DEVELOPMENT AND PHARMACOLOGICAL CHARACTERIZATION OF AN ACUTE MODEL OF ALLODYNIA IN THE ANESTHETIZED RAT

STEPHEN EDWARD SHERMAN

DEVELOPMENT AND PHARMACOLOGICAL CHARACTERIZATION OF AN ACUTE MODEL OF ALLODYNIA IN THE ANESTHETIZED RAT

by

Stephen Edward Sherman

A thesis submitted to the School of Graduate Studies
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

Specialization: Neuroscience

Division of Basic Medical Sciences
Faculty of Medicine
Memorial University of Newfoundland
St. John's, Newfoundland

1994

ABSTRACT

The acute blockade of spinal glycinergic inhibition with intrathecal strychnine (i.t. STR; a glycine antagonist) in rats produces a change in somatosensory processing which resembles allodynia, a symptom of clinical neural injury pain. In the present study, the effects of i.t. STR were examined in urethane-anesthetized rats. Noxious paw pinch (PP), or tail immersion (TI) in 55°C water, evoked a pronounced pressor response, tachycardia, a motor withdrawal reflex and desynchronization of the electroencephalogram (EEG). Non-noxious, hair deflection (HD), applied to the back, flanks, legs and tail of the rat, elicited only minor cardiovascular responses. Following i.t. STR (40 μg), an identical HD stimulus evoked responses resembling those seen with noxious stimuli: an increase in mean arterial blood pressure (M.A.P), tachycardia and a motor withdrawal reflex. All manifestations of this HD-evoked, STR-dependent allodynia were: 1) observed only with a light plane of anesthesia (as determined by EEG), 2) reversible with time (within 15-30 minutes), 3) observed without convulsions, 4) evoked only when HD was applied to cutaneous dermatomes with innervation from spinal segments near the i.t. STR injection site (segmental), and 5) i.t STR dose-dependent (10-50 μg). I.t. glycine produced dose-dependent inhibition of all indices of STR-dependent allodynia with ED_{50} and 95% C.I. values of 609(429-865), 694(548-878) and 549(458-658) μg for inhibition of heart rate (HR) increases, M.A.P. increases and motor withdrawal reflexes, respectively. I.t. betaine (a glycine derivative) also dose-dependently inhibited STR-dependent allodynia, possibly through metabolism to glycine. The ED_{50} and 95% C.I. values were 981(509-1889), 1045(740-1476) and 1083(843-1391) μg for inhibition of HR, M.A.P. and motor withdrawal responses, respectively. EEG synchrony was unaffected by i.t. glycine or i.t. betaine. Neonatal capsaicin (25 mg/kg, s.c., post-natal day (PND) 1, and 50 mg/kg, s.c., PND 2, 3, 4, 11, 25, 55 and 85) significantly attenuated responses evoked by mechanical (PP), thermal (TI) or chemical (topical xylene) noxious stimuli, but did not affect STR-dependent allodynia. All indices of STR-dependent allodynia were also unaffected by i.t. morphine at a dose (50 μg, i.t.) which completely abolished responses evoked by noxious TI or PP. STR-dependent allodynia was dose-dependently suppressed by i.t. γ-*D*-glutamylglycine (DGG; non-selective excitatory amino acid [EAA] antagonist) and i.t. 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline(NBQX, AMPA receptor antagonist). The ED_{50}'s and 95% C.I. values for DGG against HD-evoked, STR-dependent HR, M.A.P. and motor

responses were 15.6 (11.3-21.6), 16.9 (11.7-24.3) and 8.1 (5.2-12.5) μg, respectively. The corresponding values for NBQX were 12.2 (6.8-21.8), 14.4 (8.6-24.0) and 10.4 (5.5-19.6) μg. EEG synchrony was unaffected by i.t. DGG or i.t. NBQX. The results of the present study indicate that glycine plays an important role in the spinal modulation of non-nociceptive input and supports the hypothesis that a loss of glycinergic modulation may underlie allodynia. The failure of i.t. morphine and neonatal capsaicin to prevent STR-dependent allodynia indicates that this phenomenon is initiated by primary afferent neurons not normally involved in nociception, presumably Aß-fibers. The sensitivity of STR-dependent allodynia to both NMDA and non-NMDA receptor antagonists, and the failure of i.t. STR to produce hyperalgesia to mechanical, thermal or chemical noxious stimuli, confirm the independence of nociceptive pathways and STR-sensitive input in this model. The i.t. STR model allows the investigation of an important symptom of neural injury pain (opioid-resistant allodynia) in anesthetized animals, without having to inflict injury or to expose a conscious animal to aversive painful conditions, and might provide a useful alternative to chronic conscious animal models of allodynia.

Key Words: allodynia; excitatory amino acid antagonists; glycine; intrathecal; morphine; neural injury pain; rat; spinal; strychnine

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my research supervisor, Dr. Christopher Loomis, for his expert guidance and encouragement throughout my research. His patience, insight and sense of humour have been greatly appreciated. I am also grateful for his thorough review of this thesis.

I would also like to extend my gratitude to the following individuals:

Dr. Richard Neuman for his advice about recording and interpreting the electroencephalograms, his active participation on my thesis committee, and for his prompt and effective review of this thesis.

Dr. David Bryant for many helpful discussions of the statistical methods. His open door, his patience and his many insights are greatly appreciated.

Dr. Detlef Bieger for letters of support on my behalf, and gentle reminders of how much more I need to learn.

Dr. Paul Redfern for taking time from his busy schedule to serve on my thesis committee.

Dr. James Reynolds for his open door, his example and his insightful participation in my comprehensive exam.

Dr. Caroline Harley for her thought-provoking participation in my seminars and comprehensive exam.

Dr. Gerald Duncan for generously availing to me the space and services of the School of Pharmacy throughout my research.

Mrs. Eleanor Duncan for making me feel welcome at many social occasions.

Dr. Dongyuan Yao for injecting innumerable animals on my behalf, not to mention several patiently delivered Chinese lessons.

Ms. Sue Ellen Maher for her expert assistance with the histology, and her good company.

Ms. Margaret Miller, Mr. John Bautista, Mr. Robert Gilbert and Mr. Jacques Desousa for friendship and many distractions to make this degree less painful.

Ms. Heather Pope and Ms. Ferdos Roberts for secretarial assistance.

Dr. Lenka Husa and the Animal Care staff for their cooperation and excellent care of our animals.

I wish to acknowledge the use of operating funds for this research, which were furnished in the form of grants to Dr. Loomis from the Medical Research Council of Canada and the Research and Development Committee, Faculty of Medicine, Memorial University of Newfoundland.

I am grateful to the Pharmaceutical Manufacturer's Association of Canada - Health Research Foundation, and the Faculty of Medicine of Memorial University for personal funding during my degree program.

I would also like to thank Novo Nordisk for providing a sample of NBQX for use in these experiments.

for Nagwa

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

0700 h	7 o'clock in the morning
3-PYOL	5-(3-pyrrolidinyl)-3-isozolol
3,4-TAZA	2,5,6,7-tetrahydro-1*H*-azepine-4-carboxylic acid
4,5-TAZA	2,3,6,7-tetrahydro-1*H*-azepine-4-carboxylic acid
≈	approximately equal to
α	alpha, a Greek letter
ß	beta, a Greek letter
°C	degrees Celsius
δ	delta, a Greek letter, a subtype of opioid receptor
=	equal(s)
γ	gamma, a Greek letter
>	greater than
κ	kappa, a Greek letter, a subtype of opioid receptor
<	less than
μ	mu, a Greek letter, used to indicate micro in metric units, also a subtype of opioid receptor
μg	microgram(s), 10^{-6} grams, unit of mass
μL	microliter(s), 10^{-6} litres, unit of volume
μm	micrometer(s), 10^{-6} metres, unit of distance
μM	micromolar, 10^{-6} moles per litre, unit of concentration
μmol	micromole(s), 10^{-6} moles, number of molecules
%	percent
+	plus, in combination with
ρ	rho, a Greek letter
^{3}H	hydrogen atomic weight three (tritiated)
Aß	A-beta, a class of primary afferent neurons
Aδ	A-delta, a class of primary afferent neurons
AMPA	amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
AP5	*D*-2-amino-5-phosphono-valerate
betaine	common name for *N,N,N*-trimethylglycine
C	a class of primary afferent neurons
C.I.	confidence interval (about the mean)
CGRP	calcitonin gene-related peptide
Cl^-	chloride ion
cm	centimeter(s)
CNQX	6-cyano-7-nitro-quinoxaline-2,3-dione
CNS	central nervous system
Co.	company

Corp.	corporation
CSF	cerebrospinal fluid
D	dextrorotatory
DGG	γ-*D*-glutamylglycine
DL	dextrorotatory and levorotatory (racemic mixture)
DREZ	dorsal root entry zone
DRG	dorsal root ganglia
e.g.	*exempli gratia*, (for example)
EAA(s)	excitatory amino acid(s)
ED_{50}	effective dose for 50 percent response
et al.	*et alia* (and others)
EEG	electroencephalogram(s)/graph(s)
Fig.	figure
g	gram(s)
GABA	γ-aminobutyric acid, γ-aminobutyrate
GABAergic	using GABA as a neurotransmitter
h	hour(s)
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HD	hair deflection
Hg	elemental symbol for mercury
HR	heart rate
Hz	hertz, s^{-1}, reciprocal seconds, unit of frequency
I	Roman numeral one, used to denote outermost spinal lamina
i.e.	*id est*, that is
i.p.	intraperitoneal(ly)
i.t.	intrathecal(ly)
i.v.	intravenous(ly)
IgG	immunoglobulin
IASP	International Association for the Study of Pain
IC_{50}	concentration for 50 percent inhibition
II	Roman numeral two, used to denote spinal lamina
III	Roman numeral three, used to denote spinal lamina
IV	Roman numeral four, used to denote spinal lamina
IR	immunoreactivity
Inc.	incorporated
in vitro	in glassware
in vivo	in the living body
iso-THAZ	5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol
kg	kilogram(s), 10^3 grams

K_m	agonist dissociation (Michaelis-Menton) constant
kPa	kilopascal(s), unit of pressure
K_i	antagonist dissociation constant
L	levorotatory
L1	lumbar vertebra number one
LC	locus coeruleus
log	logarithm (base 10)
m	meter(s)
m/s	meter/second, unit of velocity
M.A.P.	mean arterial (blood) pressure
mg	milligram(s), 10^{-3} grams
min	minute(s), (used in figures only)
ml	milliliter(s), 10^{-3} liters
mm	millimeter(s), unit of distance
mm^2	millimeter(s) squared, unit of area
M	molar (moles/liter)
MDL27,551	4-methyl-3-methylsulphonyl-5-phenyl-4*H*-1,2,4-triazole
MK-801	(+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*) cyclo heptene-5,10-imine maleate
N	number of determinations
N	nitrogen
N.I.P.	Neural Injury Pain
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f) quinoxaline
nM	nanomolar, 10^{-9} moles per liter, unit of concentration
NMDA	*N*-methyl-*D*-aspartate
NS	no stimulus
nSTT	neospinothalamic tract
nmol	nanomole(s), 10^{-9} moles
PAG	periaqueductal gray
PAP	peroxidase-antiperoxidase
PE-10	size 10 polyethylene tubing (diameter ≈ 0.61 mm)
p	probability of error
pH	negative log of the hydrogen ion concentration
PP	paw pinch
pSTT	paleospinothalamic tract
RU 5135	3-α-hydroxy-16-imino-5ß-17-aza-androstan-11-one
s	second(s)
s.c.	subcutaneous(ly)
SAL	saline
S.E.M.	standard error of the mean
SI	sensory receiving area one of the somatosensory cortex

SII	sensory receiving area two of the somatosensory cortex
SKF10047	Smith Kline and French compound 10047 (NMDA antagonist)
SMT	spinomesencephalic tract
SRT	spinoreticular tract
STR	strychnine
STT	spinothalamic tract
T	time
THAZ	5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-d]azepin-3-ol
THIA	5,6,7,8-tetrahydro-4*H*-isoxazolo[5,4-c]azepin-3-ol
THIP	4,5,6,7-tetrahydro isoxazole[5,4-c]pyridin-3-ol
TI	tail immersion
vide infra	see below
w/v	weight per volume
V	Roman numeral five, used to denote spinal lamina
WDR	wide dynamic range
X	times (multiplied by), also Roman numeral ten

1.0 GENERAL INTRODUCTION

1.1 Statement of the Research Problem

The most common form of clinical pain is *nociceptive* pain, the type of pain caused by inflammation, a superficial injury, or a cancerous growth confined to somatic tissue. Nociceptive pain is generally regarded as a state of continuous stimulation of nociceptors, resulting in the sustained transmission of nociceptive impulses via specific neural pathways from the injury site to the somatosensory cortex. The onset of nociceptive pain is immediate, and directly related to tissue injury or exposure to a noxious stimulus. Interruption of the nociceptive stimulation or its transmission, by means of analgesic drugs (e.g., opioids), or by surgical interruption of the neural pathway, arrests the pain (Tasker, 1990).

Neural injury pain (N.I.P.) refers to a syndrome that develops in a subgroup of patients with traumatic injury to, or pathology of, the nervous system. It is a rare and idiosyncratic outcome of neural damage (Noordenbos and Wall, 1981; Arnér and Meyerson, 1988; Tasker, 1990). In those patients affected, however, it is an extremely debilitating and often intractable condition (Rowbotham *et al.*, 1991; Baron and Saguer, 1993).

The characteristics of N.I.P. are quite different from those of nociceptive pain. Pain often coexists with sensory loss (Baron and Saguer, 1993), and patients frequently describe their pain in terms of physical injury, such as burning, ripping, tearing, pressing or twisting. It may be difficult to identify or locate the inciting stimulus (Lindblom and Verrillo, 1979). In addition, the onset of N.I.P. is usually delayed for weeks or even months following the causative event (Tasker, 1990).

One of the most disturbing features of N.I.P. is its poor response to treatment. Pharmacotherapy with opioid analgesics, tricyclic antidepressants, anticonvulsants, barbiturates, local anesthetics or use-dependent sodium channel blockers is often inadequate (Shibasaki and Kuroiwa, 1974; Arnér and Meyerson, 1988; Tasker, 1990; Rowbotham *et al.*, 1991; Triggs and Beric, 1992). Indeed, certain types of N.I.P. appear to be completely refractory to current drug therapy (Triggs and Beric, 1992; Baron and Saguer, 1993). Similarly, surgical interventions intended to alleviate N.I.P. usually provide only temporary and often incomplete relief, with pain eventually returning (Tasker, 1990; Tasker *et al.*, 1992).

In addition to spontaneous pain, which can be steady or intermittent, N.I.P. is often associated with various forms of dysesthesia (see Table 1.1), including allodynia,

hyperalgesia or hyperpathia (Noordenbos and Wall, 1981; Campbell *et al.*, 1988; Nurmikko and Hietaharju, 1992; Tasker *et al.*, 1992). Allodynia is a particularly distressing form of pain, which arises from stimuli that do not normally provoke pain (Merskey, 1986). Thus, a cold draft or a light tactile stimulus, such as the touch of clothing, can evoke excruciating pain in patients with N.I.P. Thermally-evoked allodynia or hyperalgesia are infrequent symptoms of N.I.P., while mechanically-evoked allodynia is the most common type, occurring in 48% of patients with peripheral neuropathic pain and 54% of patients with central pain (Nurmikko and Hietaharju, 1992). Opioid-insensitive pain, evoked by an innocuous tactile stimulus, is a major clinical problem.

N.I.P. is believed to involve multiple pathophysiological mechanisms (Loeser, 1981; Campbell *et al.*, 1988; Price *et al.*, 1989; Price *et al.*, 1992; Dubner, 1991; Gracely *et al.*, 1992; Koltzenburg *et al.*, 1992; Baron and Saguer, 1993). Unfortunately, the precise mechanisms of N.I.P. remain unknown. The ability of a light tactile stimulus to trigger the perception of pain implicates large diameter myelinated (Aß) afferent fibers in initiating allodynia (Campbell *et al.*, 1988; Koltzenburg *et al.*, 1992; Price *et al.*, 1992; Baron and Saguer, 1993). Experimental evidence suggests that abnormal central processing of somatosensory information in the spinal dorsal horn

Table 1.1 Definitions of Terminology used to Describe Pain

Term	Definition
Pain	An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such.
Dysesthesia	An unpleasant abnormal sensation, whether spontaneous or evoked.
Allodynia	Pain due to a stimulus that does not normally provoke pain.
Hyperalgesia	An increased response to a stimulus which is normally painful.
Hyperpathia	A painful syndrome, characterized by increased reaction to a stimulus, especially a repetitive stimulus, as well as an increased threshold.
Hyperesthesia	Increased sensitivity to stimulation, excluding special senses.
Central Pain	Pain associated with a lesion of the central nervous system.

The above definitions are taken from the classification system outlined by the International Association for the Study of Pain (IASP) as presented by Merskey (1986).

