Rabito, S.F., Minshall, R.D. and Sagen, J. (1993) Lack of potent antinociceptive activity by substance P antagonist CP-96,345 in the rat spinal cord. *Life Sciences*, **52**: 353-360.

Garret, C., Carruette, A., Fardin, V., Moussaoui, S., Peyronel, J.-F., Blanchard, J.-C. and Laduron, P.M. (1991) Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc Natl Acad Sci USA*, **88**: 10208-10212.

Gearing, D.P., Gough, N.M., King, J.A., Hilton, D.J., Nicola, N.A., Simpson, R.J., Nice, E.C., Kelso, A. and Metcalf, D. (1987) Molecular cloning and expression of cdna encoding a murine myeloid leukaemia inhibitory factor (lif). *EMBO Journal*, **6**: 3995-4002.

Gearing, D.P. (1993) The leukemia inhibitory factor and its receptor. *Advances in Immunology*, **53**: 31-58.

Gerber, G. and Randic, M. (1989) Excitatory amino acid-mediated components of synaptically evoked input from dorsal roots to deep dorsal horn neurons in the rat spinal cord slice. *Neurosci Letts*, **106**: 211-219.

Gibson, S.J., Polak, J.M., Bloom, S.R. and Wall, P.D. (1981) The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (lamina X). *J Comp Neurol*, **201**: 65-80.

Giesler, G.J., Yezierski, R.P., Gerhart, K.D. and Willis, W.D. (1981) Spinothalamic tract neurons that project to medial and/or lateral thalamic nuclei: evidence for a physiologically novel population of spinal cord neurons. *J Neurophysiol*, **46**: 1285

Giesler, G.J., Nahin, R.L. and Madsen, A.M. (1984) Postsynaptic dorsal column pathway of the rat. I. Anatomical studies. *J Neurophysiol*, **51(2)**: 260-275.

Giesler, G.J. and Cliffer, K.D. (1985) Postsynaptic dorsal column pathway of the rat II. Evidence against an important role in nociception. *Brain Res*, **362(2)**: 347-356.

Go, V.L. and Yaksh, T.L. (1987) Release of substance P from the cat spinal cord. *Journal of Physiology*, **391**: 141-167.

Gobel, S. (1975) Golgi studies of the substantia gelatinosa neurones in the spinal trigeminal nucleus. *J Comp Neurol*, **162**: 367-416.

Gobel, S. (1978) Golgi studies of the neurones on laminae II of the dorsal horn of the medulla (trigeminal nucleus caudalis). *J Comp Neurol*, **180**: 395-414.

Gobel, S., Falls, W.M. and Humphrey, E. (1981) Morphology and synaptic connections of ultrafine primary axons in laminae I of the spinal dorsal horn: candidate for the terminal axon arbors of primary neurones with unmyelinated (c) axons. *J Neurosci*, **1**: 1162-1179.

Goettl, V.M., Xu, X.J., Larson, A.A. and Wiesenfeld-Hallin, Z. (1994) Substance P N-terminus inhibits C-fiber-dependent facilitation of the rat flexor reflex. *European Journal of Pharmacology*, **259**: 83-86.

Goldscheider, A. (1987) The specific energy of the sensory nerves of the skin. *Vth World Congress on Pain* (ed. by K. Brune), p.47.

Gomeza, J., Zafra, F., Olivares, L., Gimenez, C. and Aragon, C. (1995) Regulation by phorbol esters of the glycine transporter (GLYT1) in glioblastoma cells. *Biochimica et Biophysica Acta*, **1233**: 41-46.

Grafstein, B. and McQuarrie, I.G. (1978) Role of the nerve cell body in axonal regeneration. *Neuronal Plasticity* (ed. by C.W. Cotman), p.155. Raven Press, New York.

Greenamyre, J.T., Young, A.B. and Penney, J.B. (1984) Quantitative autoradiographic distribution of L-[3H]glutamate-binding sites in rat central nervous system. *Journal of Neuroscience*, **4**: 2133-2144.

Greengard, P., Jen, J., Nairn, A.C. and Stevens, C.F. (1991) Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. *Science*, **253**: 1135-1138.

Gybels, J.M. and Sweet, W.H. (1989) Neurosurgical treatment of persistent pain. Physiological and pathological mechanisms of human pain. *Pain and Headache*, **11**: 1-402.

Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordon, C.C. and Ward, P. (1991) A spirolactam conformationally-constrained analogue of physalaemin which is a peptidase-resistant, selective neurokinin NK-1 receptor antagonist. *Br J Pharmacol*, **102**: 168P

Haley, J.E., Sullivan, A.F. and Dickenson, A.H. (1990) Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. *Brain Research*, **518**: 218-226.

Handwerker, H.O., Iggo, A. and Zimmermann, M. (1975a) Segmental and supraspinal actions on dorsal horn neurones responding to noxious and non-noxious skin stimuli. *Pain*, **1**: 147-165.

Handwerker, H.O., Iggo, A. and Zimmermann, M. (1975b) Input characteristics and rostral projections of dorsal horn neurons in monkey. *J Physiol*, **244**: 76-77P.

Handwerker, H.O. and Reeh, P.W. (1991) Pain and inflammation. *Pain Research and Clinical Management.* (ed. by M.R. Bond, J.E. Charlton and C.J. Woolf), p.59. Elsevier, Amsterdam.

Hardy, J.D., Wolff, H.G. and Goodell, H. (1950) Experimental evidence on the nature of cutaneous hyperalgesia. *J Clin Invest*, **29**: 115-140.

Harlan, R.E., Garcia, M.M. and Krause, J. (1989) Cellular localization of substance P and neurokinin A-encoding preprotachykinin mRNA in the female rat brain. *J Comp Neurol*, **287**: 179-212.

Harris, J.A., Corsi, M., Quartaroli, M., Arban, R. and Bentivoglio, M. (1996) Upregulation of spinal glutamate receptors in chronic pain. *Neuroscience*, **74**: 7-12.

Harris, N.C. and Ryall, R.W. (1988) Mustard oil excites but does not inhibit nociceptive dorsal horn neurons in the rat: a presumed effect of A delta fibres. *Brit J Pharm*, **94**: 180-184.

Hashimoto, A., Nishokawa, T., Hayishi, T., Fujii, N., Harada, K., Oka, T. and Takahashi, K. (1992) The presence of free D-serine in rat brain. *FEBS Lett*, **296**: 33-36.

Hayashi, H., Suga, M., Satake, M. and Tsubaki, T. (1981) Reduced glycine receptor in the spinal cord in amyotrophic lateral sclerosis. *Annals of Neurology*, **9**: 292-294.

Hayes, A.G. and Tyers, M.B. (1979) Effects of intrathecal and intercerebroventricular injections of substance P on nociception in rat and mouse. *Brit J Pharm*, **66**: 488P

Headley, P.M. and Grillner, S. (1990) Excitatory amino acids and synaptic transmission: the evidence for a physiological function. *The Pharmacology of Excitatory Amino Acids* (ed. by D. Lodge and G. Collingridge), p.30. Elsevier, Cambridge.

Hebb, D.O. (1949) *The organisation of behaviour.* Wiley,

Helke, C.J., Charlton, C.G. and Wiley, R.G. (1986) Studies on the cellular localisation of spinal cord substance P receptors. *Neuroscience*, **19**: 523-533.

Henley, J.M., Jenkins, R. and Hunt, S.P. (1993) Localisation of glutamate receptor binding sites and mRNAs to the dorsal horn of the rat spinal cord. *Neuropharmac*, **32**: 37-41.

Henry, J.L. (1976) Effects of substance P on functionally identified units in the cat spinal cord. *Brain Res*, **114**: 439-451.

Herb, A., Burnashev, N., Werner, P., Sakmann, B., Wisden, W. and Seeburg, P.H. (1992) The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron*, **8**: 775-785.

Herbert, J.N., Augereau, J.M., Gleye, J. and Maffrand, J.P. (1990) Chelyrythrine is a potent and specific inhibitor of protein kinase C. *Biochem Biophys Res Commun*, **192**: 993-999.

Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. and Kishimoto, T. (1990) Molecular cloning and expression of an il-6 signal transducer, gp130. *Cell*, **63**: 1149-1157.

Hilton, D.J., Nicola, N.A., Gough, N.M. and Metcalf, D. (1988) Resolution and purification of three distinct factors produced by krebs ascites cells which have differentiation-inducing activity on murine myeloid leukemic cell lines. *Journal of Biological Chemistry*, **263**: 9238-9243.

Hoffman, P.N. (1989) Expression of gap-43, a rapidly transported growth-associated protein, and class ii beta tubulin, a slowly transported cytoskeletal protein, are coordinated in regenerating neurons. *Journal of Neuroscience*, **9**: 893-897.

Hokfelt, T., Kellerth, J., Nilsson, G. and Pernow, B. (1975) Experimental immunohistochemical studies on the localisation and distribution of substance P in the cat primary sensory neurons. *Brain Res*, **100**: 235-252.

Hokfelt, T., Ljungdahl, A., Terenius, L., Elder, R. and Nillson, G. (1977) Immunohistochemical analysis of peptide pathways possible related to pain and analgesia: enkephalin and substance P. *PNAS*, **74**: 3081-3085.

Hokfelt, T., Johansson, O., Ljundahl, A., Lundberg, J.M. and Schultzberg, M. (1980) Peptidergic neurons. *Nature*, **287**: 515-521.

Hokfelt, T., Zhang, X. and Wiesenfeld-Hallin, Z. (1994) Messenger plasticity in primary sensory neurons following axotomy and its functional implications. *Trends in Neurosciences*, **17**: 22-30.

Holland, L.N. and Goldstein, B.D. (1994) Examination of tonic nociceptive behavior using a method of substance P-receptor desensitization in the dorsal horn. *Pain*, **56**: 339-346.

Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W. and Heinemann, S. (1989) Clonning by functional expression of a member of the glutamate receptor family. *Nature*, **342**: 643-648.

Hollmann, M. and Heinemann, S. (1994) Cloned glutamate receptors. *Ann Rev Neurosci*, **17**: 31-108.

Hongo, T., Jankowska, E. and Lundberg, A. (1968) Postsynaptic excitation and inhibtion from primary afferents in neurones of the spinocervical tract. *J Physiol (Lond)*, 569-592.

Hope, P.J., Grubb, B.D. and Duggan, A.W. (1990a) Persistence of immunoreactive neurokinins in the dorsal horn of barbiturate anaesthetised and spinal cats, following release by tibial nerve stimulation. *Neurosci Lett*, **118**: 25-28.

Hope, P.J., Jarrott, B., Schaible, H.G., Clarke, R.W. and Duggan, A.W. (1990b) Release and spread of immunoreactive neurokinin A in the cat spinal cord in a model of acute arthritis. *Brain Research*, **533**: 292-299.

Hopkin, J.M. and Neal, M.J. (1970) The release of [14C]glycine from electrically stimulated rat spinal cord slices. *Brit J Pharm*, **40**: 136-138.

Hornfeldt, C.S., Sun, X. and Larson, A.A. (1994) The NH2-terminus of substance P modulates NMDA-induced activity in the mouse spinal cord. *Journal of Neuroscience*, **14**: 3364-3369.

Houamed, K., Kuijper, J.L., Gilbert, T.L., Haldeman, B.A., O'Hara, P.J., Mulvihil, E.R., Imors, W.A. and Hagen, F.S. (1991) Cloning, expression and gene structure of a G protein coupled glutamate receptor from the rat brain. *Science*, **252**: 1318-1321.

Hua, X.-Y., Chen, P., Polgar, E., Nagy, I., Marsala, M., Phillips, E., Wollaston, L., Urban, L., Yaksh, T.L. and Webb, M. (1998) Spinal neurokinin NK1 receptor down-regulation and antinociception: effects of spinal NK1 receptor antisense oligonucleotides and NK1 receptor occupancy. *Journal of Neurochemistry*, **70**: 688-698.

Hume, R.I., Dingledine, R. and Heinemann, S.F. (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science*, **253**: 1028-1031.

Hyatt-Sachs, H., Schreiber, R.C., Bennett, T.A. and Zigmond, R.E. (1993) Phenotypic plasticity in adult sympathetic ganglia in vivo: effects of deafferentation and axotomy on the expression of vasoactive intestinal peptide. *Journal of Neuroscience*, **13**: 1642-1653.

Hylden, J.L.K. and Wilcox, G.L. (1981) Intrathecal substance P elicits a caudally directed biting and scratching behaviour. *Brain Res*, **217**: 212-215.

Iggo, A. (1963) New specific sensory structures in hairy skin. *Acta Neuroveg*, **24**: 175-180.

Iggo, A. and Ogawa, H. (1971) Primate cutaneous thermal nociceptors. *J Physiol*, **216**: 77-78P.

Ilkjaer, S., Petersen, K.L., Brennum, J., Wernberg, M. and Dahl, J.B. (1996) Effect of systemic N-methyl-D-aspartate receptor antagonist (ketamine) on primary and secondary hyperalgesia in humans. *British Journal of Anaesthesia*, **76**: 829-834.

Islas, J.A., Astorga, J. and Laredo, M. (1985) Epidural ketamine for control of postoperative pain. *Anaesth Analg*, **64**: 1161-1162.

Jahr, C.E. and Jessell, T.M. (1985) Synaptic transmission between dorsal root ganglion and dorsal horn neurons in culture: antagonism of monosynaptic excitatory postsynaptic potentials and glutamate excitation by kynurenate. *J Neurosci*, **5**: 2281-2289.

Jansen, K.L., Faull, R.L., Dragunow, M. and Waldvogel, H. (1990) Autoradiographic localisation of NMDA, quisqualate and kainic acid receptors in human spinal cord. *Neuroscience Letters*, **108**: 53-57.

Jessell, T.M., Tsunoo A., Kanazawa, I. and Otsuka, M. (1979) Substance P: depletion in the dorsal horn of the rat spinal cord after section of the peripheral processes of primary sensory neurons. *Brain Res*, **168**: 247-259.

Jia, Y.P. and Seybold, V.S. (1997) Spinal NK2 receptors contribute to the increased excitability of the nociceptive flexor reflex during persistent peripheral inflammation. *Brain Research*, **751**: 169-174.

Johnson, J.W. and Ascher, P. (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature*, **325**: 529-531.

Jones, M.W. and Headley, P.M. (1995) Interactions between metabotropic and ionotropic glutamate receptor agonists in the rat spinal cord in vivo. *Neuropharmacology*, **34**: 1025-1031.

Kangrga, I., Larew, J.S. and Randic, M. (1990) The effects of substance P and calcitonin gene-related peptide on the efflux of endogenous glutamate and aspartate from the rat spinal dorsal horn in vitro. *Neuroscience Letters*, **108**: 155-160.

Kangrga, I. and Randic, M. (1990a) Tachykinins and calcitonin gene-related peptide enhance release of endogenous glutamate and aspartate from the rat spinal dorsal horn slice. *Journal of Neuroscience*, **10**: 2026-2038.

Kangrga, I. and Randic, M. (1990b) Tachykinins and calcitonin gene-related peptide enhance release of endogenous glutamate and aspartate from the rat spinal dorsal horn slice. *Journal of Neuroscience*, **10**: 2026-2038.

Kar, S., Rees, R.G. and Quirion, R. (1994) Altered calcitonin gene-related peptide, substance P and enkephalin immunoreactivities and receptor binding sites in the dorsal spinal cord of the polyarthritic rat. *Eur J Neurosci*, **6**: 345-354.

Kehlet, H. (1995) Synergism between analgesics. *Ann Med*, **27**: 259-262.

Kellstein, D.E., Price, D.D., Hayes, R.L. and Mayer, D.J. (1990) Evidence that substance P selectively modulates C-fibre evoked discharges of dorsal horn nociceptive neurons. *Brain Res*, **526**: 291-298.