(or higher centers) results in strengthened synaptic ties between Aß primary afferent fibers (which normally mediate touch sensation) and pain-signalling pathways in the CNS.

Low threshold, Aß primary afferent fibers relay information to second order wide dynamic range (WDR) neurons in the spinal dorsal horn. These WDR neurons also receive input from nociceptive primary afferent fibers. Why then does Aß input not normally provoke pain? It appears that the perception of a stimulus as being tactile or noxious is governed by the balance of excitatory (e.g. glutamate) and inhibitory (e.g. glycine) inputs influencing the discharge of these second-order neurons (Henry, 1989; Skilling *et al.*, 1990). Local glycinergic neurons in the spinal cord are vulnerable to damage as a result of physical trauma or ischemia (Tureen, 1936; Davidoff *et al.*, 1967). If these spinal interneurons normally regulate the discharge of second order WDR neurons, the loss of this glycinergic inhibitory regulation could result in abnormal Aß-evoked firing of WDR neurons, leading to the inappropriate perception of pain.

Studies using the glycine receptor antagonist, strychnine (STR), support the concept of glycinergic dysfunction as a mechanism of allodynia. The temporary removal of glycinergic inhibition with intrathecal (i.t.) STR induces an allodynia-like state in experimental animals, and may serve as a useful model for investigating changes in

somatosensory processing which result from glycinergic dysfunction. The problem addressed by the present thesis was the adaptation of the STR model of allodynia to an anesthetized animal preparation. This model was then used to compare the spinal pharmacology of allodynia with that of nociceptive pain.

1.2 Anatomy and Physiology of Tactile and Pain Sensation

Sensory systems consist of serial neural pathways, linking the periphery with the spinal cord, brainstem, thalamus and cerebral cortex. The sensation of pain is more complex than other sensory modalities, since it involves both a sensory component as well as an affective, or emotional, component (Henry, 1989). To distinguish the sensory component from the associated affective qualities, neurotransmission induced by noxious stimuli was termed *nociception* by Sherrington (1947).

1.2.1 Peripheral Receptors and Primary Afferent Neurons

Neurons relaying sensory information, whether peripheral or central, often exhibit remarkable specificity for a particular type of stimulus. There are five types of peripheral receptors that register information regarding

tactile stimuli and transduce this information into electrical events in neurons. In glabrous skin, Meissner's corpuscles and Merkel's receptors are found in the dermis (near the skin surface), while Pacinian and Ruffini corpuscles are deeper within the skin (Martin, 1985b). Meissner's corpuscles and Pacinian corpuscles are rapidly-adapting mechanoreceptors; they respond briefly at the onset (and sometimes at the termination) of a prolonged stimulus, but are distinguished by their differential responsiveness to variations in stimulus frequency. In hairy skin, the Meissner's corpuscle is replaced by the hair receptor, a rapidly-adapting receptor that responds to hair deflection. Merkel receptors and Ruffini corpuscles respond continuously to an enduring stimulus, and are termed slowly adapting mechanoreceptors (Martin, 1985b). When tissue is exposed to damaging, or potentially damaging stimuli, this nociceptive information is transduced into electrical signals by the least differentiated receptors of the skin: free nerve endings. Besides the skin, nociceptors are located in skeletal muscle, joints, the cornea, tooth pulp, heart, gut and cerebral blood vessels (Henry, 1989). Although each of these receptors responds to particular stimulus qualities, natural stimuli seldom trigger only one type. It is the combined activation of different groups of peripheral receptors that ultimately provides the brain with a rich and

detailed picture of each peripheral sensory event.

A variety of primary afferent neurons convey somatosensory information from the site of stimulation to the central nervous system (CNS). With some exceptions (Kumazawa, 1990), the class of afferent nerve fiber is related to the type of sensory receptor it innervates, and ultimately the type of information conveyed. Most low threshold mechanoreceptors relay information to the CNS via large diameter (12-14 μm), myelinated, Aß primary afferent fibers. These neurons conduct impulses at velocities from 80 to 85 m/s (Collins *et al.*, 1960). Aδ- and C-fibers relay different types of information about the source, intensity and quality of noxious stimuli. Small diameter (1-9 μm), myelinated, Aδ axons have conduction velocities of 5 to 30 m/s, and are primarily involved in signalling strong mechanical pressure (Henry, 1989). Other less common nociceptors, with Aδ axons, can be activated by intense heat or chemical algogenic substances (Kelly, 1985; Besson and Chaouch, 1987; Henry, 1989). Aδ fibers convey more detailed information about the quality of the stimulus than C-fibers (Adriaensen *et al.*, 1983), and their activation is associated with sharp, well-localized pain (Chapman and Bonica, 1985). C-fibers have small diameter (0.5-2 μm), unmyelinated axons and conduct impulses more slowly than other primary afferent neurons (1-2.5 m/s; Collins, 1960; Henry, 1989). They are

usually polymodal, meaning that they can be activated by a range of stimuli, including noxious heat, intense mechanical pressure or chemical mediators of inflammation such as bradykinin, serotonin, histamine or potassium ions (Levine, 1984; Kelly, 1985; Henry, 1989; Dickenson, 1991). Some C-fibers also respond to noxious cold stimuli (Henry, 1989). Pain associated with C-fiber activation is not well-localized, and is characterized as a persistent stinging, burning or aching sensation (Chapman and Bonica, 1985). An important characteristic of C-nociceptors is that they can be sensitized by a previous exposure to noxious stimuli. This sensitization is manifest as a reduction in threshold and an enhanced level of responsiveness that can persist for hours following the inciting event (Besson and Chaouch, 1987).

1.2.2 *Organization of the Sensory Spinal Cord*

All primary afferent neurons have cell bodies in the dorsal root ganglia and project axons either to the spinal dorsal horn, or through the spinal cord to the brainstem. Aδ- and C-fibers, along with some Aß afferent fibers, enter the spinal cord, primarily in the dorsal horn, where they form synapses with second order neurons. These second order neurons relay nociceptive information from the spinal cord to supraspinal structures. The majority of Aß axons involved in

low threshold tactile sensation project axons directly to the dorsal medulla, although some also have collateral axons that form synapses within the spinal cord.

The gray matter of the spinal cord is composed of 10 cytoarchitecturally distinct layers or laminae (Rexed, 1954; Steiner and Turner, 1972). The most superficial layer is lamina I, while lamina X lies adjacent to the central canal of the spinal cord. High threshold Aδ-mechanoreceptors terminate mainly, but not exclusively in lamina I, with some terminations in lamina II and V (Light and Perl, 1979; Maxwell and Réthelyi, 1987). Unmyelinated C-fibers terminate mainly in laminae I and II, although some have projections to deeper layers of the spinal gray matter (Maxwell and Réthelyi, 1987). Lamina III and IV comprise the nucleus proprius, which contains numerous large diameter myelinated axons. Cells in this region are activated predominantly by innocuous mechanical stimuli, including a major input from hair mechanoreceptors (Henry, 1989).

Second order spinal neurons are either relay cells, with axons projecting to the brainstem or thalamus, or interneurons that transfer sensory information to other interneurons or relay cells. Although a variety of classification schemes exist, second order neurons may be divided into 3 general categories, *non-nociceptive neurons*, *nociceptive specific neurons* and nociceptive *non-specific*

neurons (Besson and Chaouch, 1987; Henry, 1989). Non-nociceptive neurons arise mainly from the nucleus proprius, have large receptive fields and respond optimally to innocuous stimulation of the skin. Nociceptive non-specific neurons, sometimes called WDR neurons, carry information regarding noxious and non-noxious stimuli; their firing rate is correlated with stimulus intensity (Sorkin, 1991). WDR neurons are the most common cell type in the spinal dorsal horn, and are found mainly in laminae I, II and V (Henry, 1989). As the name implies, nociceptive specific neurons respond only to signals arising from noxious stimuli. These neurons emanate mainly from the superficial zones of the dorsal horn.

1.2.3 Spinal Sensory Neurotransmission and Neuromodulation

A variety of substances have been proposed as possible neurotransmitters or neuromodulators in primary sensory neurons. Putative afferent neurotransmitters include: substance P, neurotensin, cholecystokinin, vasoactive intestinal peptide, calcitonin gene-related peptide, somatostatin, glutamate and aspartate (Besson and Chaouch, 1987; Gibson et al., 1981; Cousins and Mather, 1984; Salt and Hill, 1983). The precise roles of many of these substances have yet to be fully elucidated.

Considerable evidence suggests that excitatory amino acids (EAAs), such as glutamate or aspartate, are important neurotransmitters in sensory pathways. Glutamate immunoreactivity has been identified in primary sensory neurons in both dorsal root ganglia (DRG) and trigeminal ganglia of the rat (Wanaka *et al.*, 1987). The unique subcellular distribution of glutamic oxaloacetic transaminase isozymes in sensory ganglia, combined with elevated levels of glutaminase (Cangro *et al.*, 1985), would provide the enhanced production and turnover of glutamate required for a neurotransmitter role. Furthermore, release of aspartate and glutamate into the spinal extracellular space of the rat spinal dorsal horn is evoked by peripheral noxious stimulation with intradermal formalin (Skilling *et al.*, 1988).

The postsynaptic actions of EAAs provide further support for a neurotransmitter role. Iontophoretic application of *L*-glutamate, *L*-aspartate, *DL*-homocysteine or *N*-methyl-*D*-aspartate (NMDA) to the spinal cord of anesthetized rats or cats produced depolarization resulting in increased interneuron firing rates (Engberg and Ryall, 1966; Anis *et al.*, 1982; Biscoe *et al.*, 1976). Investigations using specific excitatory amino acid receptor antagonists and extracellular single unit recordings revealed that both NMDA and non-NMDA receptors mediate non-noxious afferent input,

while only NMDA receptors are involved in transmission of nociceptive information (Radhakrishnan and Henry, 1993). These observations are supported by behavioral data indicating that NMDA receptor antagonists produce analgesia following i.t. administration to mice (Näsström *et al.*, 1992). However, the same study demonstrated antinociceptive effects with selective non-NMDA receptor antagonists (Näsström *et al.*, 1992), and other studies suggest that NMDA antagonism prevents hyperalgesia but not nociceptive pain (Yamamoto and Yaksh, 1992). Thus, while EAA receptor agonists appear to serve as neurotransmitters in sensory primary afferents, the specific roles of the receptor subtypes involved require further clarification.

Substance P is the most extensively studied neurotransmitter candidate for nociceptive pathways. The highest density of substance P immunoreactivity (IR) is found in regions of the spinal dorsal horn which are known to contain terminals of primary afferent neurons (Ljungdahl *et al.*, 1978; Gibson *et al.*, 1981). In addition, substance P is present in primary afferent neurons, since it is depleted following rhizotomy and released with antidromic nerve stimulation (Jessell *et al.*, 1979; Levine, 1984; Lembeck and Holtzer, 1979). Furthermore, substance P release is evoked by electrical nerve stimulation at intensities great enough to recruit Aδ- or C-fibers (Yaksh *et al.*, 1980), and

following noxious but not non-noxious mechanical stimuli (Kuraishi *et al.*, 1985). Substances that produce spinal analgesia, including morphine and norepinephrine, inhibit both the release of endogenous substance P (Pang and Vasko, 1986) and the effects of exogenous substance P on animal behaviour (Hylden and Wilcox, 1983). Although these observations have led to the proposal that substance P is a neurotransmitter subserving nociception, a more accurate description would be neuromodulator. In electrophysiological studies, i.t. substance P enhanced the excitability of spinal cord neurons in response to noxious stimuli (Wiesenfeld-Hallin, 1986). The slow onset and termination of the effect (several minutes) relative to classical neurotransmitters, and the indirect facilitating action, suggest that substance P is a neuromodulator rather than a neurotransmitter in nociceptive primary afferent neurons.

Synapses between sensory primary afferent neurons and second order neurons are subject to inhibitory modulation by a number of endogenous systems. Local inhibition of both somatosensory and nociceptive neurotransmission in the spinal dorsal horn is mediated by interneurons releasing γ-aminobutyric acid (GABA) or glycine (Game and Lodge, 1975; Désarmenien *et al.*, 1984; Duggan and Foong, 1985). This type of local inhibition will be discussed in detail in a later section. Endogenous opioids such as enkephalin are present

in spinal interneurons and mediate selective inhibition of nociceptive input (Lamotte *et al.*, 1976; Ruda, 1982; Willcockson *et al.*, 1984b). In addition, synapses in nociceptive pathways are subject to descending inhibitory modulation from supraspinal sites. Examples of descending inhibitory systems include a serotonergic system arising from the nucleus raphe magnus (Hammond *et al.*, 1980; Du *et al.*, 1984; Hammond and Yaksh, 1984; Hammond *et al.*, 1985), and a ponto-spinal noradrenergic system whose major origin is the locus coeruleus (LC; Segal and Sandberg, 1977; Westlund *et al.*, 1982; Mokha *et al.*, 1985).

1.2.4 Supraspinal Projections

1.2.4.1 Dorsal Column-Medial Lemniscal System

The dorsal column-medial lemniscal system (Fig. 1.1) mediates tactile sense and limb proprioception. The dorsal columns consist mainly of the central branches of DRG cells which ascend, without synapsing, to the dorsal column nuclei of the medulla (cuneate and gracile nuclei). A small percentage of these axons arise from second order neurons of the spinal dorsal horn (Martin, 1985a). Afferent neurons from the caudal extremities synapse in the gracile nucleus, while those of the rostral extremities form synapses in the cuneate. Within each of these tracts, somatotopy is

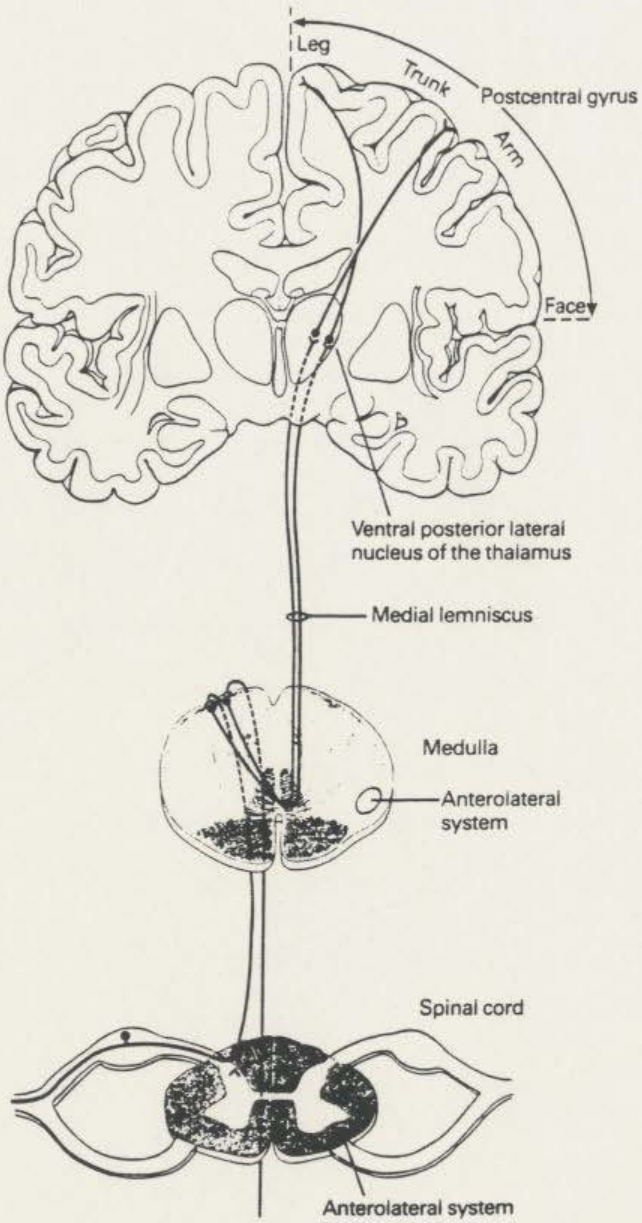


Fig. 1.1 The dorsal column-medial lemniscal system. Information about somatic sensation is carried through the spinal cord by the long axons of dorsal root ganglion cells which terminate in the dorsal column nuclei of the medulla. In the medulla, the cells of the dorsal column nuclei project axons across the midline with terminations in the contralateral ventral posterolateral nucleus of the thalamus. This nucleus, in turn, sends an extensive projection to the primary somatosensory cortex. Source: Modified from Kandel, 1985.

preserved, since fibers are added to the lateral portions of each tract in an orderly manner, and the location of a fiber in a tract is related to the region of the body it innervates. The postsynaptic fibers from the dorsal column nuclei cross the midline at the level of the medulla, as the internal arcuate fibers, and ascend through the brainstem to the ventral posterior thalamus, as the medial lemniscal system (Martin, 1985a). Third order neurons of this tract project to several discrete receiving areas of the primary somatosensory cortex in the postcentral gyrus. Here, further processing occurs that ultimately contributes to conscious perception of the features of the stimulus. Thus, under normal conditions, information about innocuous tactile stimulation is conveyed to specific sites in the brain by discrete neural pathways, which are not believed to be involved in pain perception.