Kemp, J.A. and Priestley, T. (1991) Effects of (+)-HA-966 and 7-chlorokynurenic acid on the kinetics of N-methyl-D-aspartate receptor agonist responses in rat cultured cortical neurons. *Mol Pharmacol*, **39**: 666-670.

Kenshalo, D.R., Leonard, R.B., Chung, J.M. and Willis, W.D. (1979) Responses of primate spinothalamic neurons to graded and to repeated noxious heat stimuli. *J Neurophysiol*, **42**: 1370

Kenshalo, D.R., Jr., Leonard, R.B., Chung, J.M. and Willis, W.D. (1982) Facilitation of the responses of primate spinothalamic cells to cold and to tactile stimuli by noxious heating of the skin. *Pain*, **12**: 141-152.

Kerekes, N., Landry, M., RydhRinder, M. and Hokfelt, T. (1997) The effect of ngf, bdnf and bfgf on expression of galanin in cultured rat dorsal root ganglia. *Brain Research*, **754**: 131-141.

Kevetter, G.A. and Willis, W.D. (1983) Collaterals of spinothalamic cells in the rat. *J Comp Neurol*, **215**: 453-464.

King, A.E., Thompson, S.W.N., Urban, L. and Woolf, C.J. (1988) An intracellular analysis of amino acid induced excitations of deep dorsal horn neurones in the rat spinal cord slice. *Neurosci Lett*, **89**: 286-292.

Kirchhoff, F., Mulhardt, C., Pastor, A., Becker, C.M. and Kettenmann, H. (1996) Expression of glycine receptor subunits in glial cells of the rat spinal cord. *Journal of Neurochemistry*, **66**: 1383-1390.

Kirsch, J., Malosio, M.L., Wolters, I. and Betz, H. (1993a) Distribution of gephyrin transcripts in the adult and developing rat brain. *European Journal of Neuroscience*, **5**: 1109-1117.

Kirsch, J., Wolters, I., Triller, A. and Betz, H. (1993b) Gephyrin antisense oligonucleotides prevent glycine receptor clustering in spinal neurons. *Nature*, **366**: 745-748.

Kirsch, J. and Betz, H. (1995) The postsynaptic localization of the glycine receptor-associated protein gephyrin is regulated by the cytoskeleton. *Journal of Neuroscience*, **15**: 4148-4156.

Kiryu, S., Yao, G.L., Morita, N., Kato, H. and Kiyama, H. (1995) Nerve injury enhances rat neuronal glutamate transporter expression: identification by differential display PCR. *Journal of Neuroscience*, **15**: 7872-7878.

Kitto, K.F., Haley, J.E. and Wilcox, G.L. (1992) Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. *Neurosci Lett*, **148**: 1-5.

Kiyama, H., Maeno, H. and Tohyama, M. (1993) Substance P receptor (NK-1) in the central nervous system: Possible functions from a morphological aspect. *Regul Pept*, **46**: 114-123.

Kleckner, N.W. and Dingledine, R. (1988) Requirement for glycine in acitivation of NMDA-receptors expressed in Xenopus oocytes. *Science*, **241**: 835-837.

Kleinschmidt, A., Bear, M.F. and Singer, W. (1987) Blockade of NMDA receptors disrupts experience-dependent plasticity of kitten cortex. *Science*, **238**: 355-358.

Kolaj, M., Cerne, R., Cheng, G., Brickey, D.A. and Randic, M. (1994) Alpha subunit of calcium/calmodulin-dependent protein kinase enhances excitatory amino acid and synaptic responses of rat spinal dorsal horn neurons. *J Neurophysiol*, **72**: 2525-2531.

Kolhekar, R., Meller, S.T. and Gebhart, G.F. (1993) Characterization of the role of spinal *N*-methyl-D-aspartate receptors in thermal nociception in the rat. *Neuroscience*, **57**: 385-395.

Kolhekar, R., Meller, S.T. and Gebhart, G.F. (1994) *N*-methyl-D-aspartate receptor-mediated changes in thermal nociception: Allosteric modulation at glycine and polyamine recognition sites. *Neuroscience*, **63**: 925-936.

Koo, E.H., Hoffman, P.N. and Price, D.L. (1988) Levels of neurotransmitter and cytoskeletal protein mrnas during nerve regeneration in sympathetic ganglia. *Brain Research*, **449**: 361-363.

Kotani, H., Hoshimaru, M., Nawa, H. and Nakanishi, S. (1986) Structure and gene organisation of bovine neuromedin K precursor. *PNAS*, **83**: 7074-7078.

Krause, J.E., Chirgwin, Carter, W.B., Xu and Hershey (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. *Proc Nat Acad Sci (USA)*, **84**: 881-885.

Kreeger, J.S., Kitto, K.F. and Larson, A.A. (1994) Substance P N-terminal metabolites and nitric oxide mediate capsaicin-induced antinociception in the adult mouse. *Journal of Pharmacology and Experimental Therapeutics*, **271**: 1281-1285.

Krogsgaard-Larsen, P., Honore, T., Hansen, J.J., Curtis, D.R. and Lodge, D. (1980) New class of glutamate agonist structurally related to ibotenic acid. *Nature*, **284**: 64

Kuhse, J., Laube, B., Magalei, D. and Betz, H. (1993) Assembly of the inhibitory glycine receptor: identification of amino acid sequence motifs governing subunit stoichiometry. *Neuron*, **11**: 1049-1056.

Kumazawa, T. and Perl, E.R. (1976) Differential excitation of dorsal horn marginal and substantia gelatinosa neurons by primary afferent units with fine (A-delta and C) fibres. *Sensory Functions of the Skin with Special Reference to Man* (ed. by Y. Zotterman), p.67. Pergamon, Oxford.

Kumazawa, T. and Perl, E.R. (1977) Primary cutaneous sensory units with unmyelinated (C) afferent fibres. *J Neurophysiol*, **68**: 581-595.

Kumazawa, T. and Perl, E.R. (1978) Excitation of marginal and substantia gelatinosa neurones on the primate spinal cord: indications of their place in dorsal horn functional organisation. *J Comp Neurol*, **177**: 417-434.

Kuraishi, Y., Hirota, N., Sato, Y., Hanashima, N., Takagi, H. and Satoh, M. (1989) Stimulus specificity of peripherally evoked substance P release from the rabbit dorsal horn in situ. *Neuroscience*, **30**: 241-250.

Kurek, J.B., Austin, L., Cheema, S.S., Bartlett, P.F. and Murphy, M. (1996) Up-regulation of leukaemia inhibitory factor and interleukin-6 in transected sciatic nerve and muscle following denervation. *Neuromuscular Disorders*, **6**: 105-114.

Kutsuwada, T., Kashiwabuchi, N. and Mori, H. (1992) Molecular diversity of the NMDA receptor channel. *Nature*, **358**: 36-41.

Laird, J.M., Mason, G.S., Webb, J., Hill, R.G. and Hargreaves, R.J. (1996) Effects of a partial agonist and a full antagonist acting at the glycine site of the NMDA receptor on inflammation-induced mechanical hyperalgesia in rats. *British Journal of Pharmacology*, **117**: 1487-1492.

Laird, J.M.A., Hargreaves, R.J. and Hill, R.G. (1993) Effect of RP 67580, a non-peptide neurokinin$_1$ receptor antagonist, on facilitation of a nociceptive spinal flexion reflex in the rat. *Br J Pharmacol*, **109**: 713-718.

Laird, J.M.A. and Cervero, F. (1989) A comparative study of the changes in receptive-field properties of multireceptive and nocireceptive rat dorsal horn neurones following noxious mechanical stimulation. *J Neurophysiol*, **62(4)**: 854-863.

LaMotte, C.C. (1977) Distribution of the tract of Lissauer and the dorsal root fibres in the primate spinal cord. *J Comp Neurol*, **172**: 529-562.

LaMotte, R.H., Thalhammer, J.G., Torebjork, H.E. and Robinson, C.J. (1982) Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat. *Journal of Neuroscience*, **2**: 765-781.

Langosch, D., Laube, B., Rundstrom, N., Schmieden, V., Bormann, J. and Betz, H. (1994) Decreased agonist affinity and chloride conductance of mutant glycine receptors associated with human hereditary hyperekplexia. *EMBO Journal*, **13**: 4223-4228.

Larson, A.A. and Beitz, A.J. (1988) Glycine potentiates strychnine-induced convulsions: role of NMDA receptors. *Journal of Neuroscience*, **8**: 3822-3826.

Le Bars, D., Dickenson, A.H. and Besson, J.M. (1979a) Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain*, **6**: 283-304.

Le Bars, D., Dickenson, A.H. and Besson, J.M. (1979b) Diffuse noxious inhibitory controls (DNIC). I. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. *Pain*, **6**: 305-327.

Lewin, G.R., Ritter, A.M. and Mendell, L.M. (1993) Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. *Journal of Neuroscience*, **13**: 2136-2148.

Lewin, G.R. and Barde, Y.A. (1996) Physiology of the neurotrophins. *Annual review of neuroscience*, **19**: 289-317.

Lewis, T. (1942) *Pain*, Macmillan, New York.

Li, H., Ohishi, H., Kinoshita, A., Shigemoto, R., Nomura, S. and Mizuno, N. (1997) Localization of a metabotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive, primary afferent fibers in the superficial layers of the spinal dorsal horn: an electron microscope study in the rat. *Neurosci Lett*, **223**: 153-156.

Light, A.R., Trevino, D.L. and Perl, E.R. (1979) Morphological features of functionally defined neuones in the marginal zone and substantia gelatinosa of the spinal dorsal horn. *J Comp Neurol*, **186**: 151-172.

Light, A.R. and Perl, E.R. (1979) Spinal temination of functionally identified primary afferent neurones with slowly conducting myelinated fibres. *J Comp Neurol*, **186**: 117-132.

Lin, Q., Peng, Y. and Willis, W.D. (1994) Glycine and GABAA antagonists reduce the inhibition of primate spinothalamic tract neurons produced by stimulation in periaqueductal gray. *Brain Research*, **654**: 286-302.

Lin, Q., Bo Peng, Y. and Willis, W.D. (1997) Inhibition of Primate Spinothalamic Tract Neurons by Spinal Glycine and GABA Is Reduced During Central Sensitization. *Journal of Neurophysiology*, **76**: 1005-1014.

Lippe, I.T., Stabentheiner, A. and Holzer, P. (1993) Participation of nitric oxide in the mustard oil-induced neurogenic inflammation of the rat paw skin. *Eur J Pharmacol*, **232**: 113-120.

Littlewood, N.K., Todd, A.J., Spike, R.C., Watt, C. and Shehab, S.A. (1995) The types of neuron in spinal dorsal horn which possess neurokinin-1 receptors. *Neuroscience*, **66**: 597-608.

Liu, H., Brown, J.L., Jasmin, L., Maggio, J.E., Vigna, S.R., Mantyh, P.W. and Basbaum, A.I. (1994a) Synaptic relationship between substance P and the substance P receptor: Light and electron microscopic characterization of the mismatch between neuropeptides and their receptors. *Proc Natl Acad Sci USA*, **91**: 1009-1013.

Liu, H., Brown, J.L., Jasmin, L., Maggio, J.E., Vigna, S.R., Mantyh, P.W. and Basbaum, A.I. (1994b) Synaptic relationship between substance P and the substance P receptor: light and electron microscopic characterization of the mismatch between neuropeptides and their receptors. *Proceedings of the National Academy of Sciences of the United States of America*, **91**: 1009-1013.

Liu, H., Wang, H., Sheng, M., Jan, L.Y., Jan, Y.N. and Basbaum, A.I. (1994c) Evidence for presynaptic N-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn. *Proceedings of the National Academy of Sciences of the United States of America*, **91**: 8383-8387.

Liu, H., Mantyh, P.W. and Basbaum, A.I. (1997) NMDA-receptor regulation of substance P release from primary afferent nociceptors. *Nature*, **386**: 721-724.

Liu, Q.-R., López-Corcuera, B., Mandiyan, S., Nelson, H. and Nelson, N. (1993) Cloning and expression of a spinal cord- and brain-specific glycine transporter with novel structural features. *J Biol Chem*, **268**: 22802-22808.

Liu, X.-G. and Sandkühler, J. (1997) Characterization of long-term potentiation of C-Fiber-Evoked potentials in spinal dorsal horn of adult rat: essential role of NK1 and NK2 receptors. *J Neurophysiol*, **78**: 1973-1982.

Livesey, F.J., O'Brien, J.A., Li, M., Smith, A.G., Murphy, L.J. and Hunt, S.P. (1997) A Schwann cell mitogen accompanying regeneration of motor neurons. *Nature*, **390**: 614-618.

Ludlam, W.H., Chandross, K.J. and Kessler, J.A. (1995) LIF-and IL-1β-mediated increases in substance P receptor mRNA in axotomized, explanted or dissociated sympathetic ganglia. *Brain Res*, **685**: 12-20.

Luque, J.M., Bleuel, Z., Malherbe, P. and Richards, J.G. (1994) Alternatively spliced isoforms of the *N*-methyl-D-aspartate receptor subunit 1 are differentially distributed within the rat spinal cord. *Neuroscience*, **63**: 629-635.

Luque, J.M., Nelson, N. and Richards, J.G. (1995) Cellular expression of glycine transporter 2 messenger RNA exclusively in rat hindbrain and spinal cord. *Neuroscience*, **64**: 525-535.

Lutfy, K. and Weber, E. (1996) Attenuation of nociceptive responses by ACEA-1021, a competitive NMDA receptor/glycine site antagonist, in the mice. *Brain Research*, **743**: 17-23.

Ma, Q.-P. and Woolf, C.J. (1997) The progressive tactile hyperalgesia induced by peripheral inflammation is nerve growth factor dependent. *Neuroreport*, **8**: 807-810.

Ma, Q.P. and Woolf, C.J. (1995) Involvement of neurokinin receptors in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurones. *Journal of Physiology*, **486**: 769-777.

Ma, Q.P. and Woolf, C.J. (1996) Progressive tactile hypersensitivity: an inflammation-induced incremental increase in the excitability of the spinal cord [see comments]. *Pain*, **67**: 97-106.

MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J. and Barker, J.L. (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones [published erratum appears in Nature 1986 Jun 26-Jul 2;321(6073):888]. *Nature*, **321**: 519-522.

Maehara, T., Suzuki, H., Yoshioka, K. and Otsuka, M. (1993) Substance P-evoked release of amino acid transmitters from the newborn rat spinal cord. *Regul Pept*, **46**: 354-356.

Maehara, T., Suzuki, H., Yoshioka, K. and Otsuka, M. (1995) Characteristics of substance P-evoked release of amino acids from neonatal rat spinal cord. *Neuroscience*, **68**: 577-584.

Magnusson, K.R., Larson, A.A., Madl, J.E., Altschuler, R.A. and Beitz, A.J. (1986) Co-localization of fixative-modified glutamate and glutaminase in neurons of the spinal trigeminal nucleus of the rat: an immunohistochemical and immunoradiochemical analysis. *Journal of Comparative Neurology*, **247**: 477-490.

Malmberg, A.B., Chen, C., Tonegawa, S. and Basbaum, A.I. (1997) Preserved acute pain and reduced neuropathic pain in mice lacking PKC-gamma. *Science*, **278**: 279-282.

Malmberg, A.B. and Yaksh, T.L. (1992) Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. *Science*, **257**: 1276-1279.

Malosio, M.L., Marqueze-Pouey, B., Kuhse, J. and Betz, H. (1991) Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. *EMBO Journal*, **10**: 2401-2409.

Mantyh, P.W., Allen, C.J., Ghilardi, J.R., Rogers, S.D., Mantyh, C.R., Liu, H., Basbaum, A.I., Vigna, S.R. and Maggio, J.E. (1995a) Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum in vivo.

Proceedings of the National Academy of Sciences of the United States of America, **92**: 2622-2626.

Mantyh, P.W., DeMaster, E., Malhotra, A., Ghilardi, J.R., Rogers, S.D., Mantyh, C.R., Liu, H., Basbaum, A.I., Vigna, S.R., Maggio, J.E. and et al. (1995b) Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. *Science*, **268**: 1629-1632.

Mantyh, P.W., Rogers, S.D., Honore, P., Allen, B.J., Ghilardi, J.R., Li, J., Daughters, R.S., Lappi, D.A., Wiley, R.G. and Simone, D.A. (1997) Inhibition of hyperalgesia by ablation of lamina i spinal neurons expressing the substance p receptor [see comments]. *Science*, **278**: 275-279.

Mao, J., Price, D.D., Hayes, R.L., Lu, J. and Mayer, D.J. (1992a) Differential roles of NMDA and non-NMDA receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy. *Brain Research*, **598**: 271-278.

Mao, J., Price, D.D., Hayes, R.L., Lu, J. and Mayer, D.J. (1992b) Differential roles of NMDA and non-NMDA receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy. *Brain Res*, **598**: 271-278.

Mao, J., Mayer, D.J., Hayes, R.L. and Price, D.D. (1993) Spatial patterns of increased spinal cord membrane-bound protein kinase C and their relation to increases in ^{14}C- 2-deoxyglucose metabolic activity in rats with painful peripheral mononeuropathy. *J Neurophysiol*, **70**: 470-481.

Marchand, J.E., Wurm, W.H., Kato, T. and Kream, R.M. (1994) Altered tachykinin expression by dorsal root ganglion neurons in a rat model of neuropathic pain. *Pain*, **58**: 219-231.

Marshall, G.E., Shehab, S.A., Spike, R.C. and Todd, A.J. (1996) Neurokinin1 receptors on lumbar spinothalamic neurons in the rat. *Neuroscience*, **72**: 255-263.

Martin, L.J., Blackstone, C.D., Levey, A.I., Huganir, R.L. and Price, D.L. (1993) AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience*, **53**: 327-358.

Marvizon, J.C., Martinez, V., Grady, E.F., Bunnett, N.W. and Mayer, E.A. (1997) Neurokinin 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by nmda receptors. *Journal of Neuroscience*, **17**: 8129-8136.

Masu, M., Tanabe, Y., Tsuchida, K., Shigimoto, R. and Nakanishi, S. (1991) Sequence and expression of a metabotropic glutamate receptor. *Nature*, **394**: 760-765.

Masu, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M. and Nakanishi, S. (1987) cDNA cloning of bovine substance-K receptor through oocyte expression system. *Nature*, **329**: 836-838.

Masuyama, T., Shimizu, T., Iwashita, T., Yoshimura, N. and Fukuda, T. (1996) Spinal antinociceptive effect of substance P on the responses induced by intrathecally injected NMDA in mice. *Brain Research*, **722**: 200-202.

Matsuto, T., Yanagisowa, M., Otsuka, M., Kanazawa, I. and Munekate, E. (1984) The excitatory action of the newly discovered mammalian tachykinins neurokininA and neurokininB on neurons in the isolated spinal cord of the newborn rat. *Neurosci Res*, **2**, 105-110.

Maxwell, D.J., Christie, W.M., Short, A.D., Storm-Mathisen, J. and Ottersen, O.P. (1990) Central boutons of glomeruli in the spinal cord of the cat are enriched with L-glutamate-like immunoreactivity. *Neuroscience*, **36**: 83-104.

Mayer, M.L., Vyklicky, L. and Clements, J. (1989) Regulation of NMDA receptor desensitsation in mouse hippocampal neurons by glycine. *Nature*, **338**: 425-427.

Mayer, M.L. and Westbrook, G.L. (1987) The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog Neurobiol*, **28**: 197-276.

McCarson, K.E. and Goldstein, B.D. (1991) Release of substance P into the superficial dorsal horn following nociceptive activation of the hindpaw of the rat. *Brain Research*, **568**: 109-115.

McCarson, K.E. and Krause, J.E. (1994) NK-1 and NK-3 type tachykinin receptor mRNA expression in the rat spinal cord dorsal horn is increased during adjuvant or formalin-induced nociception. *Journal of Neuroscience*, **14**: 712-720.

McCarson, K.E. and Krause, J.E. (1995) The formalin-induced expression of tachykinin peptide and neurokinin receptor messenger RNAs in rat sensory ganglia and spinal cord is modulated by opiate preadministration. *Neuroscience*, **64**: 729-739.

McGrath, P.A. (1994) Psychological aspects of pain perception. *Archives of Oral Biology*, **39**: Suppl:55S-62S.

McLachlan, E.M., Jang, W., Devor, M. and Michaelis, M. (1993) Peripheral nerve injury triggers noradrenergic sprouting within dorsal root ganglia. *Nature*, **363**: 543-546.

Meldrum, B.S. (1992) Excitatory amino acid receptors and disease. . *Current Opinion in Neurology and Neurosurgery*, **5**: 508-513.

Meller, S.T., Dykstra, C.L. and Gebhart, G.F. (1993) Acute mechanical hyperalgesia is produced by coactivation of AMPA and metabotropic glutamate receptors. *Neuroreport*, **4**: 879-882.

Melzack, R. and Wall, P.D. (1965) Pain mechanisms: a new theory. *Science*, **150**: 971-979.

Mendell, L.M. (1966) Physiological properties of unmyelinated fiber projection to the spinal cord. *Exp Neurol*, **16**: 316-332.

Menetrey, D., Giesler, G.J. and Besson, J.M. (1977) An analysis of response properties of spinal cord dorsal horn neurones to non-noxious and noxious stimuli in the spinal rat. *Exp Brain Res*, **27**: 15-33.

Menetrey, D., Chaouch, A. and Besson, J.M. (1980) Location and properties of dorsal horn neurons at origin of spinoreticular tract in lumbar enlargement of the rat. *J Neurophysiol*, **44**: 862-877.

Menetrey, D., Chaouch, A., Binder, M.D. and Besson, J.M. (1982) The origin of the spino mesencephalic tract in the rat: an anatomical study using the retrograde transport of horse radial peroxidose. *J Comp Neurol*, **206**: 193-207.

Mercadante, S., Lodi, F., Sapio, M., Calligara, M. and Serretta, R. (1995) Long-term ketamine subcutaneous continuous infusion in neuropathic cancer pain. *Journal of Pain and Symptom Management*, **10**: 564-568.

Metcalf, D., Hilton, D.J. and Nicola, N.A. (1988) Clonal analysis of the actions of the murine leukemia inhibitory factor on leukemic and normal murine hemopoietic cells. *Leukemia*, **2**: 216-221.

Meyer, R.A., Campbell, J.N. and Raja, S.N. (1994) Peripheral neural mechanisms of nociception. *Textbook of Pain* (ed. by P.D. Wall and R. Melzack), p.13. Chrchill Livingstone, Edinburgh.

Millan, M.J. and Seguin, L. (1994) Chemically-diverse ligands at the glycine B site coupled to *N*-methyl-D-aspartate (NMDA) receptors selectively block the late phase of formalin-induced pain in mice. *Neuroscience Letters*, **178**: 139-143.

Milton, I.D., Banner, S.J., Ince, P.G., Piggott, N.H., Fray, A.E., Thatcher, N., Horne, W.C.H. and Shaw, P.J. (1997) Expression of the glial glutamate transporter EAAT2 in the human CNS: an immunohistochemical study. *Molecular Brain Research*, **52**: 17-31.

Minami, M., Kuraishi, Y., Kawamura, M., Yamaguchi, T., Masu, Y., Nakanishi, S. and Satoh, M. (1989) Enhancement of preprotachykinin A gene expression by adjuvent-induced inflammation in the rat spinal cord: possible involvement of substance P- containing spinal neurons in nociception. *Neurosci Lett*, **98**: 105-110.

Minota, S., Mizayaki, T., Wang, M.Y., Read, H.L. and Din, H.J. (1989) Glycine potentiates NMDA responses in rat hippocampal CA1 neurons. *Neurosci Lett*, **178**: 139-143.

Mitchell, J.J. and Anderson, K.J. (1991) Quantitative autoradiographic analysis of excitatory amino acid receptors in the cat spinal cord. *Neuroscience Letters*, **124**: 269-272.

Mitchell, K., Spike, R.C. and Todd, A.J. (1993) An immunocytochemical study of glycine receptor and GABA in laminae I-III of rat spinal dorsal horn. *Journal of Neuroscience*, **13**: 2371-2381.

Mjellem-Joly, N., Lund, A., Berge, O.G. and Hole, K. (1991) Potentiation of a behavioural response in mice by spinal coadministration of substance P and excitatory amino acid agonists. *Neuroscience Letters*, **133**: 121-124.

Mjellem-Joly, N., Lund, A., Berge, O.G. and Hole, K. (1992) Intrathecal co-administration of substance P and NMDA augments nociceptive responses in the formalin test. *Pain*, **51**: 195-198.

Molander, C., Xu, H. and Grant, G. (1984) The cytoarchitectonic organisation of the spinal cord in the rat. I.The lower thoracic and lumbrosacral cord. *J Comp Neurol*, **230**: 133-141.

Monahan, J.B., Biesterfeldt, J.P., Hood, W.F., Compton, R.P., Cordi, A.A., Vazquez, M.I., Lanthorn, T.H. and Wood, P.L. (1990) Differential modulation of the associated glycine recognition site by competitive N-methyl-D-aspartate receptor antagonists. *Mol Pharmacol*, **37**: 780-784.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B. and Seeburg, P.H. (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science*, **256**: 1217-1221.

Morin, F. (1955) A new spinal pathway for cutaneous impulses. *Amer J Physiol*, **183**: 245-252.

Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N. and Nakanishi, S. (1991) Molecular cloning and characterization of the rat NMDA receptor [see comments]. *Nature*, **354**: 31-37.

Moroni, F., Alesiani, M., Galli, A., Mori, F., Pecorari, R., Carla, V., Cherici, G. and Pellicciari, R. (1991) Thiokynurenates: a new group of antagonists of the glycine modulatory site of the NMDA receptor. *Eur J Pharmacol*, **199**: 227-232.

Morris, R. (1989) Responses of spinal dorsal horn neurones evoked by myelinated primary afferent stimulation are blocked by excitatory amino acid antagonists acting at kainate/quisqualate receptors. *Neurosci Letts*, **105**: 79-85.

Moussaoui, S.M., Le Prado, N., Bonici, B., Faucher, D.C., Cuine, F., Laduron, P.M. and Garret, C. (1992) Distribution of neurokinin B in rat spinal cord and peripheral tissues:

comparison with neurokinin A and substance P and effects of neonatal capsaicin treatment. *Neuroscience*, **48**: 969-978.

Muller, J. (1842) *Elements of Physiology*, London:Taylor and Walton,.

Munglani, R., Harrison, S.M., Smith, G.D., Bountra, C., Birch, P.J., Elliot, P.J. and Hunt, S.P. (1996) Neuropeptide changes persist in spinal cord despite resolving hyperalgesia in a rat model of mononeuropathy. *Brain Research*, **743**: 102-108.

Munro, F.E., Fleetwood-Walker, S.M., Parker, R.M. and Mitchell, R. (1993) The effects of neurokinin receptor antagonists on mustard oil-evoked activation of rat dorsal horn neurons. *Neuropeptides*, **25**: 299-305.

Munro, F.E., Fleetwood-Walker, S.M. and Mitchell, R. (1994) Evidence for a role of protein kinase C in the sustained activation of rat dorsal horn neurons evoked by cutaneous mustard oil application. *Neuroscience Letters*, **170**: 199-202.

Murase, K., Ryu, P.D. and Randic, M. (1989) Excitatory and inhibitory amino acids and peptide-induced responses in acutely isolated rat spinal dorsal horn neurons. *Neuroscience Letters*, **103**: 56-63.

Murase, K. and Randic, M.J. (1984) Actions of substance P on rat spinal dorsal horn neurones. *J Physiol*, **346**: 203-217.

Murphy, M., Reid, K., Hilton, D.J. and Bartlett, P.F. (1991) Generation of sensory neurons is stimulated by leukemia inhibitory factor. *Proceedings of the National Academy of Sciences of the United States of America*, **88**: 3498-3501.

Murphy, M., Reid, K., Brown, M.A. and Bartlett, P.F. (1993) Involvement of leukemia inhibitory factor and nerve growth factor in the development of dorsal root ganglion neurons. *Development*, **117**: 1173-1182.

Murray, C.W., Cowan, A. and Larson, A.A. (1991) Neurokinin and NMDA antagonists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin model. *Pain*, **44**: 179-185.

Nadler, V., Kloog, Y. and Sokolovsky, M. (1988) 1-Aminocyclopropane-1-carboxylic acid (ACPC) mimics the effects of glycine on th NMDA receptor ion channel. *Eur J Pharmacol*, **157**: 115-116.

Nafe, J.P. (1997) A quantitative theory of feeling. *J Gen Psychol*, **2**: 199-210.

Nagahisa, A., Asai, R., Kanai, Y., Murase, A., Tsuchiya-Nakagaki, M., Nakagaki, T., Shieh, T.-C. and Taniguchi, K. (1992) Non-specific activity of (±)-CP-96,345 in models of pain and inflammation. *Br J Pharmacol*, **107**: 273-275.

Nagata, Y., Horiike, K. and Maeda, T. (1994) The distribution of free D-serine in certebrate brains. *Brain Res*, **623**: 291-295.

Nagy, I., Maggi, C.A., Dray, A., Woolf, C.J. and Urban, L. (1993) The role of neurokinin and *N*-methyl-D-aspartate receptors in synaptic transmission from capsaicin-sensitive primary afferents in the rat spinal cord *in vitro*. *Neuroscience*, **52**: 1029-1037.

Nagy, I., Miller, B.A. and Woolf, C.J. (1994a) NK1 and NK2 receptors contribute to C-fibre evoked slow potentials in the spinal cord. *Neuroreport*, **5**: 2105-2108.

Nagy, I., Woolf, C.J., Dray, A. and Urban, L. (1994b) Cobalt accumulation in neurons expressing ionotropic excitatory amino acid receptors in young rat spinal cord: morphology and distribution. *Journal of Comparative Neurology*, **344**: 321-335.

Nagy, J.I. and Hunt, S.P. (1982) Fluoride resistant acid phosphatase - containing neurones in dorsal root ganglia are separate from those containing substance P or somatostatin. *Neuroscience*, **7**: 89-98.

Naim, M., Spike, R.C., Watt, C., Shehab, S.A. and Todd A.J. (1997) Cells in laminae III and IV of the rat spinal cord that possess the neurokinin1 receptor and have dorsally directed dendrites receive a major synaptic input from tachykinin-containing primary afferents. *J Neurosci*, **17**: 5536-5548.

Nakajima, Y., Tsuchida, K., Negishi, M., Ito, S. and Nakanishi, S. (1992) Direct linkage of three tachykinin receptors to stimulation both phosphatidylinositol hydrolysis and cyclic AMP cascades in transfected cinese hamster ovary cells. *J Biol Chem*, **267**: 2437-2442.

Nakanishi, S. (1992) Molecular diversity of glutamate receptors and implications for brain function. *Science*, **252**: 507-603.

Nakaya, Y., Kaneko, S., Shigemoto, R., Nakanishi, S. and Mizuno, N. (1994) Immunohistochemical localisation of substance P receptor in the central nervous system of the adult rat. *J Comp Neurol*, **347**: 249-274.

Nasstrom, J., Karlsson, U. and Post, C. (1992) Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. *European Journal of Pharmacology*, **212**: 21-29.

Nathan, P.W., Smith, M.C. and Cook, A.W. (1986) Sensory effects in man of lesions of the posterior columns and of some other afferent pathways. *Brain*, **109(V)**: 1003-1041.

Nawa and et al. (1983) Nucleotide sequences of cloned cDNAs for two types of bovine SP precursor. *Nature*, **306**: 32-36.

Nawa and et al. (1984) Tissue-specific generation of two preprotackykinin mRNAs from one gene by alternative RNA slicing. *Nature*, **312**: 729-734.