1.2.4.2 The Anterolateral System

Second order neurons that carry nociceptive information are situated in several ascending tracts of the spinal cord. The anterolateral system (Fig 1.2) is the most extensively studied and well-characterized nociceptive pathway. It is also involved in thermal and crude touch sensation, but discussion of these functions is beyond the scope of this

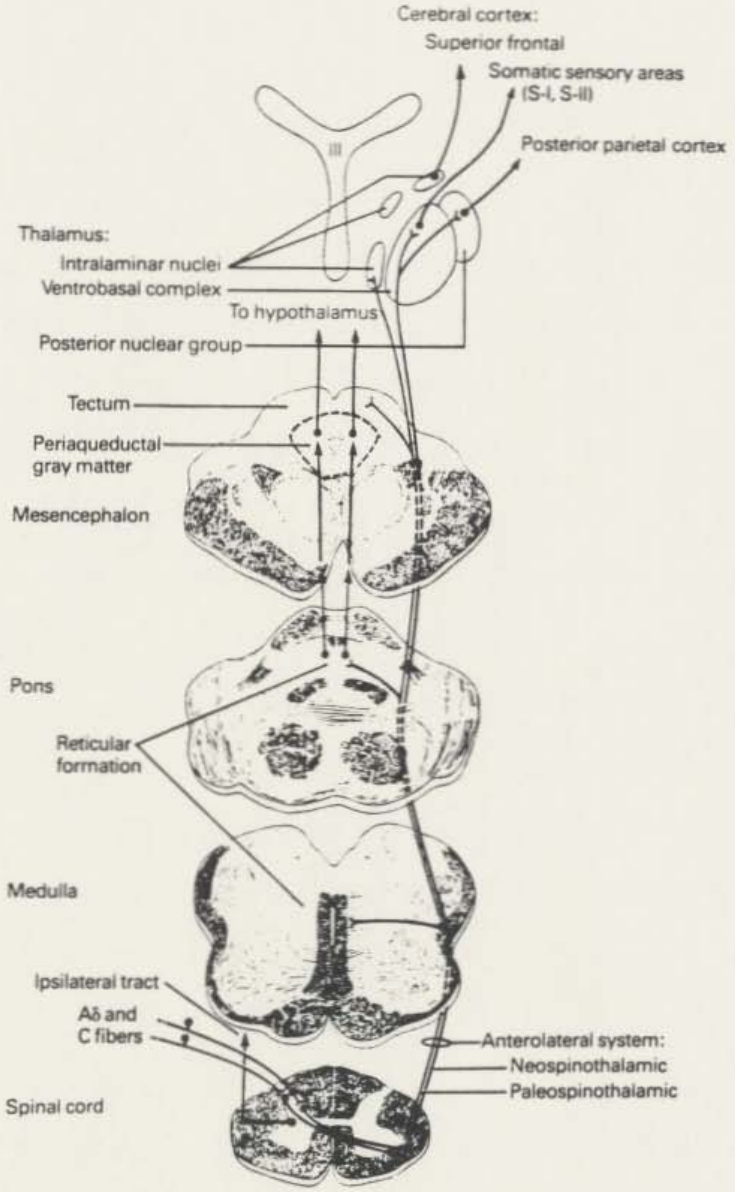


Fig. 1.2 The Anterolateral System. The neospinothalamic tract and the paleospinothalamic tract were classically considered to be the major divisions of the anterolateral system. This system conveys information about pain to a number of regions in the brainstem and diencephalon. Source: Kelly, 1985.

thesis. The anterolateral system has classically been subdivided into two major components, the neospinothalamic tract (nSTT) and the paleospinothalamic tract (pSTT; Kelly, 1985). Although the division of the anterolateral system into nSTT and pSTT is somewhat artificial and mainly of historical interest, it is used here as a reminder of the multiplicity of afferent nociceptive pathways.

The cell bodies of nSTT neurons are found primarily in lamina I and IV-VI of the dorsal horn, and receive their principal input from Aδ primary afferent fibers with terminals in the nucleus proprious (laminae III and IV). Functionally, these neurons are both WDR and nociceptive-specific (Henry, 1989). nSTT axons ascend in the lateral part of the ventrolateral funiculus. Most of these fibers cross in front of the central canal and ascend directly to the thalamus (Chapman and Bonica, 1985). This crossing results in a rough somatotopic organization, in that the lower parts of the body are represented by lateral ascending fibers and the upper parts by medial ascending fibers. Of the several thalamic nuclei receiving these inputs, most go to the ventral posterolateral nucleus, the medial part of the posterior complex, the intralaminar nuclei and the submedian nucleus (Henry, 1989). Cells from the ventral posterolateral nucleus of the thalamus project, in a somatotopic manner, to cortical areas SI and SII, which are important for sensory

discrimination (Kelly, 1985).

The pSTT is a multisynaptic system with terminations in the intralaminar and medial nuclei of the thalamus (Chapman and Bonica, 1985). Since these nuclei project to limbic regions, in addition to the frontal and parietal cortex, the pSTT is believed to be important in initiating the affective component of pain (Sorkin, 1991). This pathway is also thought to mediate diffuse, slow, burning pain (Kelly, 1985). Since many pSTT axons project to the brainstem reticular formation and the midbrain periaqueductal gray (PAG), the paleospinothalamic system has often subdivided into the pSTT proper, spinoreticular tract (SRT) and spinomesencephalic tract (SMT). SRT cells are mainly nociceptive specific, and arise predominantly from lamina V of the dorsal horn (Henry, 1989). The terminations of the SRT include the gigantocellular nucleus, the nuclei reticularis pontis, caudalis and oralis, the nucleus subcoerulus, and the cuneiform nucleus (Zemlan *et al.*, 1978). The cells of origin of the SMT are found primarily in lamina I and include nociceptive specific, WDR and non-nociceptive neurons. Fibers of this tract ascend ipsilaterally and contralaterally and terminate in the PAG, the cuneiform nucleus and other mesencephalic regions (Henry, 1989).

In addition to the nSTT and the pSTT, several other spinal tracts are believed to play a role in nociceptive

transmission, and a number of different pain modulating systems have been described. These have been the subject of several extensive reviews (Dubner and Bennett, 1983; Besson and Chaouch, 1987; Henry, 1989; Sorkin, 1991). It is evident from this cursory description, that the processing of somatosensory and nociceptive information is complex, involving several parallel but discrete systems which extend throughout the neuraxis, multiple neurotransmitters and several levels of regulation.

1.3 Neural Injury Pain

1.3.1 General Features

Neural injury pain refers to a syndrome that develops in a subgroup of patients following neural injury or pathology. Examples of N.I.P. include phantom limb pain; central post-stroke pain; diabetic, alcoholic, nutritional, traumatic or cancerous neuropathy; anterior spinal artery syndrome; post-herpetic neuralgia; reflex sympathetic dystrophy; plexus avulsion; postcordotomy dysesthesia and painful conditions associated with paraplegia and multiple sclerosis (Shibasaki and Kuroiwa, 1974; Boivie *et al.*, 1989; Tasker, 1990; Portenoy *et al.*, 1990; Tanelian and Brose, 1991; Price *et al.*, 1992; Triggs and Beric, 1992; Baron and Saguer, 1993).

N.I.P. develops in 1 of 15,000 strokes, 7-40% of traumatic spinal lesions, approximately 10% of herpes zoster infections and approximately 17% of multiple sclerosis cases (Shibasaki and Kuroiwa, 1974; Tasker, 1990; Rowbotham *et al.*, 1991). Thus, it is a relatively rare and idiosyncratic outcome of neural injury (Noordenbos and Wall, 1981; Arnér and Meyerson, 1988; Tasker, 1990). In those patients affected, however, it is extremely debilitating and often intractable (Rowbotham *et al.*, 1991; Arnér and Meyerson, 1988; Baron and Saguer, 1993).

N.I.P. is frequently described by patients as a burning, ripping, tearing, pressing or twisting pain; terms associated with physical injury. Patients are often unable to identify or locate the inciting stimulus, and radiation of sensation, abnormal temporal summation, and after-sensations are frequent sequelae of this syndrome (Lindblom and Verrillo, 1979; Noordenbos and Wall, 1981; Price *et al.*, 1992). Pain often coexists with sensory loss in the same cutaneous region (Baron and Saguer, 1993). Unlike nociceptive pain, the onset is usually delayed for weeks or months following the causative event (Tasker, 1990). For example, 82% of patients with spinal cord lesions experienced a delay in the onset of pain, which ranged from less than a month to more than one year after injury (Tasker *et al.*, 1992).

1.3.2 Current Treatments for Neural Injury Pain

N.I.P. responds poorly to all currently available pain therapies. A number of clinical studies have reported insufficient pain control, using a variety of opioid analgesics, at doses suitable for nociceptive pain (Arnér and Meyerson, 1988; Dubner, 1991; Triggs and Beric, 1992). However, the lack of opioid efficacy in the management of N.I.P. is controversial (Arnér and Meyerson, 1988; 1991; Du Pen and Williams, 1991; Portenoy *et al.*, 1990; 1991; Rowbotham *et al.*, 1991), with some authors arguing that significant analgesia can be obtained if opioid doses are carefully titrated until the appearance of either analgesia or unmanageable side effects (Portenoy *et al.*, 1990). Interpretation of studies like these is complicated by the absence of appropriate control groups (Dubner, 1991), disagreement over a suitable clinical analgesic endpoint (Arnér and Meyerson, 1991; Du Pen and Williams, 1991; Portenoy *et al.*, 1991) and an extremely high incidence (≈ 60%) of placebo responders in the N.I.P. patient population (Verdugo and Ochoa, 1991; Ochoa, 1993). The elusive nature of this problem is exemplified by a double-blind, placebo-controlled study in which patients were asked to rate their pain along sensory and affective dimensions. Morphine dose-dependently reversed the pain *affect* scores in the neuropathic pain groups, while pain sensory ratings

remained unchanged (Kupers *et al.*, 1991).

Despite the controversy, a reasonable consensus has developed concerning certain aspects of N.I.P. and the role of opioid analgesics: 1) A scientific basis exists for a rightward shift of the opioid dose-response curve in N.I.P.; neuropathic pain is generally more severe than other forms of pain (Arnér and Meyerson, 1988; Tasker, 1990; Rowbotham *et al.*, 1991). 2) There is evidence indicating that some types of N.I.P. are mediated by neural substrates not normally involved in nociception (Campbell *et al.*, 1988; Portenoy *et al.*, 1990). 3) N.I.P. is believed to have multiple pathophysiological mechanisms, which can exist separately or in various combinations in different patients (Loeser, 1981; Campbell *et al.*, 1988; Price *et al.*, 1989; Price *et al.*, 1992; Dubner, 1991; Gracely *et al.*, 1992; Koltzenburg *et al.*, 1992; Baron and Saguer, 1993). As a consequence, it is difficult to predict how individual patients will respond to opioids. 4) Most investigators now agree that opioids should be tested on a patient-to-patient basis, and that doses should be titrated appropriately before labelling pain as "refractory", and thereby denying a patient the possible benefits of opioid therapy (Arnér and Meyerson, 1988; 1991; Du Pen and Williams, 1991; Portenoy *et al.*, 1991; Rowbotham *et al.*, 1991). The fact remains however, that many patients with N.I.P. are not helped by high doses of opioids, which

cause sedation, confusion and dysphoria (Du Pen and Williams, 1991; Portenoy *et al.*, 1991). Moreover, very high doses of opioids may exacerbate, or even cause, dysesthesia (Sjøgren *et al.*, 1993).

Members of several other drug classes have been used in attempts to control N.I.P., with varying degrees of success. Tricyclic antidepressants, anticonvulsants and barbiturates are moderately effective in some forms of N.I.P. (Shibasaki and Kuroiwa, 1974; Tasker, 1990; Rowbotham *et al.*, 1991), however, the response is often only temporary (Watson *et al.*, 1988), and certain types of N.I.P. appear to be completely refractory to these agents (Triggs and Beric, 1992; Baron and Saguer, 1993). Temporary relief is usually obtained with local anesthetic blockade, but not by surgical interruption at the same site (Watson *et al.*, 1988; Tasker, 1990). Administration of acute i.v. lidocaine or chronic oral mexiletine to patients with demonstrated CNS lesions resulted in improved pain ratings and a reduction in mechanical hyperalgesia (Marchettini *et al.*, 1992; Edmondson *et al.*, 1993). The site of action of these agents is postulated to be in the CNS rather than the peripheral nerve since the dose used does not affect peripheral nerve transmission, and direct injection into peripheral nerves abolished evoked pain and sensory function, but did not relieve spontaneous pain (Marchettini *et al.*, 1992). However, adequate control groups

were not included, and some patients reported pain relief for up to 25 *days* after a single i.v. lidocaine treatment (Edmondson *et al.*, 1993). The actions of lidocaine, carbamazepine and mexiletine in N.I.P. are believed to be due to use-dependent sodium channel blockade. Use-dependent sodium channel blockers are thought to target nerves with abnormal spontaneous activity, while sparing conduction mechanisms in normal nerves (Tanelian and Brose, 1991). Considering the high incidence of placebo responders, the lack of appropriate controls, and the absence of long-term, carefully controlled trials, the true effectiveness of these putative therapies cannot be adequately assessed at the present time.

Surgical interventions intended to alleviate N.I.P., including neurectomy, rhizotomy, cordotomy, cordectomy and other destructive procedures applied to the neuraxis, usually provide temporary, incomplete pain control at best, and the pain eventually returns (Tasker, 1990; Tasker *et al.*, 1992). The short term benefit of some forms of surgery can be very misleading, but after some time the ineffectiveness of this approach becomes apparent (Noordenbos and Wall, 1981). Successful surgical interventions have been reported in patients with certain types of N.I.P. (Tasker *et al.*, 1992). However, steady pain, the most common form of pain suffered by patients with spinal injury, responded poorly to

destructive surgeries such as cordotomy, cordectomy and dorsal root entry zone (DREZ) surgery (Tasker *et al.*, 1992). Thus, a number of approaches to treating N.I.P. have been attempted with some success, but the vast majority of patients are inadequately controlled by available therapies (Tasker, 1990; Dubner, 1991; Triggs and Beric, 1992).

1.3.3 Pathophysiological Mechanisms of Neural Injury Pain

The etiology of the somatosensory dysfunction which leads to the development of dysesthesiae is unknown. Sensitization of primary nociceptors, ephaptic neural connections, spontaneous activity in a neuromata or regenerating axons, central disinhibition (due to loss of local or descending modulatory systems) and possible secondary sensitization of central nociceptive pathways have been proposed (Loeser, 1981; Wall, 1984; Ochoa, 1993). Growing evidence supports the involvement of multiple pathophysiological mechanisms (Loeser, 1981; Campbell *et al.*, 1988; Price *et al.*, 1989; Price *et al.*, 1992; Dubner, 1991; Gracely *et al.*, 1992; Koltzenburg *et al.*, 1992; Baron and Saguer, 1993).

In some patients, there is an inappropriate exaggeration of responses triggered by nociceptive afferent fibers leading to hyperalgesia. This abnormality may involve sensitized

nociceptors and/or an abnormal central facilitatory mechanism (Price *et al.*, 1992). Other patients exhibit allodynia which appears to be initiated by Aß primary afferent fibers (*vide infra*). Alterations in temporal summation of repetitive stimuli appear to be involved in both dysesthesia and spontaneous ongoing pain (Price *et al.*, 1992). Moreover, these general mechanisms may not be mutually exclusive, since Aß-fiber-initiated allodynia, heat hyperalgesia and slow temporal summation coexist in some patients or appear in various permutations in others (Price *et al.*, 1992).

1.3.3.1 Postulated Mechanisms of Allodynia

The ability of light tactile stimuli to trigger perception of pain implicates large diameter myelinated afferent fibers in initiating allodynia (Price *et al.*, 1992). This concept is supported by direct evidence from clinical studies (Campbell *et al.*, 1988; Koltzenburg *et al.*, 1992; Price *et al.*, 1992; Baron and Saguer, 1993). Following nerve block by ischemia or nerve compression, allodynia was eliminated concurrently with light touch sensation on adjacent normal skin (Campbell *et al.*, 1988; Koltzenburg *et al.*, 1992), suggesting that both sensations were mediated by the same neural elements; namely Aß-fibers. Temperature

discrimination was unaffected in these patients, signifying that functional Aδ-and C-fibers did not mediate the allodynia (Campbell *et al.*, 1988). Response latencies for detection of mechanical stimuli indicated that the conduction velocity for detection of pain in the nerve-injured limb was similar to that for detection of touch in the normal limb. Both latencies were much faster than the latency for detection of pain in the normal limb (Lindblom and Verrillo, 1979; Campbell *et al.*, 1988; Gracely *et al.*, 1992). Additional evidence for the involvement of Aß fibers in allodynia is derived from the observation that high-frequency, low-intensity electrical nerve stimulation exacerbates allodynia, rather than eliciting the analgesic effect seen with nociceptive pain (Price *et al.*, 1992). Furthermore, the degree of *impairment* of C-fiber function is positively correlated with the intensity of allodynia (Baron and Saguer, 1993).