Näsström, J., Karlsson, U. and Post, C. (1992) Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. *Eur J Pharmacol*, **212**: 21-29.

Neugebauer, V., Lucke, T. and Schaible, H.G. (1993a) N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists block the hyperexcitability of dorsal horn neurons during development of acute arthritis in rat's knee joint. *Journal of Neurophysiology*, **70**: 1365-1377.

Neugebauer, V., Lucke, T. and Schaible, H.G. (1993b) Differential effects of N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists on the responses of rat spinal neurons with joint input. *Neuroscience Letters*, **155**: 29-32.

Neugebauer, V., Lücke, T., Grubb, B. and Schaible, H.-G. (1994a) The involvement of *N*-methyl-D-aspartate (NMDA) and non- NMDA receptors in the responsiveness of rat spinal neurons with input from the chronically inflamed ankle. *Neurosci Lett*, **170**: 237-240.

Neugebauer, V., Lücke, T. and Schaible, H.-G. (1994b) Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons. *Eur J Neurosci*, **6**: 1179-1186.

Neugebauer, V., Rumenapp, P. and Schaible, H.G. (1996) The role of spinal neurokinin-2 receptors in the processing of nociceptive information from the joint and in the generation and maintenance of inflammation-evoked hyperexcitability of dorsal horn neurons in the rat. *European Journal of Neuroscience*, **8**: 249-260.

Neumann, S., Doubell, T.P., Leslie, T. and Woolf, C.J. (1996) Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. *Nature*, **384**: 360-364.

Nikolajsen, L., Hansen, C.L., Nielsen, J., Keller, J., Arendt-Nielsen, L. and Jensen, T.S. (1996) The effect of ketamine on phantom pain: a central neuropathic disorder maintained by peripheral input. *Pain*, **67**: 69-77.

Ninkovic, M., Beaujouan, J.-C., Torrens, Y., Saffroy, M., Hall, M.D. and Glowinski, J. (1984) Differential localisation of tachykinin receptors in rat spinal cord. *Eur J Pharmacol*, **106**: 463-464.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. and Prochiantz, A. (1984) Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*, **307**: 462-465.

O'Shea, R.D., Mallanack, D.T., Conway, E.L., Mercer, L.D. and Beart, P.M. (1991) Evidence for heterogenous glycine domains but conserved multiple state of the excitatory amino acid recognition site of the NMDA receptor. *Ex Br Res*, **86**: 652-662.

Ogawa, T., Karazawa, I. and Kimura, S. (1985) Regional distribution of Substance P, NKA and NKB in rat spinal cord, nerve roots and dorsal root ganglia, and the effects of dorsal root section or spinal transection. *Brain Res*, **359**: 152-157.

Okano, K., Kuraishi, Y. and Satoh, M. (1993) Pharmacological evidence for involvement of excitatory amino acids in aversive responses induced by intrathecal substance P in rats. *Biological and Pharmaceutical Bulletin*, **16**: 861-865.

Okano, K., Kuraishi, Y. and Satoh, M. (1994) Involvement of substance P and excitatory amino acids in aversive behavior elicited by intrathecal capsaicin. *Neuroscience Research*, **19**: 125-130.

Okano, K., Kuraishi, Y. and Satoh, M. (1995) Effects of intrathecally injected glutamate and substance P antagonists on repeated cold stress-induced hyperalgesia in rats. *Biological and Pharmaceutical Bulletin*, **18**: 42-44.

Olivéras, J.-L., Besson, J.M., Guilbaud, G. and Liebskind, J.C. (1974) Behavioural and electrophysiological evidence of pain inhibition from midbrain stimultion in the cat. *Exp Brain Res*, **20**: 32-44.

Onaka, M., Minami, T., Nishihara, I. and Ito, S. (1996) Involvement of glutamate receptors in strychnine- and bicuculline-induced allodynia in conscious mice. *Anesthesiology*, **84**: 1215-1222.

Otsuka, M., Yoshioka, K., Yanagisawa, M., Suzuki, H., Zhao, F.Y., Guo, J.Z., Hosoki, R. and Kurihara, T. (1995) Use of NK1 receptor antagonists in the exploration of physiological functions of substance P and neurokinin A. *Canadian Journal of Physiology and Pharmacology*, **73**: 903-907.

Palecek, J., Palecková, V., Dougherty, P.M. and Willis, W.D. (1994) The effect of phorbol esters on the responses of primate spinothalamic neurons to mechanical and thermal stimuli. *J Neurophysiol*, **71**: 529-537.

Paleckova, V., Palecek, J., McAdoo, D.J. and Willis, W.D. (1992) The non-NMDA antagonist CNQX prevents release of amino acids into the rat spinal cord dorsal horn evoked by sciatic nerve stimulation. *Neuroscience Letters*, **148**: 19-22.

Peet, M.J., Leah, J.D. and Curtis, D.R. (1983) Antagonists of synaptic and amino acid excitation of neurones in the cat spinal cord. *Brain Res*, **266**: 83-95.

Pellegrini-Giampietro, D.E., Fan, S., Ault, B., Miller, B.E. and Zukin, R.S. (1994) Glutamate receptor gene expression in spinal cord of arthritic rats. *Journal of Neuroscience*, **14**: 1576-1583.

Peng, Y.B., Lin, Q. and Willis, W.D. (1996) Effects of GABA and glycine receptor

antagonists on the activity and PAG-induced inhibition of rat dorsal horn neurons. *Brain Research*, **736**: 189-201.

Peng, Y.B., Lin, Q. and Willis, W.D. (1997) Involvement of protein kinase C in responses of rat dorsal horn neurons to mechaniscal stimuli and preiaqueductal gray descending inhibition. *Exp Brain Res*, **114**: 561-570.

Perper, J.A. (1985) Fatal strychnine poisoning--a case report and review of the literature. *Journal of Forensic Sciences*, **30**: 1248-1255.

Picard, P., Boucher, S., Regoli, D., Gitter, B.D., Howbert, J.J. and Couture, R. (1993a) Use of non-peptide tachykinin receptor antagonists to substantiate the involvement of NK1 and NK2 receptors in a spinal nociceptive reflex in the rat. *European Journal of Pharmacology*, **232**: 255-261.

Picard, P., Boucher, S., Regoli, D., Gitter, B.D., Howbert, J.J. and Couture, R. (1993b) Use of non-peptide tachykinin receptor antagonists to substantiate the involvement of NK_1 and NK_2 receptors in a spinal nociceptive reflex in the rat. *Eur J Pharmacol*, **232**: 255-261.

Piercey, M.F., Dobry, P.J.K., Schroeder L.A. and Einspahr, F.J. (1981) Behavioural evidence that substance P may be a spinal cord sensory neurotransmitters. *Brain Res*, **210**: 407-412.

Pin, J.-P. and Duvoisin, R. (1995) The metabotropic glutamate receptors: Structure and functions. *Neuropharmac*, **34**: 1-26.

Pook, P., Brugger, F., Hawkins, N.S., Clark, K.C., Watkins, J.C. and Evans, R.H. (1993) A comparison of the actions of agonists and antagonists at non-NMDA receptors of C fibres and motoneurones of the immature rat spinal cord in vitro. *Brit J Pharmac*, **108**: 179-184.

Popratiloff, A., Weinberg, R.J. and Rustioni, A. (1996) AMPA receptor subunits underlying terminals of fine-caliber primary afferent fibers. *Journal of Neuroscience*, **16**: 3363-3372.

Pribilla, I., Takagi, T., Langosch, D., Bormann, J. and Betz, H. (1992) The atypical M2 segment of the beta subunit confers picrotoxinin resistance to inhibitory glycine receptor channels [published erratum appears in EMBO J 1994 Mar 15;13(6):1493]. *EMBO Journal*, **11**: 4305-4311.

Priestley, T., Laughton, P., Myers, J., Le Bourdelles, B., Kerby, J. and Whiting, P.J. (1995) Pharmacological properties of recombinant human N-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Molecular Pharmacology*, **48**: 841-848.

Priestley, T. and Kemp, J.A. (1983) Agonist response kinetics of NMDA receptors in neurons cultured from rat cerebral cortex and cerebellum - evidence for receptor heterogeneity. *Mol Pharmacol*, **44**: 1252-1257.

Qiu, Z., Parsons, K.L. and Gruol, D.L. (1995) Interleukin-6 selectively enhances the intracellular calcium response to NMDA in developing CNS neurons. *Journal of Neuroscience*, **15**: 6688-6699.

Quirion, R., Shults, C.W., Moody, T.W., Pert, C.B., Chase, T.N. and O'Donohue, T.L. (1983) Autoradiographic distribution of substance P receptors in rat central nervous system. *Nature*, **303**: 714-716.

Radhakrishnan, V. and Henry, J.L. (1991) Novel substance P antagonist, CP-96,345, blocks responses of cat spinal dorsal horn neurons to noxious cutaneous stimulation and to substance P. *Neurosci Lett*, **132**: 39-43.

Radhakrishnan, V. and Henry, J.L. (1993a) Excitatory amino acid receptor mediation of sensory inputs to functionally identified dorsal horn neurons in cat spinal cord. *Neuroscience*, **55**: 531-544.

Radhakrishnan, V. and Henry, J.L. (1993b) L-NAME blocks responses to NMDA, substance P and noxious cutaneous stimuli in cat dorsal horn. *Neuroreport*, **4**: 323-326.

Raigorodsky, G. and Urca, G. (1987) Intrathecal N-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. *Brain Res*, **422**: 158-162.

Raigorodsky, G. and Urca, G. (1990) Spinal antinociceptive effects of excitatory amino acid antagonists: quisqualate modulates the action of N-methyl-D-aspartate. *European Journal of Pharmacology*, **182**: 37-47.

Rajan, P., Stewart, C.L. and Fink, J.S. (1995) LIF-mediated activation of STAT proteins after neuronal injury in vivo. *Neuroreport*, **6**: 2240-2244.

Ralston, H.J.I. (1968) The fine structure of neurones in the dorsal horn of the cat spinal cord. *J Comp Neurol*, **132**: 275-302.

Ralston, H.J.I. (1971) The synaptic organization in the dorsal horn of the spinal cord and in the ventrobasal thalamus in the cat. *Oral-Facial Sensory and Motor Mechanisms* (ed. by R. Dubner and Y. Kawamura), p.229. Appleton-Century-Crofts, New York.

Ralston, H.J.I. and Ralston, D.D. (1979) The distribution of dorsal root axons in laminae I,II and III of the macaque spinal cord: a quantative electron microscope study. *J Comp Neurol*, **184**: 643-684.

Ramon y Cajal, S. (1909) *Histologie du systeme nerveux de l'homme et des vertebres.* Madrid:Inst. Cajal, pp. 908-911.

Randic, M., Jeftinija, S., Urban, L., Raspantini, C. and Folkers, K. (1988) Effects of substance P analogues on spinal dorsal horn neurons. *Peptides*, **9**: 651-660.

Randic, M., Hecimovic, H. and Ryu, P.D. (1990) Substance P modulates glutamate-induced currents in acutely isolated rat spinal dorsal horn neurones. *Neuroscience Letters*, **117**: 74-80.

Randic, M. and Miletic, C.V. (1977) Effects of substance P in cat dorsal horn neurons activated by noxious stimuli. *Brain Res*, **128**: 164-169.

Rao, M.S., Sun, Y., Escary, J.L., Perreau, J., Tresser, S., Patterson, P.H., Zigmond, R.E., Brulet, P. and Landis, S.C. (1993) Leukemia inhibitory factor mediates an injury response but not a target-directed developmental transmitter switch in sympathetic neurons. *Neuron*, **11**: 1175-1185.

Rawal, N. (1995) Spinal antinociception: clinical aspects. *Annals of Medicine*, **27**: 263-268.

Reeh, P.W., Kocher, L. and Jung, S. (1986) Does neurogenic inflammation alter the sensitivity of unmyelinated nociceptors in the rat. *Brain Res*, **384**: 42-50.

Reeh, P.W., Bayer, J., Kocher, L. and Handwerker, H.O. (1987) Sensitization of nociceptive cutaneous nerve fibres from the rat's tail by noxious mechanical stimulation. *Exp Brain Res*, **65**: 505-512.

Regoli, D., D'Orleans-Juste, P., Dion, S., Rhaleb, N.E., Rouissis, N. and Drapeau, G. (1988) Neurokinin selective agonists. Discovery and pharmacological applications. *Regulatory Peptides*, **22**: 153

Ren, K., Hylden, J.L.K., Williams, G.M., Ruda, M.A. and Dubner, R. (1992a) The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. *Pain*, **50**: 331-344.

Ren, K., Williams, G.M., Hylden, J.L.K., Ruda, M.A. and Dubner, R. (1992b) The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan- induced behavioral hyperalgesia in rats. *Eur J Pharmacol*, **219**: 235-243.

Ren, K., Iadarola, M.J. and Dubner, R. (1996) An isobolographic analysis of the effects of N-methyl-D-aspartate and NK1 tachykinin receptor antagonists on inflammatory hyperalgesia in the rat. *British Journal of Pharmacology*, **117**: 196-202.

Ren, K. and Dubner, R. (1993) NMDA receptor antagonists attenuate mechanical hyperalgesia in rats with unilateral inflammation of the hindpaw. *Neurosci Lett*, **163**: 22-26.

Rethelyi, M. (1977) Preterminal and terminal axon arborization in the substantia gelatinosa of the cat's spinal cord. *J Comp Neurol*, **172**: 511-528.

Rethelyi, M., Light, A.R. and Perl, E.R. (1982) Synaptic complexes formed by functionaly defined primary afferent units with fine myelinated fibers. *J Comp Neurol*, **207**: 381-393.

Rethelyi, M., Light, A.R. and Perl, E.R. (1983) *Advances in Pain Research and Therapy*, pp. 111-118. Raven Press. New York.

Rexed, B. (1952) The cytoarchitectonic organization of the spinal cord of the cat. *J Comp Neurol*, **96**: 415-495.

Reynolds, D.V. (1969) Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science*, **164**: 444-445.

Reynolds, I.J. (1990) Arcaine is a competitive antagonists of the polyamine site of the NMDA receptor. *Eur J Pharmacol*, **177**: 215-216.

Robinson, R.C., Grey, L.M., Staunton, D., Stuart, D.I., Heath, J.K. and Jones, E.Y. (1994) The crystal structure and biological function of leukemia inhibitory factor: implications for receptor binding. *Cell*, **77**: 1101

Romano, C., Sesma, M.A., McDonald, C.T., O'Malley, K., Van den Pol, A.N. and Olney, J.W. (1995) Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *Journal of Comparative Neurology*, **355**: 455-469.

Rose, T.M. and Bruce, A.G. (1991) Oncostatin m is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6. *Proceedings of the National Academy of Sciences of the United States of America*, **88**: 8641-8645.

Rothstein, J.D., Martin, L., Levey, A.I., Dykes-Hoberg, M., Jin, L., Wu, D., Nash, N. and Kuncl, R.W. (1994) Localization of neuronal and glial glutamate transporters. *Neuron*, **13**: 713-725.

Rothstein, J.D., Van Kammen, M., Levey, A.I., Martin, L.J. and Kuncl, R.W. (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology*, **38**: 73-84.

Rothstein, J.D., Dykes-Hoberg, M., Pardo, C.A., Bristo, L.A., Jin, L., Kunkl, R.W., Kanai, Y., Hediger, M.A., Wang, Y., Shiekle, J.P. and Welty, D.F. (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron*, **16**: 675-686.

Rowley, M., Leeson, P.D., Stevenson, G.I., Moseley, A.M., Stansfield, I., Sanderson, I., Robinson, L., Baker, R., Kemp, J.A., Marshall, G.R., Foster, A.C., Grimwood, S., Tricklebank, M.D. and Saywell, K.L. (1993) 3-Acyl-4-hydroxyquinolin-2(1*H*)-ones. Systemically active anticonvulsants acting by antagonism at the glycine site of the *N*-methyl-D-aspartate receptor complex. *J Med Chem*, **36**: 3386-3396.

Rusin, K.I., Ryu, P.D. and Randic, M. (1992) Modulation of excitatory amino acid responses in rat dorsal horn neurons by tachykinins. *Journal of Neurophysiology*, **68**: 265-286.

Rusin, K.I., Bleakman, D., Chard, P.S., Randic, M. and Miller, R.J. (1993a) Tachykinins potentiate N-methyl-D-aspartate responses in acutely isolated neurons from the dorsal horn. *Journal of Neurochemistry*, **60**: 952-960.

Rusin, K.I., Bleakman, D., Chard, P.S., Randic, M. and Miller, R.J. (1993b) Tachykinins potentiate N-methyl-D-aspartate responses in acutely isolated neurons from the dorsal horn. *Journal of Neurochemistry*, **60**: 952-960.

Rusin, K.I., Jiang, M.C., Cerne, R. and Randic, M. (1993c) Interactions between excitatory amino acids and tachykinins in the rat spinal dorsal horn. *Brain Research Bulletin*, **30**: 329-338.

Ryall, R.W. and Pini, A.J. (1987) A depressant action of substance P on spinal nociceptive neurons in the rat and cat. *Substance P and Neurokinins.* (ed. by J.L. Henry, R. Couture, A.C. Cuello, G. Pelletier, R. Quiron and D. Regoli), p.239. Springer-Verlag,

Saitoh, T., Ishida, M., Maruyama, M. and Shinozaki, H. (1994) A novel antagonist, phenylbenzene omega-phosphono-α-amino acid, for strychnine-sensitive glycine receptors in the rat spinal cord. *British Journal of Pharmacology*, **113**: 165-170.

Sakurada, T., Katsumata, K., Manome, Y., Tan-No, K., Sakurada, S., Kisara, K. and Ohba, M. (1993a) Antinociceptive effects in the formalin and capsaicin tests after intrathecal administration of substance P analogues in mice. *Eur J Pharmacol*, **242**: 47-52.

Sakurada, T., Katsumata, K., Yogo, H., Tan-No, K., Sakurada, S. and Kisara, K. (1993b) Antinociception induced by CP 96,345, a non-peptide NK-1 receptor antagonist, in the mouse formalin and capsaicin tests. *Neurosci Lett*, **151**: 142-145.

Salituro, F.G., Harrison, B.L., Baron, B.M., Nyce, P.L., Stewart, K.T., Kehne, J.H., White, H.S. and McDonald, I.A. (1992) 3-(2-carboxyindol-3-yl) propionic acid based antagonists of the NMDA receptor associated glycine binding site. *J Med Chem*, **35**: 1791-1799.

Salt, T.E. (1989) Modulation of NMDA receptor mediated responses to glycine and D-serine in the rat thalamus in vivo. *Brain Research*, **481**: 403-406.

Salter, M.W. and Henry, J.L. (1991) Responses of functionally identified neurones in the dorsal horn of the cat spinal cord to substance P, neurokinin A and physalaemin. *Neuroscience*, **43**: 601-610.

Santucci, V., Gueudet, C., Edmonds-Alt, X., Breliere, J.C., Soubrie, P. and Le Fur, G. (1993) The NK2 receptor antagonist SR48968 inhibits thalamic responses evoked by thermal but not mechanical nociception. *European Journal of Pharmacology*, **237**: 143-146.

Sasai, Y. and Nakanishi, S. (1989) Molecular characterization of rat substance K receptor and its mRNAs. *Biochemical and Biophysical Research Communications*, **165**: 695-702.

Sato, K., Zhang, J.H., Saika, T., Sato, M., Tada, K. and Tohyama, M. (1991) Localization of glycine receptor alpha 1 subunit mRNA-containing neurons in the rat brain: an analysis using in situ hybridization histochemistry. *Neuroscience*, **43**: 381-395.

Sato, K., Kiyama, H., Park, H.T. and Tohyama, M. (1993) AMPA, KA and NMDA receptors are expressed in the rat DRG neurones. *Neuroreport*, **4**: 1263-1265.

Sato, K., Adams, R., Betz, H. and Schloss, P. (1995) Modulation of a recombinant glycine transporter (GLYT1b) by activation of protein kinase C. *Journal of Neurochemistry*, **65**: 1967-1973.

Satoh, O. and Omote, K. (1996) Roles of monoaminergic, glycinergic and GABAergic inhibitory systems in the spinal cord in rats with peripheral mononeuropathy. *Brain Research*, **728**: 27-36.

Scatton, B. (1993) The NMDA receptor complex. *Fundamental and Clinical Pharmacology*, **7**: 389-400.

Schaible, H.-G., Grubb, B., Neugebauer, V. and Oppmann, M. (1991) The effects of NMDA antagonists in cat spinal cord evoked by acute inflammation in the knee joint. *Eur J Neurosci*, **8**: 2062-2073.

Schaible, H.-G., Hope, P.J., Lang, C.W. and Duggan, A.W. (1992) Calcitonin gene-related peptide causes intraspinal spreading of substance P released by peripheral stimulation. *Eur J Neurosci*, **4**: 750-757.

Schaible, H.G., Jarrott, B., Hope, P.J. and Duggan, A.W. (1990) Release of immunoreactive substance P in the spinal cord during development of acute arthritis in the knee joint of the cat: a study with antibody microprobes. *Brain Research*, **529**: 214-223.

Schäfer, M.K.-H., Nohr, D., Krause, J.E. and Weihe, E. (1993) Inflammation-induced upregulation of NK1 receptor mRNA in dorsal horn neurones. *Neuroreport*, **4**: 1007-1010.

Schell, M.J., Molliver, M.E. and Snyder, S.H. (1995) D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci USA*, **92**: 3948-3952.

Schneider, S.P. and Perl, E.R. (1985a) Comparison of primary afferent and glutamate excitation of neurons in the mammalian spinal cord. *J Neurosci*, **8**: 8062-2073.

Schneider, S.P. and Perl, E.R. (1985b) Selective excitation of neurons in the mammalian spinal dorsal horn by aspartate and glutamate in vitro: correlation with location and excitatory input. *Brain Res*, **360**: 339-343.

Schouenborg, J. and Sjolund, B.H. (1986) First-order nociceptive synapses in rat dorsal horn ar blocked by an amino acid antagonist. *Brain Res*, **376**: 394-398.

Schreiber, R.C., Hyatt-Sachs, H., Bennett, T.A. and Zigmond, R.E. (1994) Galanin expression increases in adult rat sympathetic neurons after axotomy. *Neuroscience*, **60**: 17-27.

Scott, R.H., Sutton, K.G. and Dolphin, A.C. (1993) Interactions of polyamines with neuronal ion channels. *Trends in Neurosciences*, **16**: 153-160.

Segal, M. and Sandberg, D. (1977) Analgesia produced by electrical stimultion of catecholamine nuclie in the rat brain. *Brain Res*, **123**: 369-372.

Seguin, L., Le Marouille-Girardon, S. and Millan, M.J. (1995) Antinociceptive profiles of non-peptidergic neurokinin$_1$ and neurokinin$_2$ receptor antagonists: A comparison to other classes of antinociceptive agent. *Pain*, **61**: 325-343.

Seguin, L. and Millan, M.J. (1994) The glycine B receptor partial agonist, (+)-HA966, enhances induction of antinociception by RP 67580 and CP-99,994. *European Journal of Pharmacology*, **253**: R1-R3.

Seltzer, Z., Cohn, S., Ginzburg, R. and Beilin, B. (1991) Modulation of neuropathic pain behavior in rats by spinal disinhibition and NMDA receptor blockade of injury discharge. *Pain*, **45**: 69-75.

Shadiack, A.M., Hart, R.P., Carlson, C.D. and Jonakait, G.M. (1993) Interleukin-1 induces substance P in sympathetic ganglia through the induction of leukemia inhibitory factor (LIF). *Journal of Neuroscience*, **13**: 2601-2609.

Shaw, P.J., Ince, P.G., Johnson, M., Perry, E.K. and Candy, J. (1991) The quantitative autoradiographic distribution of [3H]MK-801 binding sites in the normal human spinal cord. *Brain Research*, **539**: 164-168.

Sheardown, M.J., Dreyer, J., Jensen, L.H., Stidsen, C.E. and Honore, T. (1989) A potent antagonist of the strychnine insensitive glycine receptor has anticonvulsant properties. *Eur J Pharmacol*, **174**: 197-204.

Sheardown, M.J., Neilson, E.O., Hansen, A.J., Jacobsen, P. and Honore, T. (1990) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline: a neuroprotectant for cerebral ischemia. *Science*, **247**: 571

Sher, G.D. and Mitchell, D. (1990) Intrathecal N-methyl-D-aspartate induces hyperexcitability in rat dorsal horn convergent neurones. *Neuroscience Letters*, **119**: 199-202.

Sherman, S.E. and Loomis, C.W. (1995) Strychnine-dependent allodynia in the urethane-anesthetized rat is segmentally distributed and prevented by intrathecal glycine and betaine. *Canadian Journal of Physiology and Pharmacology*, **73**: 1698-1705.

Sherman, S.E. and Loomis, C.W. (1996) Strychnine-sensitive modulation is selective for non-noxious somatosensory input in the spinal cord of the rat. *Pain*, **66**: 321-330.

Shigemoto, R., Yokota, Y., Tsuchida, K. and Nakanishi, S. (1990) Cloning and expression of a rat neuromedin K receptor cDNA. *Journal of Biological Chemistry*, **265**: 623-628.

Shigemoto, R., Nakanishi, S. and Mizuno, N. (1992) Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat. *Journal of Comparative Neurology*, **322**: 121-135.

Simone, D.A., Sorkin, L.S., Oh, U., Chung, J.M., Owens, C., LaMotte, R.H. and Willis, W.D. (1991) Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. *J Neurophysiol*, **66**: 228-246.

Simpson, R.K., Gondo, M., Robertson, C.S. and Goodman, J.C. (1996) Reduction in the mechanonociceptive response by intrathecal administration of glycine and related compounds. *Neurochemical Research*, **21**: 1221-1226.

Simpson, R.K., Gondo, M., Robertson, C.S. and Goodman, J.C. (1997) Reduction in thermal hyperalgesia by intrathecal administration of glycine and related compounds. *Neurochemical Research*, **22**: 75-79.

Sinclair, D. (1981) *Mechanisms of cutaneous sensation*, Oxford:Oxford Univ. Press.

Sinclair, D.C. (1955) Cutaneous sensation and the doctrine of specific energy. *Brain*, **78**: 584-614.

Sivilotti, L. and Woolf, C.J. (1994) The contribution of $GABA_A$ and glycine receptors to central sensitization: Disinhibition and touch-evoked allodynia in the spinal cord. *Journal of Neurophysiology*, **72**: 169-179.

Skilling, S.R., Smullin, D.H., Beitz, A.J. and Larson, A.A. (1988) Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. *J Neurochem*, **51**: 127-132.

Skilling, S.R., Smullin, D.H. and Larson, A.A. (1990) Differential effects of C- and N-terminal substance P metabolites on the release of amino acid neurotransmitters from the spinal cord: potential role in nociception. *Journal of Neuroscience*, **10**: 1309-1318.

Skilling, S.R., Harkness, D.H. and Larson, A.A. (1992) Experimental peripheral neuropathy decreases the dose of substance P required to increase excitatory amino acid release in the CSF of the rat spinal cord. *Neuroscience Letters*, **139**: 92-96.

Sladeczek, F., Pin, J.-P., Recasens, M., Bockaert, J. and Weiss, S. (1985) Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature*, **317**: 717-719.

Sluka, K.A., Milton, M.A., Willis, W.D. and Westlund, K.N. (1997a) Differential roles of neurokinin 1 and neurokinin 2 receptors in the development and maintenance of heat hyperalgesia induced by acute inflammation. *British Journal of Pharmacology*, **120**: 1263-1273.

Sluka, K.A., Rees, H., Chen, P.S., Tsuruoka, M. and Willis, W.D. (1997b) Inhibitors of g-proteins and protein kinases reduce the sensitization to mechanical stimulation and the desensitization to heat of spinothalamic tract neurons induced by intradermal injection of capsaicin in the primate. *Experimental Brain Research*, **115**: 15-24.

Sluka, K.A., Rees, H., Chen, P.S., Tsuruoka, M. and Willis, W.D. (1997c) Capsaicin-induced sensitization of primate spinothalamic tract cells is prevented by a protein kinase C inhibitor. *Brain Research*, **772**: 82-86.

Sluka, K.A. and Willis, W.D. (1997) The effects of g-protein and protein kinase inhibitors on the behavioral responses of rats to intradermal injection of capsaicin. *Pain*, **71**: 165-178.

Smith, F.L. and Dewey, W.L. (1992) Evidence that endogenous opioids mediate the antinociceptive effects of intrathecally administered calcium in mice. *J Pharmacol Exp Ther*, **262**: 995-1003.

Smith, G.D., Wiseman, J., Harrison, S.M., Elliott, P.J. and Birch, P.J. (1994) Pre treatment with MK-801, a non-competitive NMDA antagonist, prevents development of mechanical hyperalgesia in a rat model of chronic neuropathy, but not in a model of chronic inflammation. *Neuroscience Letters*, **165**: 79-83.

Smith, K.E., Borden, L.A., Hartig, P.R., Branchek, T. and Weinshank, R.L. (1992) Cloning and expression of a glycine transporter reveal colocalsization with NMDA reccptors. *Neuron*, **8**: 927-935.

Smullin, D.H., Skilling, S.R. and Larson, A.A. (1990a) Interactions between substance P, calcitonin gene-related peptide, taurine and excitatory amino acids in the spinal cord. *Pain*, **42**: 93-101.

Smullin, D.H., Skilling, S.R. and Larson, A.A. (1990b) Interactions between substance P, calcitonin gene-related peptide, taurine and excitatory amino acids in the spinal cord. *Pain*, **42**: 93-101.

Snyder S.H. (1997) D-serine: a neuromodulator at nmda receptors? *Neuroscientist*, **3**: 206

Sommer, C. and Myers, R.R. (1995) Neurotransmitters in the spinal cord dorsal horn in a model of painful neuropathy and in nerve crush. *Acta Neuropathologica*, **90**: 478-485.

Sorkin, L.S., Westlund, K.N., Sluka, K.A., Dougherty, P.M. and Willis, W.D. (1992) Neural changes in acute arthritis in monkeys. IV. Time course of amino acid release into the lumbar dorsal horn. *Brain Res*, **17**: 39-50.

Sorkin, L.S. and Puig, S. (1996) Neuronal model of tactile allodynia produced by spinal strychnine: effects of excitatory amino acid receptor antagonists and a mu-opiate receptor agonist. *Pain*, **68**: 283-292.

Sosnowski, M. and Yaksh, T.L. (1989) Role of spinal adenosine receptors in modulating the hyperesthesia produced by spinal glycine receptor antagonism. *Anesthesia and Analgesia*, **69**: 587-592.

Sosnowski, M. and Yaksh, T.L. (1990) Spinal administration of receptor-selective drugs as analgesics: new horizons. *J Pain Symptom Manage*, **5**: 204-213.

Southam, E., East, S.J. and Garthwaite, J. (1991) Excitatory amino acid receptors coupled to the nitic oxid/cyclic GMP pathway in rat cerebllum during development. *J Neurochem*, **56**: 2072-2081.

Spike, R.C. and Todd, A.J. (1992) Ultrastructural and immunocytochemical study of lamina II islet cells in rat spinal dorsal horn. *Journal of Comparative Neurology*, **323**: 359-369.

Stannard, C.F. and Porter, G.E. (1993) Ketamine hydrochloride in the treatment of phantom limb pain. *Pain*, **54**: 227-230.

Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S.J. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor [see comments]. *Nature*, **359**: 76-79.