Several mechanisms have been proposed to explain allodynia evoked by light touch. The existence of myelinated nociceptors with conduction velocities in the Aß range has led to speculation that these nociceptors might be involved in the production of allodynia. However, nociceptors do not normally have sufficiently low thresholds to be activated by light tactile stimuli (Campbell *et al.*, 1988). Another possible mechanism was suggested by a study of patients with

Aß-mediated allodynia in cutaneous regions that were physically separated from sites of spontaneous pain (Gracely *et al.*, 1992). These investigators proposed that Aß allodynia was maintained dynamically by ongoing activity from nociceptive afferent input that produced spontaneous pain in a separate cutaneous region. However, this mechanism does not account for observation that Aß-mediated allodynia can be evoked at a time when Aδ- and C-fibers are differentially blocked with local anesthetic (Campbell *et al.*, 1988), nor can it explain the occurrence of allodynia in neuropathic pain patients with normal C-fiber function (Price *et al.*, 1989). While this mechanism may be active in some cases of allodynia, it is clearly not a generally applicable mechanism. Another possibility is that allodynia is the result of cross-activation ("cross-talk") between large diameter primary afferent fibers and nociceptors at the site of injury. However, because the latency for detection of pain is short, the possibility of a link between Aß and C fibers within the peripheral nerve trunk is unlikely (Campbell *et al.*, 1988).

Another proposed mechanism of Aß allodynia, which is supported by considerable experimental evidence, is that central changes occur in the dorsal horn of the spinal cord (or higher centers) following nerve injury, leading to strengthened synaptic ties between low-threshold mechano-

receptive afferent fibers and pain-signalling pathways in the CNS. Aß afferent neurons terminate in laminae III to V of the spinal cord where they form synapses with second-order, WDR neurons. WDR neurons also receive convergent excitatory input from small diameter myelinated (Aδ) and unmyelinated, (C-fiber) nociceptive afferent neurons, and are thought to be involved in the transmission of pain (Mayer *et al.*, 1975). The perception of a stimulus as being tactile or noxious appears to be governed by the balance of excitatory (e.g. glutamate, substance P, calcitonin gene related peptide) and inhibitory (glycine, GABA, taurine) inputs influencing the discharge of these second-order neurons (Skilling *et al.*, 1990; Henry, 1989; Kangrga *et al.*, 1990). Disturbances in synaptic activity between low threshold afferent fibers and spinal WDR neurons could lead to the miscoding of this Aß afferent input and ultimately to the perception of pain. These synaptic links might ordinarily be present but not effective enough to activate central pathways under normal conditions (Campbell *et al.*, 1988). Alternatively, changes in dorsal horn circuitry have been observed following both central and peripheral nerve lesions (Woolf, 1983; Woolf *et al.*, 1992; Bennett and Xie, 1988; Tureen, 1936; Davidoff *et al.*, 1967), and this new circuitry might provide an inappropriate link between low threshold input and pain processing mechanisms in the CNS.

1.3.3.2 The Role of Inhibitory Neurotransmitters in Allodynia

GABA and glycine are inhibitory neurotransmitters which are important in sensory processing in the spinal dorsal horn (Nistri, 1983; Willcockson, 1984a; Désarmenien *et al.*, 1984). Glycine-containing cells are most abundant in lamina III and IV of the rat spinal cord, while some are distributed in laminae II and V. Glycine appears to co-exist with GABA in many, but not all, GABAergic cells in the dorsal horn (Todd and Sullivan, 1990). It has been demonstrated that glycinergic neurons in laminae II and III of the rat spinal cord receive a major monosynaptic input from myelinated low threshold mechanoreceptive primary afferent neurons (Todd, 1990), providing anatomical evidence for the hypothesis that these fibers activate local glycinergic neurons in the dorsal horn (Fig. 1.3). If glycinergic interneurons normally regulate the discharge of second order WDR neurons, the loss of this inhibitory regulation could result in abnormal Aß-fiber evoked firing of these WDR neurons.

Exogenously applied glycine has been shown to inhibit the discharge of identified spinothalamic tract (STT) neurons in lamina V (Willcockson *et al.*, 1984a, Game and Lodge, 1975), consistent with the identification of glycinergic terminals in the dorsal horn (Ribeiro-Da-Silva and Coimbra, 1980). Although STT neurons in lamina I may also be inhibited by glycine (Willcockson *et al.*, 1984a), the glycine

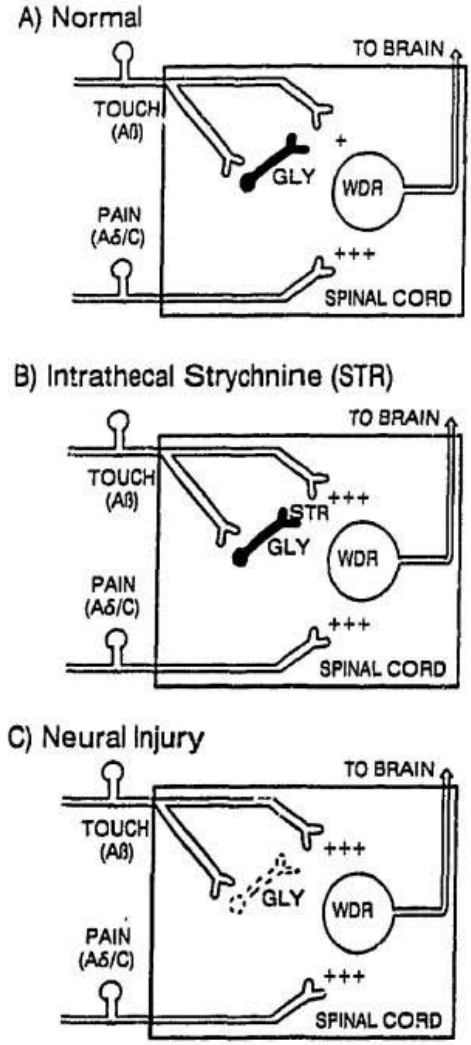


Fig. 1.3 Schematic Diagram of the Spinal Dorsal Horn Illustrating the Postulated Role of Glycine. Aß primary afferent neurons (mediating touch) and Aδ/C primary afferent neurons (mediating pain) converge on spinal wide dynamic range (WDR) neurons and their combined input determines the final message relayed to the brain. Normally (A) glycinergic interneurons modulate tactile-evoked afferent input to WDR neurons; however, following i.t. strychnine (STR; B) or neural injury (e.g. from ischemia; C) this glycinergic modulation is lost. The resulting enhanced synaptic efficacy, as indicated by the number of plus (+) signs, might cause Aß afferent input to be misinterpreted as pain.

antagonist, STR, had no effect on nociceptive neurons (presumably nociceptive specific) in this lamina (Yokota *et al.*, 1979). A difference in glycinergic control of nociceptive neurons in lamina I as compared to lamina V was suggested (Yokota *et al.*, 1979). In other studies, application of STR onto dorsal horn neurons facilitated the post-synaptic discharge evoked by afferent input, and enlarged the receptive fields for innocuous tactile (brush) stimulation (Game and Lodge, 1975; Zieglgänsberger and Herz, 1971). These data are consistent with the hypothesis that activation of glycinergic neurons in the spinal cord by low threshold primary afferent fibers regulates the discharge of WDR neurons.

Local glycinergic neurons in the spinal cord are vulnerable to ischemic and excitotoxic damage. For example, temporary aortic occlusion reduced the number of interneurons in the central gray matter of the cat spinal cord (Tureen, 1936). Changes in the content of GABA and glutamine in the spinal cord were not detected after ischemia. In contrast, the content of glycine, aspartate and glutamate was decreased, and the reductions in glycine and aspartate were reported to correlate significantly with the interneuron count (Davidoff *et al.*, 1967). These data suggest an association between glycine, aspartate, and the interneurons destroyed by ischemia, as well as a greater sensitivity of

glycine-, as compared to GABA-containing neurons. This differential sensitivity may be related to the relative abundance of GABAergic neurons when compared with those containing glycine in the spinal dorsal horn (Todd and Sullivan, 1990). The sensitivity of glycinergic spinal neurons to ischemia, and their probable role in sensory processing, is consistent with pain reported in patients having anterior spinal artery syndrome following infarction of the anterior spinal artery (Triggs and Beric, 1992). These patients exhibit painful burning dysesthesia, which develops below the level of the spinal cord lesion, and is refractory to opioid, anticonvulsant and antidepressant therapy.

A localized ischemic injury can be produced experimentally in the rat spinal cord by injecting the dye, Erythrosin B, intravenously and focusing a tunable argon laser on a discrete spinal segment. The laser irradiation causes a localized photochemical reaction with the dye, leading to a focal ischemic spinal injury. This procedure results in necrosis of laminae I-V of the affected spinal segments, hypersensitivity of WDR neurons, and cutaneous mechanical allodynia which is resistant to morphine, but sensitive to baclofen (Hao *et al.*, 1991c; 1991d).

Following peripheral nerve transection, histological evidence of post-synaptic necrosis is also present in the

dorsal horn (Sugimoto *et al.*, 1987; 1989; 1990). This effect has been attributed to massive injury discharge and subsequent excitotoxicity to postsynaptic cells. Injury discharge is a high frequency burst of activity in sensory fibers following acute injury (Wall *et al.*, 1974), the post-synaptic effects of which are blocked by NMDA-receptor antagonists (Seltzer *et al.*, 1991). EAAs are known to rise to toxic concentrations following injury to the rat spinal cord (Liu *et al.*, 1991). Thus, there is ample evidence of prominent morphological changes in the dorsal horn after nerve injury which reflect trans-synaptic changes associated with primary afferent terminal degeneration (Kapadia and LaMotte, 1987).

Collectively, the above studies demonstrate that experimentally-induced spinal cord or peripheral nerve injury in animals results in allodynia, neuropathic pain behaviour and morphological damage in the dorsal horn, including the loss of interneurons and reduction in glycine content (Davidoff *et al.*, 1967; Sugimoto *et al.*, 1990; Hao *et al.*, 1991c,d). Injury to the spinal cord or peripheral nerves is the most common event preceding clinical N.I.P. (Tasker, 1990).

1.4 The Strychnine Model of Allodynia

1.4.1 Experimental Basis for the Strychnine Model

Studies using the glycine receptor antagonist, STR, provide evidence for glycinergic dysfunction in allodynia. Microinjection of STR into the nucleus caudalis of anesthetized cats resulted in augmented responses of second order neurons to non-noxious, tactile stimuli (Khayyat, *et al.*, 1975). In naive conscious rats, light tactile stimuli such as hair deflection elicited no more than an orientation response. Following i.t. STR, however, light tactile stimuli evoked vigorous scratching and biting of the stimulation site, vocalization, attempts to escape and aggressive behaviour (Beyer *et al.*, 1985; Beyer *et al.*, 1988; Sosnowski and Yaksh, 1989; Yaksh, 1989). These observations suggest that the temporary removal of glycinergic inhibition with i.t. STR results in an allodynic state, and provide a basis for the use of i.t. STR as an experimental model of allodynia.

1.4.2 Development of an Anesthetized Animal Model of Strychnine-Dependent Allodynia

The effectiveness of i.t. STR in eliciting allodynia in conscious animals is well-documented (Beyer *et al.*, 1985; 1988; Sosnowski and Yaksh, 1989; Yaksh, 1989). In contrast, the sensory effects of i.t. STR in anesthetized animals have

yet to be fully characterized. A study of the effects of i.t. STR in urethane-chloralose anesthetized rats reported a pronounced pressor response to a normally innocuous air-jet stimulus (Yaksh, 1989), comparable to that evoked by noxious stimuli applied to the skin of normal rats. However, this investigation of STR-dependent allodynia relied on evoked pressor responses as the sole measure of allodynia, in a very limited number of animals.

STR-like, tactile-evoked motor responses have been described with anesthetic and subanesthetic doses of chloralose in the absence of STR (Alvord and Fuortes, 1954; Lees, 1972; Angel, 1986). As the level of anesthesia may have fluctuated in the Yaksh (1989) study, and the effect of repeated application of the air jet stimulus was not determined, a change in the level of arousal could account for the enhancement of the pressor response following i.t. STR with repeated application of the stimulus. An important objective of the present study was to characterize the effects of i.t. STR on responses to innocuous tactile stimuli in urethane-anesthetized rats. A major consideration was the effect of anesthesia on STR-dependent allodynia.

STR has been reported to have a number of actions on biological systems unrelated to the antagonism of glycine receptors (Barron and Guth, 1987; Bertolino and Vicini, 1988), including direct actions on Cl^- channels (Pritchard,

1971; Barron and Guth, 1987). High doses of STR have also been reported to block the actions of GABA, noradrenaline and dopamine (Curtis, *et al.*, 1971). Although these other actions of STR are observed only at concentrations several orders of magnitude greater than those required for glycine antagonism (Becker and Betz, 1987), the injection of concentrated STR solutions into the relatively small volume of rat cerebrospinal fluid (CSF) might permit some of these effects to occur following i.t. administration. Thus, the role of glycine receptors in STR-dependent allodynia was investigated in the present study using the endogenous agonist, glycine.

The present study also investigated the effects of the glycine derivative, betaine (*N,N,N*-trimethylglycine) on STR-dependent allodynia. A previous study indicated that i.t. betaine (800 μg) selectively eliminated the convulsive actions of i.t. STR without affecting STR-dependent allodynia (Beyer *et al.*, 1988). The ability of a single agent to discriminate between the sensory and convulsive actions of STR is intriguing, but imponderable since both of these actions of STR are believed to be the result of glycine receptor antagonism (Freed, 1985; Beyer *et al.*, 1985; 1988; Yaksh, 1989).

In contrast to the detailed study of altered tactile sensation after i.t. STR (Beyer *et al.*, 1985; 1988; Yaksh,

1989), the effects of i.t. STR on noxious inputs have received only cursory examination (Beyer *et al.*, 1985). By comparing the effects of STR on responses to nociceptive and non-nociceptive stimuli, the relative importance of glycinergic modulation on these types of input can be ascertained. The present study investigated the effects of i.t. STR on responses to noxious thermal, mechanical and chemical stimuli.

In view of the pivotal role of Aß fibers in clinical allodynia, the present study investigated the neural substrates involved in STR-dependent allodynia by selectively destroying C-fiber primary afferent neurons with capsaicin. Given neonatally, capsaicin destroys from 60-95% of C-fibers and from 10-30% of Aδ-fibers, with no demonstrable effect on Aß-fibers (Jancsó *et al.*, 1977; Jancsó *et al.*, 1980; Lawsen and Nickels, 1980; Jancsó and Kiraly, 1981). The only previous study examining the effects of capsaicin on STR-dependent allodynia (Yaksh, 1989) used acute i.t. capsaicin. While this capsaicin regimen does elevate nociceptive thresholds (Yaksh, 1980), the neurotoxic outcome is much less predictable than with neonatal capsaicin. For example, some animals are totally unaffected by i.t. capsaicin (Nagy *et al.*, 1981), and there is considerable variation in the onset and duration of those that do respond (Palermo, *et al.*, 1981; unpublished observations). More importantly, generalized,

non-specific damage has been described following acute spinal administration of capsaicin (Nagy *et al.*, 1981). Normally, spinal morphine blocks nociception, without affecting non-painful sensation (Lamotte *et al.*, 1976; Cousins *et al.*, 1979). This is consistent with the selective effect of morphine on Aδ- and C-fibers as determined in electrophysiological studies (Le Bars *et al.*, 1976; Kellstein *et al.*, 1990), and the ineffectiveness of opioid analgesics in managing clinical N.I.P. (Arnér and Meyerson, 1988; Triggs and Beric, 1992). To further assess the role of Aß-fibers in STR-dependent allodynia, as well as the utility of this model in mimicking opioid-resistant pain, the sensitivity of STR-dependent allodynia to acute i.t. morphine was examined.

Excitatory amino acids have a well-established role in normal sensory neurotransmission (Hill and Salt, 1982; Aanonsen and Wilcox, 1986; 1990; Jessell *et al.*, 1986; Morris, 1989; Dickenson, 1991; Näsström *et al.*, 1992; Radhakrishnan and Henry, 1993) and their involvement in pathological pain states continues to be investigated (Dickenson, 1991; Hao *et al.*, 1991b; Näsström *et al.*, 1992; Xu *et al.*, 1993). There is growing evidence that non-NMDA receptors play a principal role in allodynia but not hyperalgesia, while NMDA receptors play a role in both of these conditions. Thus, allodynia to light tactile stimuli following focal spinal ischemia is sensitive to blockade with

the amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX; Xu *et al.*, 1993) as well as the NMDA receptor antagonist MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cycloheptene-5,10-imine maleate; Hao *et al.*, 1991a; 1991b). In contrast, hyperalgesia is reversed by i.t *D*-2-amino-5-phosphono-valerate (AP5; NMDA antagonist), but not i.t. 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; non-NMDA antagonist; Coderre and Melzack, 1991). The role of NMDA receptors in STR-dependent allodynia has been demonstrated in conscious animals. STR-dependent, touch-evoked agitation was dose-dependently attenuated with MK-801, AP5, kynurenic acid, SKF10047 and ketamine (Yaksh, 1989). However, the effects of non-NMDA receptor antagonists on STR-dependent allodynia remain to be determined. The present study examined the effects of i.t. γ-*D*-glutamylglycine (DGG, a non-selective excitatory amino acid antagonist) as well as the actions of the AMPA-selective antagonist NBQX, on STR-dependent allodynia in anesthetized rats.

1.5 Rationale and Specific Research Objectives

The pathophysiology of N.I.P. is unknown. Convergent lines of evidence suggest that loss of glycinergic modulation can result in the miscoding of low threshold somatosensory

input, leading inappropriately to a nociceptive message. This mechanism might explain allodynia, an important clinical symptom of N.I.P. I.t. STR, which blocks spinal glycine receptors, induces an allodynia-like state in experimental animals, and may serve as a useful model for investigating changes in somatosensory processing which result from glycinergic dysfunction. Such a model, incorporating the use of an anesthetized animal, would mitigate the sensitive ethical issues arising from animal studies of chronic pain (Casey and Dubner, 1989). In addition, the use of anesthesia would permit invasive surgical and experimental procedures (e.g. lesioning, implantation of electrodes, use of neurotoxins, use of intense electrical or chemical stimuli).

While the effectiveness of i.t. STR in eliciting allodynia in conscious animals is well-documented (Beyer *et al.*, 1985; 1988; Sosnowski and Yaksh, 1989; Yaksh, 1989), the sensory effects of i.t. STR in anesthetized animals have received little attention. The overall goal of the present research was to characterize the spinal pharmacology of abnormal sensory processing induced by i.t. STR in the anesthetized rat.

The specific research objectives were:

1. To determine the effects of anesthesia on cardiovascular and motor responses evoked by tactile stimuli following i.t. STR.

2. To determine the dose-response relationship and time course of i.t. STR-dependent tactile-evoked responses.

3. To determine the dose-response relationships of i.t. glycine receptor agonists in reversing STR-dependent tactile-evoked responses.

4. To determine the effects of neonatal capsaicin on responses to noxious thermal, mechanical and chemical stimuli, as well as, the combination of (normally innocuous) tactile stimuli plus i.t. STR.

5. To determine the effects of i.t. STR on responses to noxious thermal, mechanical and chemical stimuli.

6. To compare the i.t. morphine sensitivity of STR-dependent responses evoked by tactile stimuli to (STR-independent) responses evoked by nociceptive stimuli.

7. To determine the dose-response relationships of i.t. administered EAA receptor antagonists in reversing STR-dependent tactile-evoked responses.

2.0 CHARACTERISTICS OF STRYCHNINE-DEPENDENT ALLODYNIA IN THE ANESTHETIZED RAT

2.1 Introduction

As described in Chapter 1 (1.4), i.t. STR blocks spinal glycinergic inhibition and produces a reversible allodynia-like state. Thus, when conscious rats were treated with i.t. STR, light tactile stimuli provoked vocalization, aggression and other nocifensive behaviours, which are usually elicited only by a noxious stimulus (Beyer *et al.*, 1985; Beyer *et al.*, 1988; Sosnowski and Yaksh, 1989; Yaksh, 1989).

The actions of i.t. STR on somatosensory functions have not been completely characterized in anesthetized animals. A study of the effects of i.t. STR in urethane-chloralose anesthetized rats reported a pronounced pressor response to a normally innocuous air-jet stimulus (Yaksh, 1989). However, this study involved a small number of animals (1-2/treatment) and relied on the evoked pressor response as the sole measure of allodynia. Moreover, STR-like, tactile-evoked motor responses have been described with anesthetic and subanesthetic doses of chloralose in the absence of STR (Alvord and Fuortes, 1954; Lees, 1972; Angel, 1986). The present study used urethane-anesthetized rats (no chloralose) to investigate the effects of i.t. STR on responses to

innocuous tactile stimuli. Both cardiovascular and motor responses to innocuous hair deflection (HD) were determined in the presence and absence of i.t. STR.

A number of important questions have been addressed in these experiments. A major consideration was the effect of anesthesia on STR-dependent, tactile-evoked cardiovascular and motor responses. Except where the effect of anesthetic depth on evoked responses was specifically examined, a light plane of anesthesia was maintained using cortical electroencephalographic (EEG) monitoring. The time course of i.t. STR-dependent responses was determined, with particular attention given to changes in the distribution of cutaneous sensitivity with time. The current data also demonstrate the reversibility of i.t. STR-dependent responses with time, and their reproducibility with repeated i.t. STR injections. In addition, the relationship between the i.t. STR dose and the magnitude of the HD-evoked, STR-dependent responses was investigated.

STR-dependent allodynia is believed to result from the temporary removal of spinal glycinergic inhibition due to glycine receptor blockade by STR. If this is correct, then STR-dependent allodynia should be reversible with glycine receptor agonists. In the present study, the dose-response relationships for i.t. glycine and the glycine derivative,

betaine, were determined against STR-dependent allodynia. Betaine was selected for these experiments based on its reported ability to selectively eliminate the convulsive actions of i.t. STR without affecting STR-dependent allodynia (Beyer *et al.*, 1988). The ability to discriminate between the sensory and convulsive actions of STR is significant, since both of these actions of STR are believed to be due to glycine receptor antagonism (Freed, 1985; Beyer *et al.*, 1985; 1988; Yaksh, 1989).

2.2 Methods

2.2.1 Animals

All experiments were conducted using male, Sprague-Dawley rats (330-450 g at the time of experiment), obtained from Charles River (St. Constant, Canada). Animals were housed in the Animal Care Facility, with a room temperature of 22°C, a 12-h light-dark cycle (lights on 0700 h), and free access to rat chow and tap water. All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Memorial University Animal Care Committee.

2.2.2 Implantation of Intrathecal Catheters

Under halothane anesthesia, rats were fitted with i.t. catheters prepared from stretched polyethylene tubing (PE-10 pulled to ≈1.5X the original length). As previously described (Sherman *et al.*, 1988), the catheters were filled with sterile saline, inserted through the cisterna magna into the spinal subarachnoid space and guided 8.5 cm caudally (L1 termination). A fixed loop in the rostral end of the catheter was sutured to the overlying muscle and the incision closed. The rostral tip was externalized and sealed with a stainless steel plug. Animals were permitted to recover for a period of at least 3 days following the surgery and only those animals without overt signs of neurological impairment were used for experimentation.

2.2.3 Drug Administration

All drugs used for i.t. administration were dissolved in 0.9% sterile saline (Astra Pharma, Inc.), injected with a hand-held Hamilton syringe and flushed through the i.t. catheter with 8-10 μL of sterile saline. Strychnine hemisulfate (Sigma Chemical, Inc.) was administered in a volume of 4 μL to reduce rostro-caudal spread in the CSF. Betaine and glycine (Sigma Chemical, Inc.) were injected in volumes of 5 and 10 μL, respectively.

2.2.4 Acute Anesthetized Animal Preparation

On the day of the experiment, surgical anesthesia was induced with halothane, the left jugular vein was cannulated, and thereafter, anesthesia was maintained using i.v. urethane (10% w/v in saline; Sigma Chemical, Inc.). The initial urethane dose (1.1 g/kg) was infused slowly over 5-10 minutes as the anesthetic effect of halothane declined. Throughout the experiment, anesthesia was supplemented with i.v. urethane as required. The left carotid artery was cannulated for continuous monitoring of blood pressure and HR with a pressure transducer (P23XL) and polygraph (Model 79E, Grass Instruments). The incision was sutured and the animal placed in a stereotaxic frame. The incision and contact points with the ear bars were coated with 2% lidocaine gel (Astra Pharma, Inc.) to reduce basal sensory input. Cortical EEG was monitored with 2 subcutaneous needle electrodes (E2, Grass Instruments) placed 2 mm left of midline, one extending rostrally entering the skin near bregma, the other extending caudally entering the skin about 2 mm caudal to the first. Body temperature was maintained at 37°C with a thermostatically-regulated blanket (Harvard Apparatus). The animal was permitted to stabilize for 1 h following the surgical procedure. During this time, anesthesia was adjusted if required.

With urethane anesthesia, a reliable relationship has been observed between the proportion of time that the EEG is "synchronized" and the depth of anesthesia (Angel *et al.*, 1976, Lincoln *et al.*, 1980). For our purposes, light anesthesia was defined as the presence of an EEG pattern which fluctuates between high amplitude ("synchronized") and low amplitude ("desynchronized"), with high amplitude activity present for not more than 60% of the time. It has been assumed that EEG synchrony is associated with high amplitude activity. Although this is not strictly true in all cases, it is consistent with the observations of previous studies (Angel *et al.*, 1976, Lincoln *et al.*, 1980), and provides a reliable method for assessing the depth of anesthesia. The basis for the 60% cut-off is illustrated in the results section (Fig. 2.2).

2.2.5 Application of Stimuli

Two types of noxious stimuli were applied to the anesthetized animals; tail immersion (TI) and paw pinch (PP). TI involved holding a segment of the animal's tail in a 55°C water bath for a period of 10 seconds. Reflex withdrawal from the bath was prevented until the end of the 10-second interval. To apply the PP stimulus, both hind paws were gripped with hemostats at the post axial border (on the

medial surface, distal to the tarsal joint), such that the region of skin covered by each hemostat was ≈20 mm^2. The force of the pinch was produced by 500 gram weights attached to the handles of each hemostat. This resulted in a final pressure on each paw of approximately 0.7 kPa. Reflex withdrawal of the hind paws was prevented until the end of the 10-second stimulus period.

The innocuous tactile stimulus used in this study was HD, which involved repeatedly stroking the hair with a cotton-tipped applicator. By itself, this stimulus produced little or no change in heart rate (HR) or blood pressure and was not associated with any type of motor withdrawal. Following i.t. STR, the same stimulus resulted in elevation of blood pressure and HR, and an abrupt motor withdrawal response when the HD was applied to a cutaneous dermatome innervated by the spinal region affected by STR (see results). The HD stimulus was applied for a period of 2 minutes, during which the legs, flanks, lower back and tail were sequentially stroked with a cotton-tipped applicator. When a dermatome was found where this stimulus evoked a withdrawal response (after i.t. STR), the region was stroked in an oscillating motion. Oscillating stimuli evoke cardiovascular responses more effectively than a stationary stimulus (unpublished observation; Yaksh, 1989).

2.2.6 Experimental Protocols

Anesthetized rats, prepared as described above, were used for one of the following experimental protocols. In one group of animals, the level of anesthesia was purposely varied according to a number of criteria, including: presence of whisker movement, response to tactile stimuli, response to noxious PP, basal HR, basal blood pressure, and the percentage of time that the EEG was synchronized (in the absence of stimuli). Innocuous HD was applied to the legs, flanks, lower back and tail of the animal 5 minutes prior to i.t. STR (40 μg) and at 5-minute intervals for 30 minutes after i.t. STR. Maximum evoked increases in HR and mean arterial pressure (M.A.P.) during the 30-minute post-STR period were recorded. The total period of time after STR during which a motor withdrawal response could be evoked by HD was also determined (withdrawal duration). EEG was monitored for a 1-h period beginning 15 minutes prior to i.t. STR injection. The number of 1-minute intervals with synchronous EEG in each h (as determined by visual examination) was expressed as a percentage. The data were divided into groups based on the percentage of synchrony in the EEG, and these data were then used to examine the relationship between %synchrony and responses to HD in the presence of i.t. STR. Time-course data from animals

categorized as lightly anesthetized (< 60% synchrony) are also presented.

To determine the relationship between i.t. STR dose and responses evoked by HD, the above procedure was repeated with varying doses of STR (10,20,30,40 or 50 μg) or saline, and the maximum evoked increase in HR, the maximum evoked increase in M.A.P. and the duration for which a withdrawal response could be evoked were used to determine the dose-response relationship. Animals were categorized as either deeply or lightly anesthetized based on EEG synchrony. Each animal received 3-6 doses of i.t. STR (or saline) with either light or deep anesthesia, but no animal was given the same treatment twice, and subsequent doses were not administered until the effects of the previous dose had completely subsided.

As a control for experiments using i.t. glycine or i.t. betaine, each rat received i.t. saline followed either 20 or 30 minutes later by i.t. STR. Approximately 1 h after this i.t. saline-i.t STR control, each rat received either i.t. glycine (300, 600 or 1000 μg) or i.t. betaine (300, 600, 1000, 2000 μg). Twenty minutes after each betaine treatment, or 30 minutes after each glycine treatment, i.t. STR (40 μg) was administered. HD was applied to the legs, flanks, lower back and tail of the animal 5 minutes prior to i.t. STR and

at 5-minute intervals for 30 minutes after each STR administration. The pretreatment times enabled the drugs to reach their peak effects based on results of preliminary experiments. The pretreatment time for the saline-STR control was always the same as the drug being tested in the same animal. Most animals received 2 doses of either glycine or betaine. Before administration of a second dose, the i.t. saline-i.t. STR control was repeated; a second dose was only administered if the effects of the initial dose were no longer present as evidenced by a return of all responses to the level of the first i.t. saline-i.t. STR control. In animals receiving 2 doses of drug, the first dose selected was always lower than the second to reduce the time required for recovery between doses and to minimize the possibility of a carryover effect.

2.2.7 Data Analysis

All blood pressure data are presented as changes in M.A.P. calculated from the following equation:

M.A.P. = systolic blood pressure + 1/3 pulse pressure

Since we were interested in the responses *evoked* by different stimuli, the *change* in M.A.P. or HR has been reported relative to the immediate pre-stimulus control (*not* relative to T=0) for each point in the time-course. More

precisely, maximum HR or M.A.P. observed in the 1-minute interval before stimulus application was subtracted from the maximum value observed during stimulus application, and this difference was reported.

Statistically significant differences across several independent treatment groups (Fig. 2.2) were detected by completely randomized 1-way analysis of variance (ANOVA). For changes in M.A.P and HR occurring in the same animals over time, a one-way repeated measures ANOVA was employed (Fig. 2.4). Significant differences were identified using a Neuman-Keuls test. Time-course data for the incidence of motor withdrawal were compared with the control group by means of a modified Dunnett's test. Dose-response data (Fig. 2.5, 2.6 and 2.7) were analyzed by regression ANOVA and a modified t-test was used to determine if regression lines had significant slopes. Variability associated with single measurements is indicated by standard errors of the mean (S.E.M.), while variability associated with blocks of data is indicated by pooled 95% confidence intervals (C.I.). ED_{50} values and 95% C.I. were determined for the glycine and betaine dose-response curves. Methods of data analysis were based on general texts (Box *et al.*, 1978; Tallarida and Murray, 1987; Winer *et al.*, 1991).

2.3 Results

2.3.1 General Observations Following i.t. Strychnine

Stroking the legs, flanks, lower back and tail of an anesthetized rat with a cotton-tipped applicator is an innocuous event which did not elicit cardiovascular or motor responses. After i.t. STR (40 μg), the same tactile stimulus evoked an increase in HR, an elevation in M.A.P. and an abrupt motor withdrawal (Fig. 2.1). During each 2-minute stimulus period (Fig 2.1), HD was applied sequentially to the legs, flanks, lower back and tail of the anesthetized rat. Cardiovascular and motor responses could only be evoked at a few discrete sites, presumably corresponding to cutaneous dermatomes innervated by spinal segments near the STR injection site (Fig. 2.2). The sensitivity and distribution of cutaneous sites changed with time after i.t. STR administration, probably reflecting the rostro-caudal spread of STR in the subarachnoid space, and/or the elimination of the drug from the CSF.