Sucher, N.J., Akbarian, S., Chi, C.L., Leclerc, C.L., Awobuluyi, M., Deitcher, D.L., Wu, M.K., Yuan, J.P., Jones, E.G. and Lipton, S.A. (1995) Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. *Journal of Neuroscience*, **15**: 6509-6520.

Sucher, N.J., Awobuluyi, M., Choi, Y.B. and Lipton, S.A. (1996) NMDA receptors: from genes to channels. *Trends in Pharmacological Sciences*, **17**: 348-355.

Sugimoto, T., Bennett, G.J. and Kajander, K.C. (1989) Strychnine-enhanced transsynaptic degeneration of dorsal horn neurons in rats with an experimental painful peripheral neuropathy. *Neuroscience Letters*, **98**: 139-143.

Sugimoto, T., Bennett, G.J. and Kajander, K.C. (1990) Transsynaptic degeneration in the superficial dorsal horn after sciatic nerve injury: effects of a chronic constriction injury, transection, and strychnine. *Pain*, **42**: 205-213.

Sugiura, Y., Lee, C.L. and Perl, E.R. (1986) Central projections of identified, unmyelinated (C) afferent fibres innervating mammalian skin. *Science*, **234**: 358-361.

Sugiura, Y., Terui, N., Hosoya, Y. and Kohno, K. (1989) Distribution of unmyelinated primary afferent fibres in the dorsal horn. *Processing of Sensory Information in the Superficial Dorsal Horn of the Spinal Cord.* (ed. by F. Cervero, G.J. Bennett and P.M. Headley), p.15. Life Sci,

Sugiyama, H., Ito, I. and Hirono, C. (1987) A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature*, **325**: 531-533.

Sun, Y., Landis, S.C. and Zigmond, R.E. (1994a) Signals that induce expression of leukemia inhibitory factor (LIF) in rat superior cervical ganglion (SCG) and their nerves after axonal transection. *Soc Neurosci Abstr*, **20**, 1304(Abstract)

Sun, Y., Rao, M.S., Zigmond, R.E. and Landis, S.C. (1994b) Regulation of vasoactive intestinal peptide expression in sympathetic neurons in culture and after axotomy: the role of cholinergic differentiation factor/leukemia inhibitory factor. *Journal of Neurobiology*, **25**: 415-430.

Sun, Y., Landis, S.C. and Zigmond, R.E. (1996) Signals triggering the induction of leukemia inhibitory factor in sympathetic superior cervical ganglia and their nerve trunks after axonal injury. *Molecular and Cellular Neurosciences*, **7**: 152-163.

Sun, Y. and Zigmond, R.E. (1996) Leukaemia inhibitory factor induced in the sciatic nerve after axotomy is involved in the induction of galanin in sensory neurons. *European Journal of Neuroscience*, **8**: 2213-2220.

Symes, A., Lewis, S., Corpus, L., Rajan, P., Hyman, S.E. and Fink, J.S. (1994) STAT proteins participate in the regulation of the vasoactive intestinal peptide gene by the ciliary neurotrophic factor family of cytokines. *Molecular Endocrinology*, **8**: 1750-1763.

Tachibana, M., Wenthold, R.J., Morioka, H. and Petralia, R.S. (1994) Light and electron microscopic immunocytochemical localization of AMPA-selective glutamate receptors in the rat spinal cord. *J Comp Neurol*, **344**: 431-454.

Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. and Kishimoto, T. (1989) Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell*, **58**: 573-581.

Taga, T. (1996) Gp130, a shared signal transducing receptor component for hematopoietic and neuropoietic cytokines. *Journal of Neurochemistry*, **67**: 1-10.

Taga, T. and Kishimoto, T. (1997) Gp130 and the interleukin-6 family of cytokines. *Annual Review of Immunology*, **15**: 797-819.

Takahashi, M., Billups, B., Rossi, D., Sarantis, M., Hamann, M. and Attwell, D. (1997) The role of glutamate transporters in glutamate homeostasis in the brain. *J Exp Biol*, **200**: 401-409.

Takeda, Y. and Krause, J.E. (1991) Pharmacological and molecular biological studies on the diversity of rat tachykinin NK2 receptor subtypes in rat central nervous, duodenum, vas deferens and urinary bladder. *Annu New York Acad Sci*, **632**: 479-482.

Tanabe, Y., Masu, M., Ishii, T., Shigemoto, R. and Nakanishi, S. (1992) A family of glutamate receptors. *Neuron*, **8**: 169-179.

Tang, C., Dichter, M. and Morad, M. (1990) Modulation of the N-methyl-D-aspartate channel by extracellular H+. *PNAS*, **87**: 6445-6449.

Tasker, R.R. and Dostrovsky, J.O. (1989) Deafferentation and central pain. *Textbook of Pain* (ed. by P.D. Wall and R. Melzack), p.154. Churchill Livingston, London.

Thompson, S.W., Urban, L. and Dray, A. (1993a) Contribution of NK1 and NK2 receptor activation to high threshold afferent fibre evoked ventral root responses in the rat spinal cord in vitro. *Brain Research*, **625**: 100-108.

Thompson, S.W., Urban, L. and Dray, A. (1993b) Contribution of NK1 and NK2 receptor activation to high threshold afferent fibre evoked ventral root responses in the rat spinal cord in vitro. *Brain Res*, **625**: 100-108.

Thompson, S.W., Dray, A. and Urban, L. (1994) Injury-induced plasticity of spinal reflex activity: NK1 neurokinin receptor activation and enhanced A- and C-fiber mediated responses in the rat spinal cord in vitro. *Journal of Neuroscience*, **14**: 3672-3687.

Thompson, S.W., Dray, A. and Urban, L. (1996) Leukemia inhibitory factor induces mechanical allodynia but not thermal hyperalgesia in the juvenile rat. *Neuroscience*, **71**: 1091-1094.

Thompson, S.W.N., Gerber, G., Sivilotti, L.G. and Woolf, C.J. (1990) Activity dependent changes in rat ventral horn neurons in vitro: summation of prolonged evoked postsynaptic depolarisations produce D-APV sensitive windup. *Eur J Neurosci*, **2**: 638-649.

Thompson, S.W.N., Dray, A. and Urban, L. (1994) Injury-induced plasticity of spinal reflex activity: NK1 neurokinin receptor activation and enhanced A- and C-fiber mediated responses in the rat spinal cord *in vitro*. *J Neurosci*, **14**: 3672-3687.

Thompson, S.W.N., Dray, A. and Urban, L. (1996) Leukemia inhibitory factor induces mechanical allodynia but not thermal hyperalgesia in the juvenile rat. *Neuroscience*, **71**: 1091-1094.

Thompson, S.W.N., Vernallis, A.B., Heath, J.K. and Priestley, J.V. (1997) Leukaemia inhibitory factor is retrogradely transported by a distinct population of adult rat sensory neurons: co-localization with trka and other neurochemical markers. *European Journal of Neuroscience*, **9**: 1244-1251.

Thompson, S.W.N. and Majithia, A. (1998) LIF and NGF induce noradrenergic sprouting in the intact Dorsal Root Ganglion of Adult Rats in vivo. *Journal of Physiology*, (In Press)

Thomson, A.M., Walker, V.E. and Flynn, D.M. (1989) Glycine enhances NMDA receptor-mediated synaptic potential in neocortical slices. *Nature*, **338**: 422-424.

Todd, A.J. (1990) An electron microscope study of glycine-like immunoreactivity in laminae I-III of the spinal dorsal horn of the rat. *Neuroscience*, **39**: 387-394.

Todd, A.J., Maxwell, D.J. and Brown, A.G. (1991) Relationships between hair-follicle afferent axons and glycine-immunoreactive profiles in cat spinal dorsal horn. *Brain Research*, **564**: 132-137.

Todd, A.J., Spike, R.C., Brodbelt, A.R., Price, R.F. and Shehab, S.A. (1994) Some inhibitory neurons in the spinal cord develop c-fos-immunoreactivity after noxious stimulation. *Neuroscience*, **63**: 805-816.

Todd, A.J., Spike, R.C., Chong, D. and Neilson, M. (1995) The relationship between glycine and gephyrin in synapses of the rat spinal cord. *Eur J Neurosci*, **7**: 1-11.

Todd, A.J. (1996) GABA and glycine in synaptic glomeruli of the rat spinal dorsal horn. *European Journal of Neuroscience*, **8**: 2492-2498.

Todd, A.J., Watt, C., Spike, R.C. and Sieghart, W. (1996) Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord. *Journal of Neuroscience*, **16**: 974-982.

Tolle, T.R., Berthele, A., Zieglgansberger, W., Seeburg, P.H. and Wisden, W. (1993) The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. *Journal of Neuroscience*, **13**: 5009-5028.

Torebjörk, H.E., Lundberg, L.E.R. and LaMotte, R.H. (1992) Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. *J Physiol (Lond)*, **448**: 765-780.

Torebjörk, H.E. and Ochoa, J.L. (1980) Specific sensations evoked by activity in singly identified sensory units in man. *Acta Physiol Scand*, **110**: 445-447.

Toullec, R., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boissin, P., Boursier, E., Loriolle, F., Duhamel, L., Charon, G. and Kirilovsky, J. (1990) The bisindoylmaleimide GF109203X is a potent and selective inhibitor of protein kinase C. *J Biol Chem*, **266**: 15771-15781.

Traub, R.J. (1996) The spinal contribution of substance P to the generation and maintenance of inflammatory hyperalgesia in the rat. *Pain*, **67**: 151-161.

Treede, R.-D., Meyer, R.A., Raja, S.N. and Campbell, J.N. (1992) Peripheral and central mechanisms of cutaneous hyperalgesia. *Prog Neurobiol*, **38**: 397-421.

Treede, R.-D. and Magerl, W. (1995) Modern concepts of Pain and Hyperalgesia: beyond the polymodal C-nociceptor. *New Insights in Physiological Science*, **10**: 216-228.

Tsuchida, K., Shigemoto, R., Yokota, Y. and Nakanishi, S. (1990) Tissue distribution and quantification of the mRNAs for three rat tachykinin receptors. *Eur J Biochem*, **193**: 751-757.

Umehara, F., Izumo, S., Ronquillo, A.T., Matsumuro, K., Sato, E. and Osame, M. (1994) Cytokine expression in the spinal cord lesions in htlv-i-associated myelopathy. *Journal of Neuropathology and Experimental Neurology*, **53**: 72-77.

Urban, L., Dray, A., Nagy, I. and Maggi, C.A. (1993) The effects of NK-1 and NK-2 receptor antagonists on the capsaicin evoked synaptic response in the rat spinal cord in vitro. *Regulatory Peptides*, **46**: 413-414.

Urban, L., Naeem, S., Patel, I.A. and Dray, A. (1994a) Tachykinin induced regulation of excitatory amino acid responses in the rat spinal cord in vitro. *Neuroscience Letters*, **168**: 185-188.

Urban, L., Thompson, S.W. and Dray, A. (1994b) Modulation of spinal excitability: co-operation between neurokinin and excitatory amino acid neurotransmitters. *Trends in Neurosciences*, **17**: 432-438.

Urban, L. and Randic, M. (1984) Slow excitatory transmission in the rat dorsal horn: possible mediation by peptides. *Brain Res*, **290**: 336-342.

Vaello, M.L., Ruiz-Gomez, A., Lerma, J. and Mayor, F., Jr. (1994) Modulation of inhibitory glycine receptors by phosphorylation by protein kinase C and cAMP-dependent protein kinase. *Journal of Biological Chemistry*, **269**: 2002-2008.

Vidnyanszky, Z., Hamori, J., Negyessy, L., Reugg, D., Knopfel, T., Kuhn, R. and Gorcs, T.J. (1994) Cellular and subcellular loaclisation of the MGluR5a metabotropic glutamate receptor in rat spinal cord. *Neuroreport*, **6**: 209-213.

Villanueva, L., Chitour, D. and Le Bars, D. (1986a) Involvement of the dorsolateral funiculus in the descending spinal projections responsible for diffuse noxious inhibitory controls in the rat. *J Neurophysiol*, **56**: 1185-1195.

Villanueva, L., Peschanski, M., Calvino, B. and Le Bars, D. (1986b) Ascending pathways in the spinal cord involved in triggering diffuse noxious inhibitory controls in the rat. *J Neurophysiol*, **55**: 35-55.

Von Frey, M. (1906) The distribution of afferent nerves in the skin. *J A M A*, **47**: 645-648.

Wadiche, J.I., Amara, S.G. and Kavanaugh, M.P. (1995a) Ion fluxes associated with excitatory amino acid transport. *Neuron*, **15**: 721-728.

Wadiche, J.I., Arriza, J.L., Amara, S.G. and Kavanaugh, M.P. (1995b) Kinetics of a human glutamate transporter. *Neuron*, **14**: 1019-1027.

Wall, P.D. (1967) The laminar organisation of dorsal horn and effects of descending impulses. *J Physiol (Lond)*, **188**: 403-423.

Wall, P.D., Merrill, E.G. and Yaksh, T.L. (1979) Response of single units in laminae II and III of cat spinal cord. *Brain Res*, **160**: 245-260.

Wall, P.D. (1996) Pain in the brain and lower parts of the anatomy. *Pain*, **62**, 389-390.

Wanaka, A., Shiotani, Y., Kiyama, H., Matsuyama, T., Kamada, T., Shiosaka, S. and Tohyama, M. (1987) Glutamate-like immunoreactive structures in primary sensory neurons in the rat detected by a specific antiserum against glutamate. *Experimental Brain Research*, **65**: 691-694.

Wang, R.A. and Randic, M. (1996) alpha-subunit of CaM-KII increases glycine currents in acutely isolated rat spinal neurons. *Journal of Neurophysiology*, **75**: 2651-2653.

Wang, Y.T. and Salter, M.W. (1995) Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature*, **369**: 233-235.

Warden, M.K. and Young, W.S., 3d (1988) Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. *Journal of Comparative Neurology*, **272**: 90-113.

Ware, C.B., Horowitz, M.C., Renshaw, B.R., Hunt, J.S., Liggitt, D., Koblar, S.A., Gliniak, B.C., McKenna, H.J., Papayannopoulou, T., Thoma, B. and et al. (1995) Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development*, **121**: 1283-1299.

Watanabe, M., Mishina, M. and Inoue, Y. (1994) Distinct spatiotemporal distributions of the *N*-methyl- D-aspartate receptor channel subunit mRNAs in the mouse cervical cord. *J Comp Neurol*, **345**: 314-319.

Watkins, J.C. and Evans, R.H. (1981) Excitatory amino acid transmitters. *Ann Rev Pharmacol Toxicol*, **21**: 165-204.

Watson, S.P. and Downes, C.P. (1983) Substance P induced hydolysis of inositol phopholipids in guinea pig ilem and rat hypothalamus. *Eur J Pharmacol*, **93**: 245-253.

Weddell, G. and Miller, S. (1962) Cutaneous sensibility. *Ann Rev Physiol*, **24**: 199-222.

Weiya Ma, Ribeiro-da-Silva, A., De Koninck, Y., Radhakrishnan, V., Henry, J.L. and Cuello, A.C. (1996) Quantitive analysis of substance P-immureactive boutons on physiologically characterised dorsal horn neurons in the cat lumbar spinal cord. *J Comp Neurol*, **376**: 45-64.

Werner, P., Voigt, M., Keinanen, K., Wisden, W. and Seeburg, P.H. (1991) Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. *Nature*, **351**: 742-744.

Westbrook, G.L. and Mayer, M.L. (1987) Micromolar concentrations of Zn2+ antagonize NMDA and GABA responses of hippocampal neurons. *Nature*, **328**: 640-643.

Westlund, K.N., McNeill, D.L. and Coggeshall, R.E. (1989) Glutamate immunoreactivity in rat dorsal roots. *Neurosci Lett*, **96**: 13-17.

White, J.C. and Sweet, W.H. (1969) *Pain and the Neurosurgeon*, Thomas, Springfield.