In some animals, HD-sensitive sites were observed unilaterally. Autopsy of these animals invariably revealed that the tip of the i.t. catheter, and hence the site of STR injection, was laterally positioned in the subarachnoid space on the most responsive side (Fig. 2.2). These observations indicate that STR-dependent, HD-evoked responses were

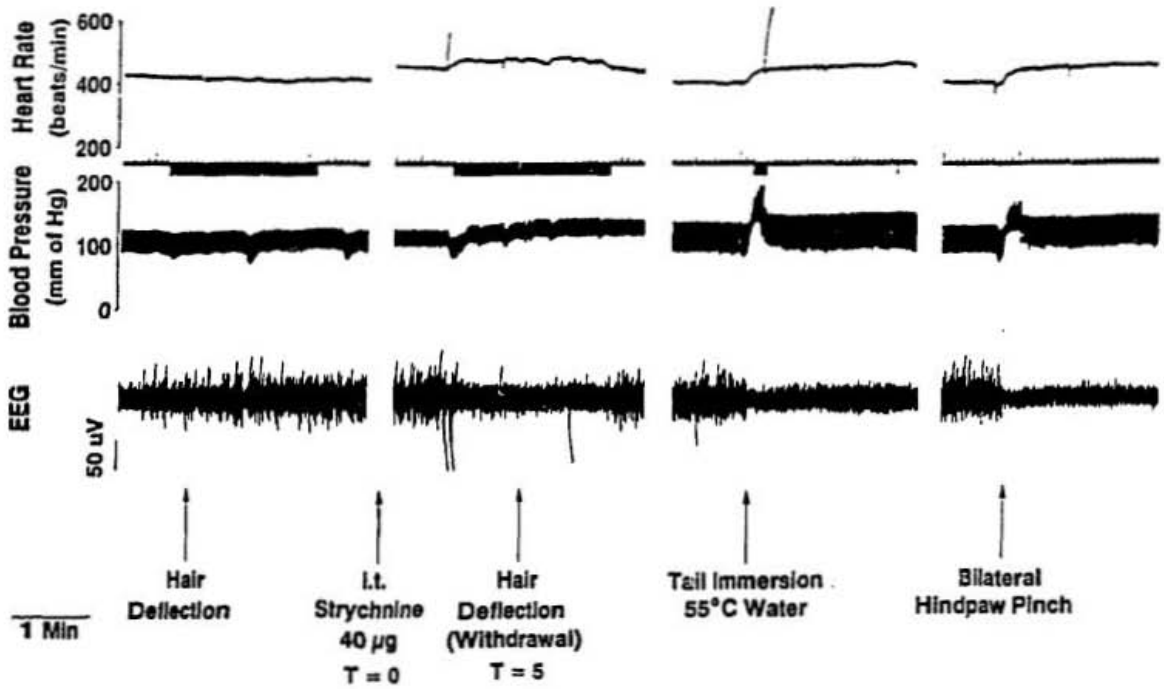


Fig. 2.1. Sample polygraph tracings of heart rate (HR), blood pressure and EEG in urethane-anesthetized rats. The 2 panels on the left demonstrate responses to (non-noxious) hair deflection (HD) applied to the legs, flanks and back of the rat before and after 40 µg of intrathecal strychnine (i.t. STR). HD in the absence of i.t. STR had little effect on HR, blood pressure, or EEG. Note the increase in HR and blood pressure, and the desynchrony of the EEG evoked by the HD stimulus 5 minutes after i.t. STR (panel 2). For comparative purposes, typical responses to noxious stimuli have been depicted in the 2 panels on the right. Both noxious paw pinch and noxious tail immersion resulted in an increase in HR, a rise in blood pressure and desynchronization of the EEG. The horizontal bars below the HR tracing indicate the time of application of each stimulus.

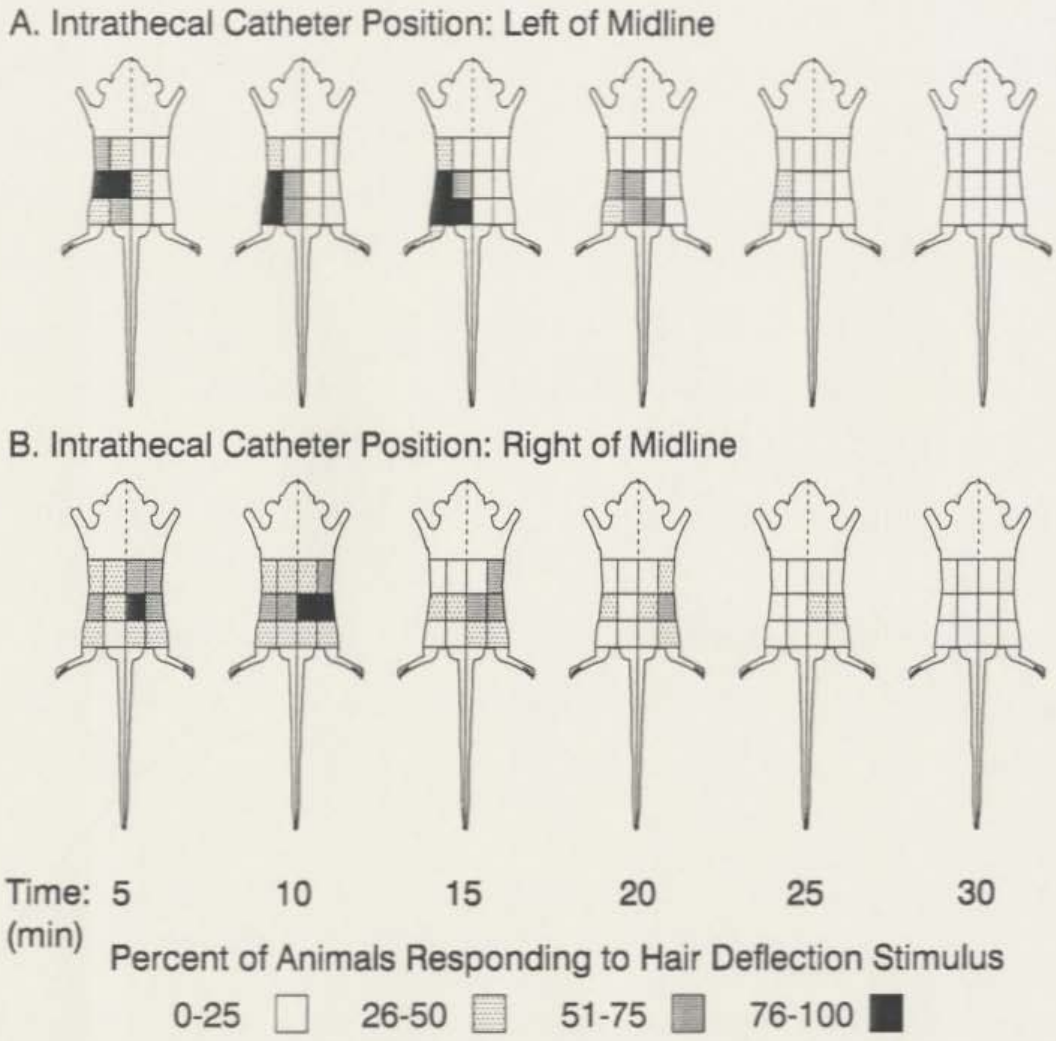


Fig. 2.2 Time-course of intrathecal (i.t.) strychnine (STR)-dependent motor withdrawal responses evoked by hair deflection (HD) as determined by the area of abnormal cutaneous sensitivity. The percent of anesthetized rats (shading) exhibiting a motor withdrawal response to HD applied within a (rectangular) cutaneous region is shown as a function of time after i.t STR (40 μg). The rostro-caudal distribution of sensitivity to HD suggests that i.t. STR affects discrete spinal segments, rather than eliciting generalized spinal disinhibition. The data presented are from rats with i.t. catheter tips located either to the left (A.; N = 9) or right (B.; N = 18) of midline at the lumbar enlargement (> 2 mm from the midline). Note that a greater percentage of animals exhibit sensitivity to HD in cutaneous regions ipsilateral to the i.t. catheter tip (which presumably corresponds to the site of STR delivery at T = 0).

segmental, and that the segmental nature of these responses was a result of the distribution of STR in the spinal subarachnoid space. When 40 μg of STR was injected i.v., HD was ineffective in evoking cardiovascular or motor withdrawal responses (unpublished observations). Doses up to 200 μg of i.v. STR produced similar outcomes, while 300 μg elicited convulsions. These i.v. data provide further evidence for a spinal action of i.t. STR, rather than a generalized disinhibition of the CNS.

The cardiovascular and motor responses dissipated rapidly when the HD stimulus was applied continuously over one cutaneous site. This effect did not appear to be related to the distribution of STR. Sustained cardiovascular and motor responses were observed using intermittent or oscillating HD stimuli. The rapid decline in responsiveness with continuous stimulation suggests that some form of adaptation was occurring to prolonged application of the HD stimulus.

2.3.2 Effects of the Depth of Anesthesia

To ensure that responses to HD were reproducible over the course of the experiment, the cortical EEG was monitored throughout all experiments to assess the depth of anesthesia

(Angel *et al.*, 1976; Lincoln *et al.*, 1980). The depth of anesthesia was adjusted empirically (see Methods), and the resulting %synchrony quantified. As illustrated in Fig. 2.3, the magnitude of each of the STR-dependent HD-evoked responses was relatively constant, provided that the EEG was synchronous for less than 60% of the test period. When the EEG exhibited synchrony for greater than 60% of the test period, responses evoked by HD in the presence of STR were significantly suppressed. On the basis of these observations, we used 60% synchrony in the EEG as an arbitrary border between "deep" and "light" anesthesia.

2.3.3 Time-Course of Strychnine-Dependent Hair Deflection-Evoked Responses

Fig. 2.4 illustrates the time-course of HD-evoked responses after 40 μg i.t. STR in lightly anesthetized animals. While spontaneous increases in HR and blood pressure were observed in some STR-treated animals prior to the application of the HD stimulus, only the *changes* in HR and M.A.P. *evoked* by HD, relative to the immediate pre-stimulus control, are presented. After 40 μg of i.t. STR, the HD stimulus evoked significant changes in HR for 20

minutes and in M.A.P. for 10 minutes. A withdrawal response could be evoked in a significant percentage of animals for 30 minutes after i.t. STR. In 6 animals, HD was applied up to 45 minutes after 40 μg i.t. STR; no statistically significant responses were observed beyond 35 minutes (data not shown).

2.3.4 Effects of Intrathecal Strychnine Dose on Responses Evoked by Hair Deflection

The relationship between the dose of i.t. STR and the magnitude of HD-evoked responses is shown in Fig. 2.5. In lightly anesthetized animals, a significant ($p < 0.01$) correlation was observed between the dose of i.t. STR and both the magnitude of cardiovascular responses and the duration of motor withdrawal. In deeply anesthetized animals, no significant correlation was found between the dose of i.t. STR and any of the evoked responses; the slopes of the regression lines did not differ significantly from zero. Within the categories of light and deep anesthesia, the mean % synchrony was quite stable across all doses of i.t. STR. These data confirm the usefulness of the 60% synchrony cut-off between light and deep anesthesia.

Spontaneous motor activity, in the form of isolated movements of the trunk and hind limbs, were often observed in

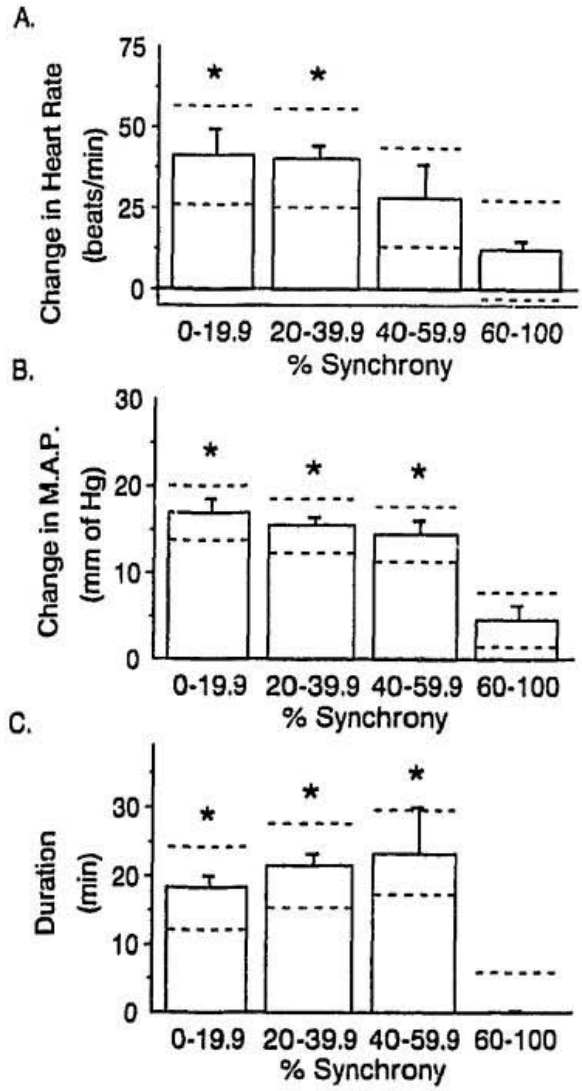


Fig. 2.3. Relationship between EEG synchrony and responses evoked by innocuous hair deflection (HD) after 40 μg of intrathecal strychnine (i.t. STR). Responses to innocuous HD after 40 μg i.t STR were determined at various depths of anesthesia (see methods). Maximum evoked increases in HR (A) and mean arterial pressure (M.A.P.; B), and the duration for which a motor withdrawal response could be evoked by HD (C) were grouped according to the percentage of synchrony in the EEG: 0-19.9% (N = 7), 20-39.9% (N = 17), 40-59.9% (N = 3) and 60-100% (N = 4). An asterisk indicates a significant difference from the 60-100% group. Error bars are S.E.M. and horizontal lines are the pooled 95% confidence intervals.

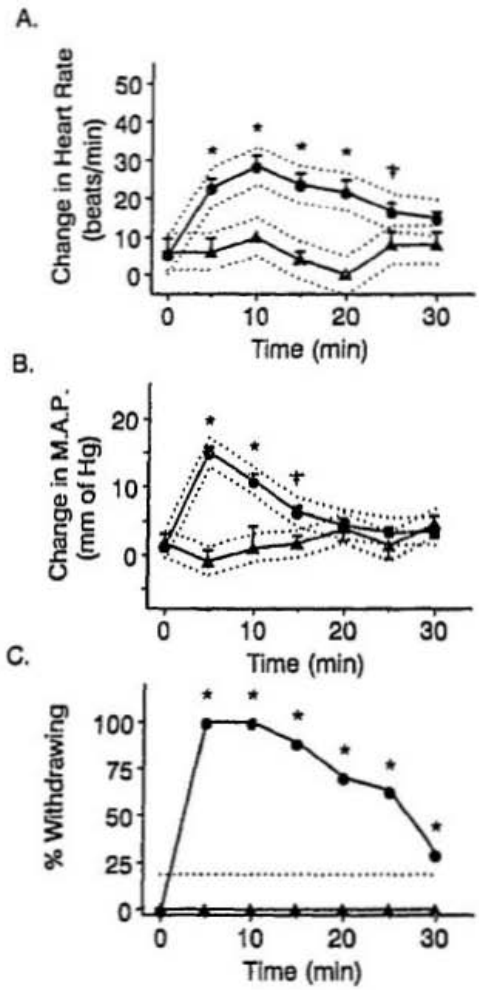


Fig. 2.4. Time-course of cardiovascular and motor responses evoked by hair deflection (HD) following intrathecal (i.t.) strychnine (STR) in lightly anesthetized rats. At t = 0, rats received either i.t. STR (● ; 40 μg, N = 28) or i.t. saline (▲ ; 15 μL, N = 5). HD was applied over the legs, flanks and back of the animal at 5-min intervals for 30 min. HD-evoked changes in heart rate (A) and mean arterial pressure (M.A.P.; B), and the % of animals exhibiting a motor withdrawal response (C) are illustrated. Asterisks indicate points significantly different from both t = 0 and from the saline control group at the corresponding time point. Daggers (ǂ) indicate a significant difference from responses at t = 0 only. Error bars depict S.E.M. and dotted lines indicate the pooled 95% confidence intervals.