Wiesenfeld-Hallin, Z. (1986) Substanc P and somatostatin modulate spinal cord excitability via physiologically different sensory pathways. *Brain Res*, **372**: 172-175.

Willcockson, W.S., Chung, J.M., Hori, Y., Lee, K.H. and Willis, W.D. (1984) Effects of iontophoretically released peptides on primate spinothalamic tract cells. *Journal of Neuroscience*, **4**: 741-750.

Willis, W.D., Trevino, D.L., Coulter, J.D. and Maunz, R.A. (1974) Responses of primate spinothalamic tract neurons to natural stimulation of hindlimb. *J Neurophysiol*, **37**: 358-372.

Willis, W.D. and Coggeshall, R.E. (1991) *Sensory mechanisms of the spinal cord.* Plenum, New York.

Willis, W.D. and Leonard, R.B. (1978) Spinothalmic tract neurones in the substantia gelantinosa. *Sci*, **202**: 986-988.

Wo, Z.G. and Oswald, R.E. (1994) Transmembrane topology of two kainate receptor subunits revealed by N-glycosylation. *Proceedings of the National Academy of Sciences of the United States of America*, **91**: 7154-7158.

Wong, C.S., Cherng, C.H. and Ho, S.T. (1995) Clinical applications of excitatory amino acid antagonists in pain management. *Acta Anaesthesiol*, **33**: 227-232.

Wong, E.H., Kemp, J.A., Priestley, T., Knight, A.R., Woodruff, G.N. and Iversen, L.L. (1986) The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proceedings of the National Academy of Sciences of the United States of America*, **83**: 7104-7108.

Woodley, S.J. and Kendig, J.J. (1991) Substance P and NMDA receptors mediate a slow nociceptive ventral root potential in neonatal rat spinal cord. *Brain Research*, **559**: 17-21.

Woolf, C.J. (1984) Long term alterations in the excitability of the flexion reflex produced by a peripheral tissue injury in the chronic decerebrate rat. *Pain*, **18**: 325-343.

Woolf, C.J. and King, A.E. (1990) Dynamic alterations in the cutaneous mechanoreceptive fields of dorsal horn neurons in rat spinal cord. *J Neuroscience*, **10**: 2727-2736.

Woolf, C.J. and Thompson, S.W.N. (1991) The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation; Implications for the treatment of post-injury pain hypersensitivity states. *Pain*, **44**: 293-299.

Woolf, C.J. and Wall, P.D. (1986) Relative effectiveness of C primary afferent fibres of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. *J Neurosci*, **6**: 1433-1442.

Woolf, C.J. and Wiesenfeld-Hallin, Z. (1985) Substance P and calcitonin gene related peptide synergistically modulate the excitability of the spinal cord. *Neurosci Lett Suppl*, **22**: S239

Wrathall, J.R., Teng, Y.D., Choiniere, D. and Mundt, D.J. (1992) Evidence that local non-NMDA receptors contribute to functional deficits in contusive spinal cord injury. *Brain Res*, **586**: 140-143.

Xu, X.-J., Dalsgaard, C.-J. and Wiesenfeld-Hallin, Z. (1992a) Spinal substance P and *N*-methyl-D-aspartate receptors are coactivated in the induction of central sensitization of the nociceptive flexor reflex. *Neuroscience*, **51**: 641-648.

Xu, X.-J., Dalsgaard, C.-J. and Wiesenfeld-Hallin, Z. (1992b) Intrathecal CP-96,345 blocks reflex facilitation induced in rats by substance P and C-fiber-conditioning stimulation. *Eur J Pharmacol*, **216**: 337-344.

Yaksh, T.L. (1989) Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonists. *Pain*, **37**: 111-123.

Yamamori, T. (1991) Localization of cholinergic differentiation factor/leukemia inhibitory factor mRNA in the rat brain and peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America*, **88**: 7298-7302.

Yamamoto, T., Shimoyama, N. and Mizuguchi, T. (1993a) Effects of FK224, a novel cyclopeptide NK1 and NK2 antagonist, and CP-96,345, a nonpeptide NK1 antagonist, on development and maintenance of thermal hyperesthesia evoked by carrageenan injection in the rat paw. *Anesthesiology*, **79**: 1042-1050.

Yamamoto, T., Shimoyama, N. and Mizuguchi, T. (1993b) The effects of morphine, MK-801, an NMDA antagonist, and CP-96, 345, an NK1 antagonist, on the hyperesthesia evoked by carageenan injection in the rat paw. *Anesthesiology*, **78**: 124-133.

Yamamoto, T. and Yaksh, T.L. (1991) Stereospecific effects of a nonpeptidic NK1 selective antagonist, CP-96,345: Antinociception in the absence of motor dysfunction. *Life Sci*, **49**: 1955-1963.

Yamamoto, T. and Yaksh, T.L. (1992) Effects of intrathecal capsaicin and an NK-1 antagonist, CP,96-345, on the thermal hyperalgesia observed following unilateral constriction of the sciatic nerve in the rat. *Pain*, **51**: 329-334.

Yamamoto, T. and Yaksh, T.L. (1993) Effects of intrathecal strychnine and bicuculline on nerve compression-induced thermal hyperalgesia and selective antagonism by MK-801. *Pain*, **54**: 79-84.

Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T. and Kishimoto, T. (1988) Cloning and expression of the human interleukin-6 (bsf-2/ifn beta 2) receptor. *Science*, **241**: 825-828.

Yang, C.Y., Wong, C.S., Chang, J.Y. and Ho, S.T. (1996) Intrathecal ketamine reduces morphine requirements in patients with terminal cancer pain. *Canadian Journal of Anaesthesia*, **43**: 379-383.

Yashpal, K., Dam, T.-V. and Quirion, R. (1990) Quantitative autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord. *Brain Res*, **506**: 259-266.

Yashpal, K., Dam, T.V. and Quirion, R. (1991a) Effects of dorsal rhizotomy on neurokinin receptor sub-types in the rat spinal cord: a quantitative autoradiographic study. *Brain Research*, **552**: 240-247.

Yashpal, K., Radhakrishnan, V. and Henry, J.L. (1991b) NMDA receptor antagonist blocks the facilitation of the tail flick reflex in the rat induced by intrathecal administration of substance P and by noxious cutaneous stimulation. *Neuroscience Letters*, **128**: 269-272.

Yashpal, K., Radhakrishnan, V., Coderre, T.J. and Henry, J.L. (1993) CP-96,345, but not its stereoisomer, CP-96,344, blocks the nociceptive responses to intrathecally administered substance P and to noxious thermal and chemical stimuli in the rat. *Neuroscience*, **52**: 1039-1047.

Yashpal, K., Kar, S., Quirion, R., Hui-Chan, C.W.Y. and Henry, J.L. (1994) Noxious stimulation decreases substance P binding in rat spinal dorsal horn: Competition by endogenous ligand. *Neuroreport*, **5**: 2101-2104.

Yashpal, K., Pitcher, G.M., Parent, A., Quirion, R. and Coderre, T.J. (1995) Noxious thermal and chemical stimulation induce increases in ^{3}H-phorbol 12,13-dibutyrate binding in spinal cord dorsal horn as well as persistent pain and hyperalgesia, which is reduced by inhibition of protein kinase C. *J Neurosci*, **15**: 3263-3272.

Yashpal, K., Hui-Chan, C.W. and Henry, J.L. (1996) SR 48968 specifically depresses neurokinin A- vs. substance P-induced hyperalgesia in a nociceptive withdrawal reflex. *European Journal of Pharmacology*, **308**: 41-48.

Yezierski, R.P. and Broton, J.G. (1991) Functional properties of spinomesencephalic tract (SMT) cells in the upper cervical spinal cord of the cat. *Pain*, **45**: 187-196.

Yezierski, R.P. and Mendez, C.M. (1991) Spinal distribution and collateral projections of rat spinomesencephalic tract cells. *Neuroscience*, **44**: 113-130.

Yokota, Y., Sasai, Y., Tanaka, K., Fujiwara, T., Tsuchida, K., Shigemoto, R., Kakizuka, A., Ohkubo, H. and Nakanishi, S. (1989) Molecular characterization of a functional cDNA for rat substance P receptor. *Journal of Biological Chemistry*, **264**: 17649-17652.

Yoshida, K., Taga, T., Saito, M., Suematsu, S., Kumanogoh, A., Tanaka, T., Fujiwara, H., Hirata, M., Yamagami, T., Nakahata, T., Hirabayashi, T., Yoneda, Y., Tanaka, K., Wang, W.Z., Mori, C., Shiota, K., Yoshida, N. and Kishimoto, T. (1996) Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proceedings of the National Academy of Sciences of the United States of America*, **93**: 407-411.

Yoshimura, M. and Jessell, T. (1990) Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord. *J Physiol (Lond)*, **430**: 315-335.

Young, M.R., Fleetwood-Walker, S.M., Mitchell, R. and Munro, F.E. (1994) Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons. *Neuropharmacology*, **33**: 141-144.

Young, M.R., Fleetwood-Walker, S.M., Birch, P.J., Bountra, C. and Bowers, J. (1995a) The role of neurokinin and metabotropic glutamate receptors in thermal and mechanical paw withdrawal responses in normal and carrageenan-treated rats. *Brit J Pharm*, 316P

Young, M.R., Fleetwood-Walker, S.M., Mitchell, R. and Dickinson, T. (1995b) The involvement of metabotropic glutamate receptors and their intracellular signalling pathways in sustained nociceptive transmission in rat dorsal horn neurons. *Neuropharmacology*, **34**: 1033-1041.

Yukhananov, R.Y. and Larson, A.A. (1994) An N-terminal fragment of substance P, substance P(1-7), down-regulates neurokinin-1 binding in the mouse spinal cord. *Neuroscience Letters*, **178**: 163-166.

Zafra, F., Aragon, C., Olivares, L., Danbolt, N.C., Gimenez, C. and Storm-Mathisen, J. (1995a) Glycine transporters are differentially expressed among CNS cells. *Journal of Neuroscience*, **15**: 3952-3969.

Zafra, F., Gomeza, J., Olivares, L., Aragon, C. and Gimenez, C. (1995b) Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. *European Journal of Neuroscience*, **7**: 1342-1352.

Zarbin, M.A., Wamsley, J.K. and Kuhar, M.J. (1981) Glycine receptor: light microscope autoradiographic localisation with [3H] strychnine. *J Neurosci*, **1**: 532-537.

Zieglgansberger, W. and Tulloch, I.F. (1979) Effects of substance P on neurones in the dorsal horn of the spinal cord of the cat. *Brain Research*, **166**: 273-282.

Zieglgänsberger, W. and Herz, A. (1971) Changes of cutaneous receptive fields of spinocervical tract neurones and other dorsal horn neurones by microelectrophorectically administered amino-acids. *Exp Brain Res*, **13**: 111-126.

Zigmond, R.E., HyattSachs, H., Mohney, R.P., Schreiber, R.C., Shadiack, A.M., Sun, Y. and Vaccariello, S.A. (1996) Changes in neuropeptide phenotype after axotomy of adult peripheral neurons and the role of leukemia inhibitory factor. *Perspectives On Developmental Neurobiology*, **4**: 75-90.

Zigmond, R.E. (1997) LIF, NGF, and the cell body response to axotomy. *Neuroscientist*, **3**: 176-185.

APPENDIX

Publications arising from research

Some of the work from this thesis has been published:

1. P.A. Heppenstall, S.M. Fleetwood-Walker and T. Dickinson (1995). Antagonists at the glycine modulatory site of the NMDA receptor reverse neurokinin$_1$ receptor agonist facilitation of NMDA receptor-evoked activity in the rat dorsal horn. *Soc. Neurosci. Abstr.* **21**: 541.6

2. P.A. Heppenstall and S.M. Fleetwood-Walker (1996). Interaction between neurokinin$_1$ and NMDA receptors mediated by the glycine site of the NMDA receptor in rat dorsal horn. *Congress Abstracts, VIII World Congress on Pain.*

3. P.A. Heppenstall (1996). Mediators of tachykinin/NMDA interactions in nociception. *Proceedings of the Physiological Society Symposium on Interactions in Somatosensory Processing.* (Invited Speaker).

4. P.A. Heppenstall and S.M. Fleetwood-Walker (1996) The glycine site of the NMDA receptor contributes to neurokinin$_1$ receptor agonist facilitation of NMDA receptor agonist evoked activity in rat dorsal horn neurons. *Brain Res* **744**: 235-245

5. P.A. Heppenstall and S.M. Fleetwood-Walker (1997) Glycine receptor regulation of neurokinin$_1$ receptor function in rat dorsal horn neurones. *Neuroreport* **8**: 3109-3112

6. P.A. Heppenstall, S.M. Fleetwood-Walker, S.W.N. Thompson and H.J. Spiers (1997) LIF mRNA distribution in lumbar spinal cord and its modification by peripheral inflammation and nerve injury. *J. Physiol* (in press)

7. P.A. Heppenstall, S.W.N. Thompson, H. Spiers and S.M. Fleetwood-Walker (1997) Injury induced increases in leukaemia inhibitory factor expression in the spinal cord: consequences for spinal expression of galanin, neurokinin$_1$ receptor and glutamate transporter GLT-1. *J. Neurosci* (submitted)

8. S.W.N. Thompson, S.M. Fleetwood-Walker, P.A. Heppenstall, M. Jones and D. Wynick (1998) New insights on the regulation and function of Galanin following peripheral axotomy. *Neuropeptides* (in press)

ANTAGONISTS AT THE GLYCINE MODULATORY SITE OF THE NMDA RECEPTOR REVERSE NEUROKININ$_1$ RECEPTOR AGONIST FACILITATION OF NMDA RECEPTOR EVOKED ACTIVITY IN THE RAT DORSAL HORN.

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Evidence that substance P (SP) and glutamate coexist in fine primary afferent terminals, and that NK_1 and NMDA receptors interact synergistically (Randic, M. et al., J. Neurophysiol. 63: 265-286 (1992)) suggests a potential role for these receptors in the central hyperexcitability that is bought about by sustained activity in nociceptive afferents. Indications that SP may have more than a simple direct action in acute nociception are supported by evidence that glycine is released from local inhibitory interneurons by SP (Maehara, T. et al., Regul. Peptides Suppl. 1, S102. (1992)). Following sustained activity, glycine might now co-activate the glycine recognition site of the NMDA receptor (Gly_{NMDA}) and contribute to the NK_1 and NMDA interaction. We have investigated this hypothesis using extracellular recordings from laminae III-V dorsal horn neurons of anaesthetised rats.

Ionophoresis of the selective NMDA agonist 1-aminocyclobutane-*cis*-1,3-dicarboxylic acid (ACBD) produced a 945±343% increase in the firing rate. The highly selective NK1 receptor agonist acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-SP_{6-11} facilitated this response by 223±37% percent, but had no effect when applied without ACBD at the same current. In the presence of the Gly_{NMDA} antagonists 7-chlorothiokynurenic acid or 7-chloro-3-(cyclopropylcarbonyl)-4-hydroxy-2(1H)-quinoline (L701,252) this increment was reduced by 89±30% and 98±29% while the antagonists had no effect on ACBD evoked activity alone.

This suggest that the co-operation between NMDA and NK_1 receptors is mediated, in part, by the glycine site of the NMDA receptor, perhaps by means of glycine, released from an interneuron in response to SP.

INTERACTION BETWEEN NEUROKININ$_1$ AND NMDA RECEPTORS MEDIATED BY THE GLYCINE SITE OF THE NMDA RECEPTOR IN RAT DORSAL HORN

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Aim of Investigation: Neurokinin$_1$ (NK_1) receptors may contribute to spinal nociceptive processing by modulating excitatory amino acid-mediated transmission, particularly through potentiation of NMDA receptor activity [Randic, M. *et al.*, J. Physiol. **63**: 265-286 (1992)]. There is also evidence that substance P acting at NK_1 receptors increases release of glycine from local interneurons [Maehara, T. *et al.*, Regul. Peptides Suppl. **1**, S102 (1992)]. The present experiments were carried out to investigate the effects of NK_1-induced glycine release on the glycine site of the NMDA receptor (Gly_{NMDA}) and to establish the role of the Gly_{NMDA} site in the NK_1/NMDA interaction.

Methods: Extracellular recordings were made from laminae III-V dorsal horn neurons of anaesthetised rats. NMDA agonist 1-aminocyclobutane-cis-1,3-dicarboxylic acid (ACBD) was ionophoresed either alone, or followed by NK_1 agonist acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-SP_{6-11}. When the activity had stabilised Gly_{NMDA}, antagonists 2-carboxy-4,6-dichloro-(1H)-indole-3-propanoic acid (MDL 29951), 7-chloro-3-(cyclopropylcarbonyl)-4-hydroxy-2(1H)-quinoline (L701,252), 5,7-dinitroquinaxoline-2,3-dione (MNQX) or 7-chlorothiokynurenic acid (7-CTK) were applied. The Gly_{NMDA} antagonists were also tested on ACBD activity alone.

Results: Ionophoresis of ACBD produced a 945±343% increase in the firing rate. Acetyl-[Arg^6,Sar^9, $Met(O_2)^{11}$]-SP_{6-11} facilitated this response by 223±37% but had no effect when applied without ACBD at the same current. The Gly_{NMDA} antagonists reduced this increment to 49± 21% (MDL 29951), 111± 30% (L701,252), 129± 62% (MNQX) or 102± 29% (7-CTK) of activity elicited by ACBD alone, while the Gly_{NMDA} antagonists had no effect on ACBD-evoked activity alone at these currents. Inhibitory effects were seen at currents more than 2 fold higher.

Conclusions: This study provides evidence for the involvement of the Gly_{NMDA} site in the NK_1/NMDA interaction. The relative contribution of pre- and postsynaptic effects of NK_1 receptor activation, with respect to the influence of the Gly_{NMDA} site on NMDA receptor complex function, is yet to be clarified.

Extracellular recording were made from lamina III–V dorsal horn neurons from sixty-two male Wistar rats (250–400 g) anaesthetized with intravenous α-chloralose (60 mg kg^{-1}) and urethane (1·2 g kg^{-1}). Ionophoresis of the selective NMDA agonist 1-aminocyclobutane-cis-1,3-dicarboxylic acid (ACBD) produced a steady increased firing rate. The NK_1 agonist acetyl-[Arg^6, Sar^9, $Met(O_2)^{11}$]-SP_{6-11} was then applied and facilitated the ACBD activity by 223 ± 37% (mean± S.E.M.), but had no effect when applied alone. This enhanced activity was inhibited by the Gly_{NMDA} antagonists (2-carboxy-4,6-dichloro-(1H)-indole-3-propanoic acid (MDL 29951), 7-chloro-3-(cyclopropylcarbonyl)-4-hydroxy-2(1H)-quinoline (L701,252), 5,7-dinitro-quinaxoline-2,3-dione (MNQX) or 7-chlorothiokynurenic acid (7-CTK), but not by the AMPA receptor antagonist 6-nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione (NBQX). AMPA-evoked activity was inhibited by NBQX, but not unaffected by L701,252. The Gly_{NMDA} antagonists had no effect on activity evoked by ACBD alone until higher currents were applied and were without effect on activity evoked by AMPA. The role of the Gly_{NMDA} site was further investigated by applying Gly_{NMDA} agonist 1-aminocyclo-propanecarboxylic acid (ACPC) instead of the NK_1 agonist. This potentiated the ACBD response by 88 ± 11% and ejection of L701,252 reversed the increment in activity. The selective PKC inhibitors chelerythrine and GF109203X inhibited the enhanced activity due to the combination of NK_1 and NMDA agonists, but not that evoked by AMPA or by ACBD alone.

These results indicate that the facilitation of NMDA activity by NK_1 agonist is dependent on both an intracellular pathway involving PKC and the status of the Gly_{NMDA} site.

REFERENCES

Battaglia, G. & Rustioni, A. (1988). *J. Comp. Neurol.* **277**, 302–312.
Chen, L. & Huang, L.Y. (1992). *Nature* **356**, 521–523.
Dougherty, P.M. & Willis, W.D. (1990). *J. Physiol.* **425**, 38P.
Johnson, J.W. & Ascher, P. (1987). *Nature* **325**, 529–531.
Randic, M., Jiang, M.C., Rusin, K.I., Cerne, R. & Kolaj, M. (1993). *Regul. Peptides* **46**, 418–420.
Smullin, D.H., Skilling, S.R. & Larson, A.A. (1990). *Pain* **42**, 93–101.

Mediators of tachykinin/NMDA interactions in nociception

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Neurokinin$_1$ (NK_1) and NMDA receptors are thought to be important mediators of the central hyperexcitability that follows sustained activity in nociceptive afferents. Evidence that substance P (SP) and glutamate exist in primary afferent terminals (Battaglia & Rustioni, 1988) and that NMDA receptor activity is augmented by NK_1 receptor activation (Dougherty & Willis, 1990) implies that an interaction between the two receptors may account for the altered excitability. A number of potential means exist whereby NK_1 receptor-mediated effects might facilitate NMDA receptor function. One is suggested by evidence that SP increases the levels of glycine in spinal cord (Smullin *et al.* 1990). Glycine is a co-agonist at the NMDA receptor (Johnson & Ascher, 1987); therefore we have investigated the role of the glycine site of the NMDA (Gly_{NMDA}) receptor during interaction of NK_1 and NMDA receptors. Alternatively, the intracellular signalling pathways activated by NK_1 receptors, including protein kinase C, may act to enhance the activation of NMDA channels (Chen & Huang, 1992; Randic *et al.* 1993).

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The glycine site of the NMDA receptor contributes to neurokinin$_1$ receptor agonist facilitation of NMDA receptor agonist-evoked activity in rat dorsal horn neurons

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Glycine receptor regulation of neurokinin$_1$ receptor function in rat dorsal horn neurones

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ACTIVATION of spinal neurokinin$_1$ (NK$_1$) receptors leads to increases in the extracellular concentration of glycine in the dorsal horn. We have investigated the role of the inhibitory glycine receptor as a regulator of NK$_1$ receptor-mediated effects on dorsal horn neurones. Iontophoretic application of GR82334, a selective NK$_1$ antagonist, did not alter dorsal horn neuronal activity evoked by cutaneous applications of mustard oil. However, in the presence of the glycine antagonists, strychnine or phenylbenzene-ω-phosphono-α-amino acid (PMBA), GR82334 displayed inhibitory properties. Therefore inhibitory glycine receptors may mask the contribution made by NK$_1$ receptors to nociceptive processing. This is discussed with reference to the role of NK$_1$ receptors during brief and long duration nociceptive transmission.

Key words: Dorsal horn; Glycine receptor; Mustard oil; Neurokinin$_1$ receptor; Pain; Substance P

Introduction

The neurokinin$_1$ (NK$_1$) receptor does not have a well-defined role in spinal nociceptive transmission. From electrophysiological and behavioural experiments, the receptor appears to be involved only after severe or chronic stimulation. For example, while behavioural responses to a variety of sustained/inflammatory models of pain are effectively reduced by NK$_1$ antagonists, brief noxious inputs remain unaffected.[1] Similarly, in electrophysiological experiments, these antagonists will inhibit responses to long-term noxious stimulation such as intraplantar injection of formalin without affecting acute C-fibre responses[2] or short-term neuronal responses to the sensitizing agent mustard oil.[3] Therefore, both the intensity and mode of sustained nociceptive stimulation may be crucial in defining an NK$_1$ receptor involvement.

There could be a number of factors contributing to the apparent lack of NK$_1$ receptor involvement in acute responses. One possibility is the co-activation of an inhibitory mechanism by substance P (SP). Indeed an inhibitory effect of NK$_1$ receptors has previously been reported for cat dorsal horn neurones, where [Met-OMe^{11}]SP (SPOMe), an NK$_1$ agonist, reduced responses to innocuous brushing,[4] and in mouse using intrathecal drug application, where very low doses of SP reduced the behavioural responses to NMDA.[5] Furthermore, glycine is released following bath application of SP *in vitro*[6] and following microdialysis of SP into the spinal cord *in vivo*,[7] and it can have two opposing actions: an excitatory effect via its co-agonist binding site on the NMDA receptor and an inhibitory effect through the strychnine-sensitive glycine receptor.[8] We have previously reported that NK$_1$ receptor agonists elicit a glycine-mediated facilitation of NMDA receptor function,[9] but we now propose that the strychnine-sensitive receptor offers an alternative inhibitory target for the released glycine (which is more obvious during short-term nociception).

NK$_1$ receptor activation of a glycine interneurone would initially obscure an NK$_1$ excitatory input until the point was reached where the accumulation of lasting intracellular changes such as phosphorylations and NMDA receptor activation overcame the acute inhibitory modulation. Effects of NK$_1$ antagonists would therefore not be apparent in models with a shorter time course such as mustard oil application. However in those situations where the stimulus was more severe, the combination of intracellular changes and increased SP release would eventually lead to a direct, measurable NK$_1$ receptor effect. We investigated this hypothesis using ionophoresis of selective receptor agonists and antagonists and recording extracellularly from dorsal horn neurones of anaesthetized rats.

Materials and Methods

Fifteen male Wistar rats (210–400 g) were anaesthetized with halothane followed by α-chloralose (60 mg/kg, i.v.) and urethane (1.2 g/kg). Subsequent doses of α-chloralose were given when required. The trachea was cannulated and a light flow of oxygen passed over the cannula. Body temperature was maintained at 37°C using a thermostatically controlled heat blanket. The spinal column was exposed and

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LIF MRNA DISTRIBUTION IN LUMBAR SPINAL CORD AND ITS MODIFICATION BY PERIPHERAL INFLAMMATION AND NERVE INJURY

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Leukaemia inhibitory factor (LIF) is a neuroactive cytokine that is associated with peripheral nerve injury. It is upregulated and retrogradely transported to the DRG by a specific population of nociceptive specific neurones (Thompson, S.W.N. *et al.* (1997) *Eur. J. Neurosci.* **9** 1244-1251) where it may selectively influence neuropeptide expression (Sun, A.U. and Zigmond, R.E. (1996) *Eur. J. Neurosci.* **8** 2213-2220). The present study examines the distribution of LIF mRNA in the spinal cord and investigates its altered expression in the superficial dorsal horn following peripheral inflammation or nerve injury.

Experiments were performed on male Wistar rats (200-400g) anaethetised with halothane. Inflammation (n=3) was induced over a 52 hour period with a single unilateral intraplantar injection of 150µl of Complete Freund's Adjuvant (CFA). In a second group (n=3), the L3 spinal nerve was tightly ligated (5/0 Mersilk) for 7 days and a third control group (n=3) received no treatment. Spinal cords comprising segments L2-L4 were then removed from these animals and sectioned at 15µm on a cryostat. LIF expression was investigated using *in situ* hybridisation histochemistry (ISHH) with two [^{35}S]-labelled oligonucleotide probes complimentary to base sequences 86-133 and 434-478 of the LIF mRNA and which showed corresponding patterns of distribution in control animals.

In control animals, dorsal horn expression of LIF was high in superficial laminae I-II with only light expression in the deeper laminae III-V. There was also expression around the central canal that extended into the ventral horn and was observed on motorneurones. Both peripheral inflammation and neuropathy significantly increased levels of LIF mRNA in the dorsal horn by greater than 3 fold compared to control animals. This was most evident in the lateral superficial laminae where the number of cells expressing this cytokine increased by 18±10% (mean±SEM) ($P<0.04$; T-test) for inflammed animals and 44±5% ($P<0.0001$) for the neuropathic group. Corresponding sense probe or RNAse treated sections showed no hybridisation.

These results show that LIF is normally expressed in the spinal cord in a specific laminar pattern and this expression increases dramatically following peripheral inflammation or nerve damage. LIF may therefore be a critical regulator of the central changes that occur following peripheral injury and could contribute to the behavioural hyperalgesia associated with these changes.

New insights on the regulation and function of Galanin following peripheral axotomy

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Following the initial characterisation in the early seventies of substance P as a undecapeptide present in sensory neurons, numerous studies have expanded our knowledge upon the wealth of neuropeptides present in these cells. Whilst the action of many of these peptides remains uncertain, there appears to be a general consensus that many function under normal circumstances as neuromodulators of synaptic transmission within the spinal cord (see Urban et al.,1994).

It is also now widely appreciated that the neurochemical phenotype of sensory neurons does not remain constant but displays considerable plasticity following peripheral nerve injury. Thus following axotomy, small diameter dorsal root ganglion (DRG) neurons, which under normal circumstances are considered to be nociceptive in function, lose substance P (SP), somatostatin (SOM) and calcitonin gene-related peptide (CGRP). Conversely peptides which are constitutively expressed at very low levels or not at all in these cells such as vasoactive intestinal polypeptide (VIP), galanin (GAL) and neuropeptide Y (NPY) are upregulated. Different changes occur in neurones with larger diameter cell bodies and which under normal circumstances transmit low threshold non-noxious information to the spinal cord. Thus, similar to small diameter neurons, they acquire NPY, VIP and GAL whilst in addition a significant portion also upregulate SP.

The factors which control the *de-novo* expression of these neuropeptides within sensory neurons and their function following peripheral axotomy is not well understood. Galanin is of particular interest. Following peripheral axotomy, GAL gene expression within primary sensory neurons is partially dependent upon the upregulation of the neuroactive cytokine Leukemia Inhibitory Factor (LIF) at the site of nerve trauma (Sun and Zigmond,1997, Thompson et al.,1997; Thompson et al.,1998). LIF mRNA expression is also increased within the spinal cord following peripheral axotomy (Heppenstall et al.,1998). LIF in turn also influences GAL mRNA expression within the superficial dorsal horn of the spinal cord (Heppenstall et al.,1998). The function of GAL within sensory neurons and the spinal cord remains equivocal. Early studies showed that GAL significantly depressed spinal reflex activity *in vivo* , an effect which was more pronounced following axotomy (Wiesenfeld-Hallin et al.,1989). However the effect of GAL upon reflex excitability is biphasic with an excitatory effect at low concentrations and an inhibitory effect only at higher concentrations. We have studied behavioural responses to nerve injury in mice lacking a functional galanin gene. Following sciatic axotomy, autotomy of the deafferented hindlimb is abolished in the knockout mice whilst all wild type animals exhibit severe autotomy following a control lesion (Holmes et al.,1997). The excitability of chronically axotomised primary sensory neurons to an intracellular depolarizing current pulse measured in an acutely extirpated DRG preparation *in vitro* is significantly lower in GAL KO mice compared to wild types. It is a possibility therefore that GAL regulates sensory neuron excitability following peripheral axotomy. The excitability of spinal reflex activity following peripheral axotomy is currently being assessed in wild type and knockout mice.

Heppenstall,P.A., Thompson,S.W.N., Spiers,H.J. and Fleetwood-Walker,S.M. (1998) in preparation.
Holmes,F.E., McMahon,S.B. Murphy, D. and Wynick,D. (1997) *Soc. Neurosci. Abstr.* **23** 760.4
Sun,Y. and Zigmond,R. (1996). *Europ.J.Neurosci.* **8**: 2213-2220.
Thompson, S.W.N. Vernallis, A E., Heath, J.K. and Priestley, J.V. (1997) *Eur.J Neurosci.* **9** 1244-1251.
Thompson, S.W.N. Priestley,J.V. and Southall,A. (1998) *Neuroscience, 000-000* in press.
Urban,L. Thompson,S.W.N. & Dray,A (1994). *Trends Neurosci.* **17**, 432-438.
Wiesenfeld-Hallin Z. Xu XJ. Langel U. Bedecs K. Hokfelt T. Bartfai T. (1992) *Proc.Natl. Acad. Sci. USA.* **89.** 3334-3337.