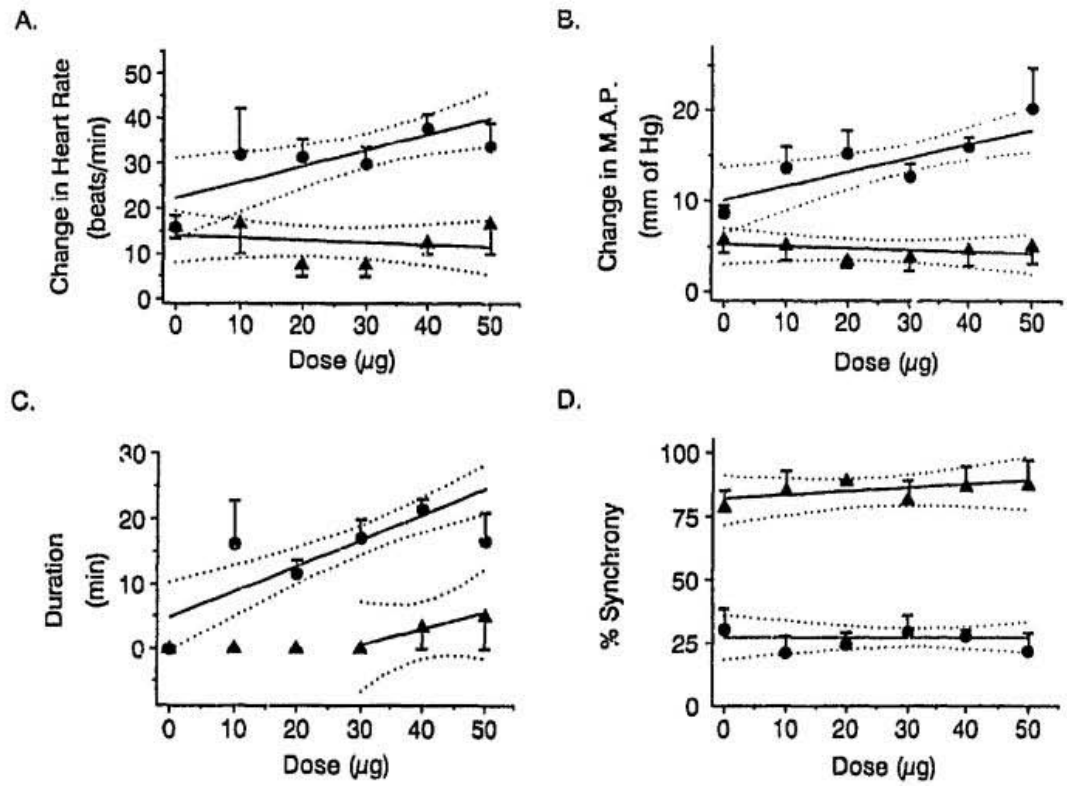


Fig. 2.5. Effect of the level of anesthesia on the relationship between intrathecal strychnine (i.t. STR) dose and responses evoked by non-noxious hair deflection (HD) in anesthetized rats. Rats which were lightly (●) or deeply (▲) anesthetized with urethane, were given one of several doses of i.t. STR and the HD stimulus was applied to the legs, flanks and back of the rat at 5-min intervals for 30 min. The maximum evoked increase in heart rate (A), the maximum evoked increase in mean arterial pressure (M.A.P.; B), the duration for which a motor withdrawal response could be evoked (C) and the % synchrony in the EEG (D) were determined for each dose and level of anesthesia. Each point represents the mean ± S.E.M. of 5-28 rats for light anesthesia and 3-5 rats for deep anesthesia. The least squares regression lines and corresponding 95% confidence intervals (dotted lines) are shown.

the first 5-10 minutes after STR injection. These responses did not appear to be convulsions as they differed considerably from the tonic extensions normally seen with high doses of STR. Rather, they resembled responses evoked by HD, and may simply have been responses evoked by contact with the bench top during respiratory movements. These responses occurred with relatively low frequency and were easily distinguished from stimulus-evoked responses which are time-linked to HD. In all instances, the HD-evoked cardiovascular and motor responses persisted for 10-20 minutes after spontaneous motor movements had ceased. Spontaneous movements occurred at doses of i.t. STR as low as 10 μg, which is well below the threshold for convulsions reported by others (Yaksh, 1989), but above the threshold for spontaneous STR-induced scratching in conscious animals (Beyer *et al.*, 1985). With doses of i.t. STR between 10 and 30 μg, tactile-evoked responses were less pronounced and tended to be of shorter duration than with the 40-μg dose. A dose of 50 μg yielded convulsive movements in a substantial number of animals. These jerking movements occurred with greater frequency and continuity than those observed with lower doses and often interfered with the measurement of sensory-evoked responses. STR doses > 50 μg could not be tested. The 40-μg dose of i.t. STR resulted in the clearest

separation of sensory-evoked responses from spontaneous motor movements and was used in the remainder of the experiments.

2.3.5 Effects of Intrathecal Glycine and Intrathecal Betaine

As shown in Fig. 2.6 and 2.7, both i.t. glycine and i.t. betaine produced dose-dependent inhibition of all indices of STR-dependent allodynia. The ED_{50} values and 95% C.I. for i.t. glycine were 609 (429-865) μg for heart rate responses, 694 (548-878) μg for elevation of M.A.P. and 549 (458-658) μg for motor withdrawal responses. With i.t. betaine, the corresponding values were 981 (509-1889) μg, 1045 (740-1476) μg and 1083 (843-1391) μg for inhibition of heart rate, M.A.P. and motor responses, respectively. It is noteworthy that the 95% confidence limits associated with both the regression lines and their ED_{50} values were reasonably narrow indicating the utility and sensitivity of this anesthetized preparation for dose-response studies. These results are the first demonstration that reproducible, touch-evoked, STR-dependent cardiovascular and motor responses can be induced in lightly-anesthetized rats with sufficient magnitude and accuracy to permit quantitative dose-response analysis.

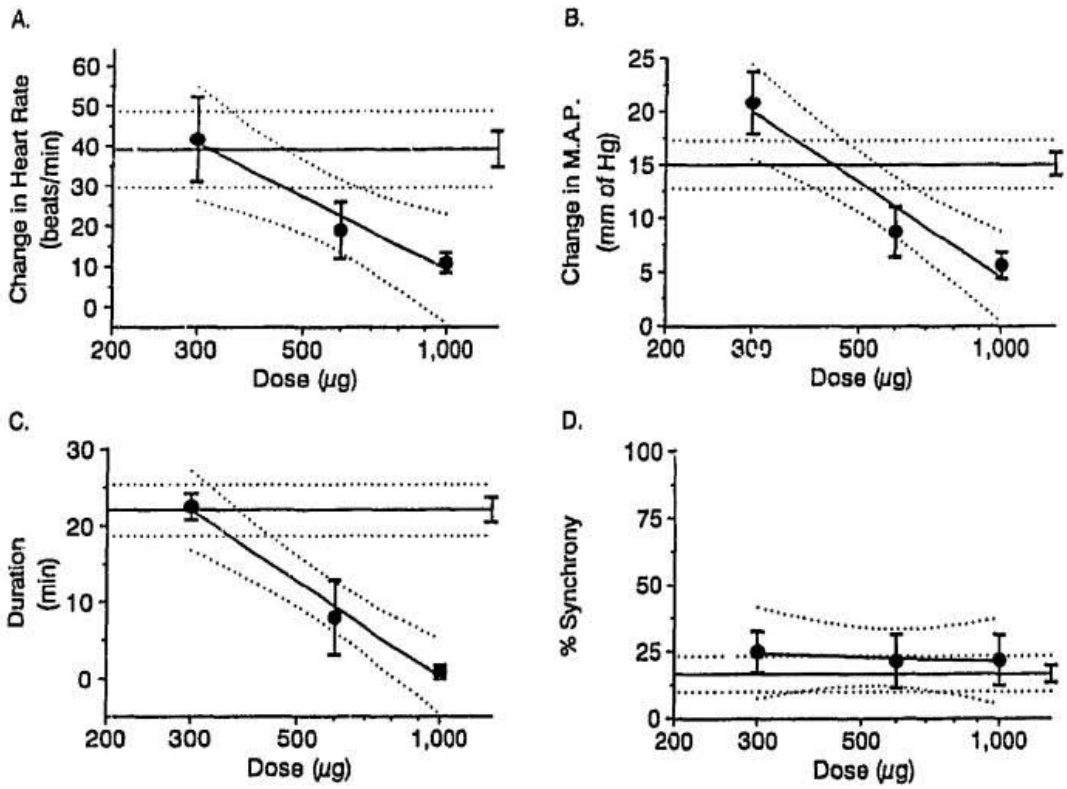


Fig. 2.6. Log dose-response relationship for intrathecal (i.t.) glycine inhibition of responses evoked by hair deflection (HD) after i.t. strychnine (STR). Responses to normally innocuous HD were determined in the presence of i.t. STR (40 μg). The maximum evoked increase in heart rate (A), the maximum evoked increase in mean arterial pressure (M.A.P.; B), the duration for which a motor withdrawal response could be evoked (C) and the % synchrony in the EEG (D) were determined following i.t. pretreatment with either saline or one of several doses of glycine. Each point represents the mean ± S.E.M. of 5 or 6 animals. Least squares regression lines and corresponding 95% confidence intervals (C.I.; dotted lines) are shown. The horizontal solid lines and adjacent dotted lines indicate the mean ± 95% C.I. of all saline treatments (N = 17).

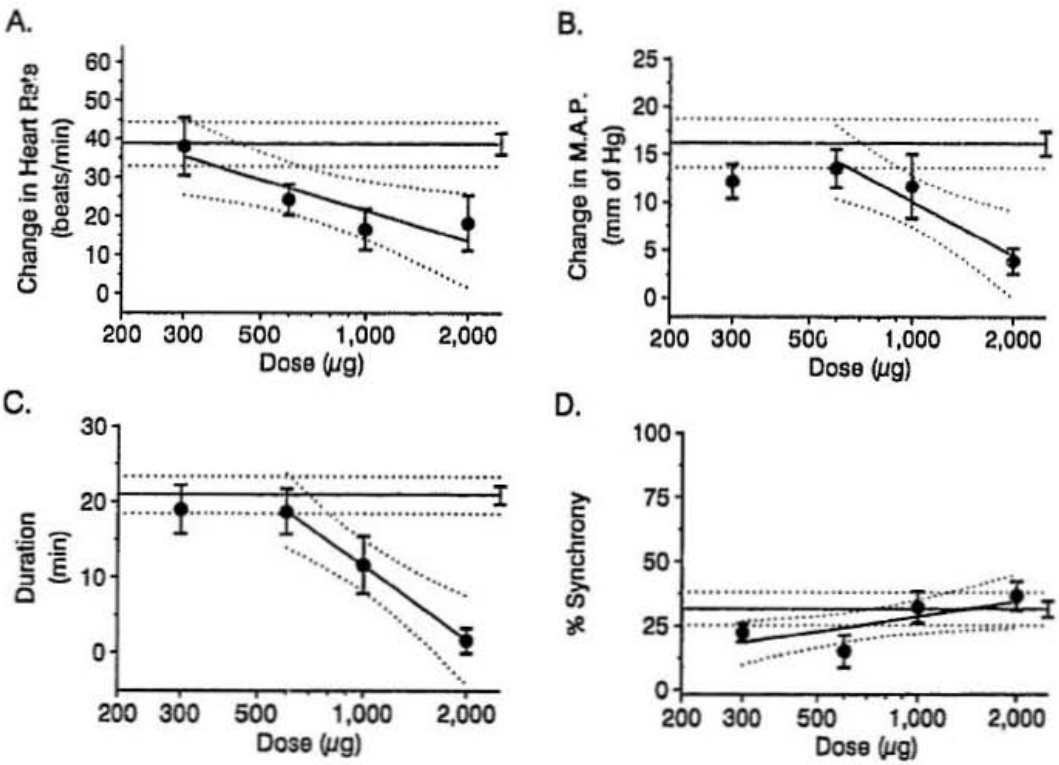


Fig. 2.7. Log dose-response relationship for intrathecal (i.t.) betaine inhibition of responses evoked by hair deflection (HD) after i.t. strychnine (STR). Responses to normally innocuous HD were determined in the presence of i.t. STR (40 μg). The maximum evoked increase in heart rate (A), the maximum evoked increase in mean arterial pressure (M.A.P.; B), the duration for which a withdrawal response could be evoked (C) and the % synchrony in the EEG (D) were determined following i.t. pretreatment with either saline or one of several doses of betaine. Each point represents the mean ± S.E.M. of 6-10 animals. Least squares regression lines and corresponding 95% confidence intervals (C.I.; dotted lines) are shown. The horizontal solid lines and adjacent dotted lines indicate the mean ± 95% C.I. of all saline treatments (N = 30).

2.4 Discussion

2.4.1 Intrathecal Strychnine Produces Acute Reversible Allodynia

Allodynia is defined by the International Association for the Study of Pain (IASP) as: "pain due to a stimulus that does not normally provoke pain" (Merskey, 1986), and is a characteristic feature of neural injury pain. The temporary blockade of spinal glycinergic inhibition with i.t. STR provides a rapid and reversible method of inducing an allodynic state resembling that seen in N.I.P. Thus, behavioral studies in rats demonstrated that after i.t. STR, a light tactile stimulus, normally eliciting only an orientation response, evokes vigorous scratching and biting of the stimulation site, vocalization, attempts to escape and aggressive behaviour (Beyer *et al.*, 1988; Sosnowski and Yaksh, 1989, Yaksh, 1989). The present study characterized the effects of i.t. STR on tactile-evoked responses in urethane-anesthetized rats. Under light anesthesia, i.t. STR, but not saline, altered responses to innocuous tactile stimulation such that HD evoked a brisk motor withdrawal response, pronounced tachycardia and hypertension. These evoked responses, suggestive of a noxious event, and consistent with the IASP definition of allodynia, were: 1) STR dose-dependent (10-50 μg, i.t.); 2) reversible with

time (over a period of 15-30 minutes); 3) reversible with i.t. glycine or betaine; 4) observed in the absence of convulsions; and 5) only evoked by HD applied to the cutaneous dermatomes whose afferent inputs enter the spinal cord near the STR injection site (segmentally localized).

2.4.2 Effects of Anesthesia on Strychnine-Dependent Responses to Tactile Stimulation

We systematically analyzed the effects of urethane anesthesia on HD-evoked, STR-dependent responses using the cortical EEG as an index of CNS depression by the anesthetic. Motor and cardiovascular responses of relatively constant magnitude could be evoked with HD in the presence of i.t. STR, provided that the EEG was synchronous for < 60% of the test period. When > 60% synchrony was present, these responses were significantly suppressed. Using this criterion, we were able to maintain an appropriately "light" (< 60% synchrony) and stable plane of anesthesia throughout all subsequent experiments. It is noteworthy that the mean %synchrony in the EEG was stable across all doses of i.t. STR, indicating that the enhanced responsiveness to tactile stimuli was not due to a non-specific increase in the level

of arousal, but rather, was the result of a local spinal action of i.t. STR. This is consistent with a previous study in which continuous i.v. STR failed to produce arousal from anesthesia with thiopentone, methohexitone, propofol or etomidate before the onset of convulsions (Al-Muhandis *et al.*, 1991). In contrast, laudanosine caused arousal from anesthesia at doses well below those causing convulsions (Al-Muhandis *et al.*, 1991). These data reinforce the spinal selectivity of STR and agree with a vast literature indicating that glycine is relatively less important in more rostral CNS regions (Curtis *et al.*, 1968; Aprison *et al.*, 1969; Logan and Snyder, 1972; Zarbin *et al.*, 1981).

Yaksh (1989) reported that an innocuous air jet applied to the flanks of urethane-chloralose anesthetized rats evoked a pronounced pressor response after i.t. STR. In light of the allodynia observed in conscious STR-treated rats, this pressor effect was interpreted as a response to an aversive nociceptive event. However, EEG recordings during chloralose anesthesia have suggested that this agent is both a CNS stimulant and depressant (Lees, 1972). STR-like, tactile-evoked motor responses have been described at anesthetic and subanesthetic doses of chloralose (Alvord and Fuortes, 1954; Lees, 1972; Angel, 1986). Tactile-evoked motor responses under chloralose anesthesia decline unpredictably after

several hours, but can be markedly facilitated by STR (Alvord and Fuortes, 1954). It is not known how these chloralose effects may have influenced the tactile-evoked, STR-dependent pressor response in the Yaksh (1989) study, since abrupt mild stimuli are very effective in evoking chloralose-dependent responses, while strong stimuli often leave the animal refractory to further stimuli (Alvord and Fuortes, 1954). As no objective measurement of the depth of anesthesia was used, and the effect of repeated application of the air jet stimulus was not determined, a change in the level of arousal may have accounted for the enhancement of the pressor response following i.t. STR with repeated application of the stimulus.

In the present study, chloralose was omitted from the anesthetic regimen and the cortical EEG was continuously monitored during the experiment to maintain a light plane of anesthesia. This yielded reproducible responses to tactile stimuli, and avoided the potential complication of a chloralose-STR interaction. Separate, well-spaced i.t. STR injections were used, and comparisons were made between peak effects before and after each drug treatment, thereby avoiding the possibility that a temporal decline in STR blockade would be misinterpreted as a drug effect. We also recorded three different evoked responses during the

experiment: HR and blood pressure which increase together in response to noxious stimuli (or HD+STR), and a motor withdrawal response.

2.4.3 Time-Course and Segmental Localization of Strychnine-Dependent Allodynia

The allodynic effect of i.t. STR had a rapid onset and short duration; the characteristic time-course profile seen with a lipophilic drug after i.t. administration (Yaksh and Rudy, 1977; Cousins and Mather, 1984). While pharmacokinetic studies of i.t. STR have not been performed, indirect evidence suggests that STR is swiftly cleared from the subarachnoid space. When STR was delivered by continuous i.t. infusion, rapid delivery rates (5-8 μg/minute) were required to initiate and maintain allodynia, and a brief interruption (1-2 minutes) of the i.t. STR infusion led to a swift diminution of the allodynic effect (unpublished observations). Even with high rates of i.t. infusion, a total STR dose of 700 μg could be administered without inducing spontaneous motor activity. In contrast, an i.v. bolus of only 300 μg caused convulsions. Once in the periphery, STR is metabolized in the liver to inactive metabolites, and rapidly excreted in urine and feces; 80% of

a 0.5 mg/kg (s.c., adult rat) dose of [^{3}H]STR was eliminated within 24 h (Oguri *et al.*, 1989).

Beyer and colleagues (1988) reported that i.t. STR-enhanced responses to innocuous pressure were restricted to hairy skin. However, as shown in Fig. 2.2, motor withdrawal responses were occasionally elicited by stroking the tail or hind paws with the cotton-tipped applicator. These differences may be related to the length of i.t. catheter or the endpoint used. The Beyer study used a 7.5-cm i.t. catheter as compared with the 8.5-cm catheter employed in the present study. The latter would be expected to yield a higher concentration of STR in the sacral region of the spinal cord, thereby inducing abnormal sensitivity of the tail. Alternatively, the tactile-evoked, caudally-directed scratching and biting endpoint used in the Beyer study may be a less reliable measure of allodynia in the tail region. Regardless, the results of the present study suggest that STR-dependent allodynia is not restricted to hairy skin and is related mainly to the STR-distribution within the spinal cord.

Johnston, 1973; Fagg and Lane, 1979). Efficient glycine removal maintains a low basal glycine concentration in spinal tissue (4 μmol/g wet weight; Aprison *et al.*, 1969) and CSF (60 μM; Semba and Patsalos, 1993). When all of these factors are taken into account, competition with STR at glycine receptors remains the most plausible mechanism for glycine inhibition of STR-dependent allodynia.

Paradoxical STR-like actions of glycine at low i.t. doses may also obligate the use of higher glycine doses to inhibit STR-dependent allodynia. Behavioral allodynia has been reported with i.t. glycine (5-400 μg) in conscious rats, as evidenced by vocalization in response to light stroking of the hair (Beyer *et al.*, 1985). Vocalizations were not as prevalent or intense as those seen after i.t. STR, they were not produced by light pressure with von Frey fibers (<2 g) and they became *less* prevalent as the dose of glycine was increased from 5-400 μg (Beyer *et al.*, 1985). A later study from the same laboratory found no sensory or motor manifestations with i.t. glycine (400 μg) and reported that this dose significantly reduced cutaneous sensitivity following i.t. STR (Beyer *et al.*, 1988). These results are consistent with the dose-response relationship of i.t. glycine presented in Fig. 2.6.

2.4.4 Intrathecal Glycine Suppresses Strychnine-Dependent Allodynia

In the present work, i.t. glycine produced dose-dependent inhibition of all indices of STR-dependent allodynia, demonstrating that spinal glycine receptors are involved in this phenomenon. In comparison to other spinally administered drugs (e.g. see Chapter 3.0), the doses of i.t. glycine required to block STR-dependent allodynia were relatively high, with ED_{50} values near 600 μg. However, when the receptor affinity, physical properties and rapid cellular uptake mechanisms for glycine are considered, these high doses are still consistent with a selective action at spinal glycine receptors. For example, studies using rat spinal synaptosomal membranes have linked STR to a single class of binding sites with an affinity constant in the range of 3-10 nM (Young and Snyder, 1974). Glycine has much lower affinity and inhibits STR binding with an IC_{50} of approximately 6 μM. (Johnson *et al.*, 1992). In addition, tissue penetration is impeded by the polarity of this molecule, and it is efficiently removed from its site of action by a high affinity uptake system (estimated K_m between 26.5-121 μM; Logan and Snyder, 1972; Balcar and Johnston, 1973). Glycine uptake is not inhibited by STR or depressant amino acids, such as GABA or ß-alanine (Logan and Snyder, 1972; Balcar and

2.4.5 Intrathecal Betaine Suppresses Strychnine-Dependent Allodynia

Betaine, an endogenous glycine derivative and putative glycine receptor agonist, has been reported to discriminate between sensory and motor effects of STR. In a previous study, i.t. betaine (800 μg) selectively blocked i.t. STR-induced convulsions without affecting skin hyperalgesia or other sensory manifestations of i.t STR (Beyer *et al.*, 1988). In the present study, using dose-response analysis, i.t. betaine blocked all HD-evoked responses after i.t. STR, consistent with the effect of glycine. No distinction could be made between cardiovascular and motor endpoints, suggesting that i.t. betaine did not exhibit selectivity for spinal motor efferent pathways.

As with glycine, the doses of i.t. betaine required to prevent STR-dependent allodynia were high (ED_{50} values near 1 mg). In contrast to glycine, however, a high affinity uptake system has not been described for betaine. Furthermore, betaine, *N,N*-dimethylglycine and sarcosine (*N*-methylglycine) given systemically (i.p.) were equipotent in reducing the incidence of STR-induced seizures and death, while i.p. glycine was ineffective (Freed, 1985). These data demonstrate the facility with which methylated glycine derivatives cross tissue barriers and also highlight their

lack of structural specificity in blocking STR-induced convulsions. This is in marked contrast to the stringent structural requirements for glycine receptor agonism (Young and Snyder, 1973; Drummond *et al.*, 1989). These results suggest that the effects of betaine observed in the present study were not the result of a direct action at glycine receptors.

Receptor binding studies have not been carried out to determine if betaine binds to STR-sensitive glycine receptors; however, a structurally similar glycine derivative, *N,N*-dimethylglycine, neither reduced nor potentiated the depressant effects of glycine on cat spinal interneurons (Curtis *et al.*, 1968). In the liver, betaine is metabolized to dimethylglycine, sarcosine and ultimately glycine (Barak and Tuma, 1983), however, it is unknown if conversion to glycine occurs in the CNS. The high doses of i.t. betaine required in the present experiments suggest that competition between the (unmetabolized) betaine molecule and STR at glycine receptors may not have been its primary mechanism of action. Betaine may act as a prodrug, yielding the metabolic product glycine (Barak and Tuma, 1983), which then displaces STR from spinal glycine receptors.

2.4.6 Summary

We have provided evidence that i.t. STR-dependent, HD-evoked responses in urethane-anesthetized rats may be a useful model of allodynia. Provided that a light plane of anesthesia is maintained, tactile stimuli, applied in the presence of i.t. STR, evoke autonomic and motor responses resembling those evoked by noxious stimuli. All of the STR-dependent responses can be dose-dependently inhibited by i.t. glycine or i.t. betaine. The narrow 95% confidence limits determined in the present study indicate that responses to HD following i.t. STR can be reproduced with sufficient magnitude and accuracy to permit quantitative dose-response analysis.

3.0 SPINAL PHARMACOLOGY OF STRYCHNINE-DEPENDENT ALLODYNIA

3.1 Introduction

Psycho-physical studies of patients with N.I.P. have shown that sensory input from Aß primary afferent neurons (rather than Aδ/C-fibers) initiates the abnormal perception of pain in clinical allodynia (Campbell *et al.*, 1988; Koltzenburg *et al.*, 1992; Baron and Saguer, 1993). Therefore, the neural substrates involved in STR-dependent allodynia were investigated in the present study by selectively destroying C-fiber primary afferent neurons with the neurotoxin, capsaicin. Since analgesic doses of morphine selectively inhibit Aδ- and C-nociceptor input (Le Bars *et al.*, 1976), without affecting non-painful (Aß-mediated) sensation (Cousins *et al.*, 1979), the effect of acute i.t. morphine on STR-dependent allodynia was also determined.

The effect of i.t. STR on behavioral responses to non-noxious (light tactile) stimulation has been reported in several studies (Beyer *et al.*, 1985; 1988; Yaksh, 1989). These data are consistent with the hypothesis that, under normal conditions, glycinergic neurons are important modulators of non-nociceptive (Aß) input in the spinal cord. However, the influence of STR on responses evoked by noxious

stimulation has received little attention (Beyer *et al.*, 1985). Thus, the extent to which glycine interneurons modulate Aδ/C-fibers in the spinal cord, and the selectivity of glycinergic modulation for non-nociceptive versus nociceptive input is unclear. In this study, the responses to noxious thermal, mechanical and chemical stimuli were investigated in the absence and presence of i.t. STR, for comparison with STR's effect on HD-evoked responses.

Excitatory amino acids have a well-established role in normal sensory neurotransmission (Salt and Hill, 1983; Aanonsen and Wilcox, 1986; Jessell *et al.*, 1986; Morris, 1989; Dickenson, 1991; Näsström *et al.*, 1992), and their involvement in pathological pain states continues to be investigated (Dickenson, 1991; Hao *et al.*, 1991b; Näsström *et al.*, 1992; Xu *et al.*, 1993). There is growing evidence that non-NMDA receptors play a principal role in allodynia but not hyperalgesia, while NMDA receptors appear to be involved in both conditions (Hao *et al.*, 1991b; Coderre and Melzack, 1992; Xu *et al.*, 1993). Indeed, NMDA receptor antagonists have been shown to attenuate STR-dependent allodynia in conscious rats (Yaksh, 1989). However, the effect of non-NMDA receptor antagonists on STR-dependent allodynia has not been determined. The present study investigated the effect of i.t. DGG, a non-selective EAA antagonist, and the AMPA-

selective antagonist, NBQX, on i.t. STR-dependent allodynia in anesthetized rats.

3.2 Methods

3.2.1 Animals

All experiments were conducted using male Sprague-Dawley rats (300-475 g) at the time of the acute experiment. Animals receiving neonatal treatments (see below) were the offspring of pregnant rats obtained from Charles River (St. Constant, Canada); the remaining animals were purchased as adults. Rats were housed in the Animal Care Facility, with a room temperature of 22°C, a 12-h light/dark cycle (lights on 07:00 h) and free access to rat chow and tap water. All experiments were conducted in accordance with the Guidelines of the Canadian Council on Animal Care and were approved by the Memorial University Animal Care Committee.

3.2.2 Implantation of Intrathecal Catheters

Under halothane anesthesia, all rats were fitted with i.t. catheters (as described in Chapter 2) and allowed to recover for at least 4 days prior to the acute experiment.

3.2.3 Drug Administration

A subset of animals was pretreated with s.c. capsaicin or vehicle (Tween 80: Ethanol: Saline 2:2:16) on postnatal days 2, 3, 4, 11, 25, 55 and 85. All injections were given under halothane anesthesia. The dose of capsaicin was 25 mg/kg on postnatal day 2 and 50 mg/kg on all subsequent days.

With the exception of NBQX, drugs used for i.t. administration were dissolved in 0.9% sterile saline (Astra Pharma, Inc.). Strychnine hemisulfate (Sigma Chemical, Inc.) was administered in a volume of 4 μL, while morphine sulfate (B.D.H. Chemicals) and DGG (Sigma Chemical, Inc.) were each delivered in a volume of 5 μL. NBQX (Novo Nordisk) was prepared in accordance with the manufacturer's recommendations as a pH-adjusted dextrose solution, and injected in a volume of 10-15 μL. For all injections, the drug solution was flushed through the i.t. catheter with 8 μL of sterile saline.

3.2.4 Acute Experiments

Animals were prepared for acute anesthetized experiments as described in Chapter 2.0. Several types of stimuli were applied to the anesthetized animals. PP was used as a

noxious mechanical stimulus. Both hind paws were gripped with hemostats such that the region of skin covered by each hemostat was ≈20 mm^2. The force of the pinch was produced by 500 gram weights attached to the handles of each hemostat. This resulted in final pressure on each paw of approximately 0.7 kPa. Although withdrawal attempts were recorded, withdrawal of the hind paws was prevented until the end of the 10-second stimulus period. TI in a 55°C water bath was used as a noxious thermal stimulus. This stimulus consistently elicited attempts to withdraw the tail, but reflex withdrawal was prevented until the end of the 10-second stimulus interval. Topical xylene (50 μL) delivered to the dorsal surface of the hind paw was employed as a chemical noxious stimulus (Olsen and Lund, 1991). This stimulus did not result in a consistent motor response, but cardiovascular responses began immediately and persisted for approximately 20 minutes. Hair deflection with a cotton-tipped applicator was used as an innocuous tactile stimulus, as described in Chapter 2.0.

All rats treated neonatally with capsaicin or vehicle received each of the following treatments consecutively: 1) i.t. saline+HD; 2) i.t. STR with no stimulus (NS; see Fig 3.1); 3) i.t. STR+HD; 4) i.t. saline+PP; 5) i.t. STR+PP; 6) i.t. saline+TI; 7) i.t. STR+TI; 8) i.t. saline+topical xylene

and 9) i.t. STR+topical xylene. The HD stimulus was applied at 5-minute intervals for 30 minutes after i.t. STR or saline as described in the previous chapters. TI or PP stimuli were applied 3 times at 10-minute intervals after i.t. saline or STR, and the maximum evoked cardiovascular responses were used in the analysis. For treatments 8 and 9, xylene was applied only once to each hind paw and the peak cardiovascular response was recorded.

Separate groups of animals, which had *not* been pretreated neonatally, were used to examine the effects of i.t. morphine and EAA antagonists on STR-dependent responses to HD. For the i.t. morphine experiments, each 30-minute determination of responses to HD in the presence of i.t. STR was bracketed by determination of responses to noxious stimuli. The noxious stimuli (TI and PP as described above) were applied in the absence of STR --either before or 40 minutes after STR, when the effects of STR on responses to tactile stimuli were no longer evident. All rats used in this protocol received i.t. saline (15 μL) as the first treatment and either i.t. morphine (50 μg) or i.t. saline (15 μL) as the second treatment. The exact timing of drug administration and stimulus application is illustrated in Fig. 3.6. The protocol was designed so that the application of stimuli corresponded with the peak effect of i.t. morphine

as established in preliminary experiments.

To determine the dose-response relationships of DGG and NBQX for inhibition of STR-dependent allodynia, each rat received i.t. saline followed 1 h later by either i.t. DGG (5, 10, 20, 30, 40 or 50 μg) or NBQX (1, 5, 30, or 45 μg). Twenty minutes after each treatment (saline, DGG or NBQX), i.t. STR (40 μg) was administered. HD was applied to the legs, flanks and lower back of the animal 5 minutes prior to i.t. STR and at 5-minute intervals for 30 minutes after each STR administration. The 20-minute pretreatment time enabled the drugs to reach their peak effects based on results of preliminary experiments. Most animals received 2 doses of either DGG or NBQX. Before administration of a second dose, the i.t. saline-i.t. STR control was repeated; a second dose was only administered if the effects of the initial dose were no longer present as evidenced by a return of all responses to the level of the first i.t. saline-i.t. STR control. In animals receiving 2 doses of drug, the first dose selected was always lower than the second to reduce the time required for recovery between doses and to minimize the possibility of a carryover effect.

3.2.5 Immunohistochemistry

Spinal cords from animals receiving neonatal treatments with capsaicin or vehicle were processed for substance P- or calcitonin gene-related peptide (CGRP)-like IR. Following the acute experiment, the depth of anesthesia was increased with i.v. urethane and rats were perfused transcardially with ≈50 mL of ice-cold heparinized saline followed by ≈200 mL of 4% paraformaldehyde at 120 mm of Hg (Loomis *et al.*, 1992). Spinal cords were excised and examined for signs of inflammation and haemorrhage, and the position of the i.t. catheter was determined. Animals were excluded from the study if the catheter tip was not found in the subarachnoid space near the lumbar enlargement.

Spinal cords were post-fixed in the same fixative solution at 4°C for 16-24 h and the segment of the cord at the tip of the catheter was taken for immunohistochemical staining. Forty μm thick transverse sections of the spinal cord were processed for substance P- or CGRP-like IR employing the peroxidase-antiperoxidase (PAP) method (Sternberger, 1979).

Sections were sequentially incubated in: 1) 10% normal goat serum containing 0.3% H_2O_2 and 4% triton X-100; 2) either rabbit antiserum to substance P (1:2000 dilution, Incstar Corp.) or CGRP (1:10,000 dilution, Amersham); 3) 1:150

dilution of goat-anti-rabbit-IgG serum (Boehringer Mannheim Biochemicals); 4) 1:300 dilution of rabbit PAP (Sternberger Meyer Immunochemicals); and 5) staining medium containing 3',3' diamino-benzidine HCl (0.5 mg/mL), glucose oxidase (3.8 U/mL, Aspergillus niger type V, Sigma Chemicals) and ß-*D*-glucose (2 mg/mL) in 0.1 M phosphate buffer (pH 7.2). Between incubations, sections were washed 4 times (20 minutes each time) in phosphate buffered saline.

3.2.6 Data Analysis

All blood pressure data are presented as changes in M.A.P. calculated from the following equation:

M.A.P. = systolic blood pressure + 1/3 pulse pressure

This study focused on responses *evoked* by the different stimuli. Thus, the *change* in M.A.P. or HR has been reported relative to the immediate pre-stimulus control (*not* relative to T=0) for each point in the time course. More precisely, maximum HR or M.A.P. observed in the 1-minute interval before stimulus application was subtracted from the maximum value observed during stimulus application, and this difference was reported. The *same* method of calculation was used for the STR+NS condition, which makes this measurement different from a measure of the effects of STR *alone*, since responses were compared to the *pre-stimulus control* (not T=0; see Fig. 3.1).

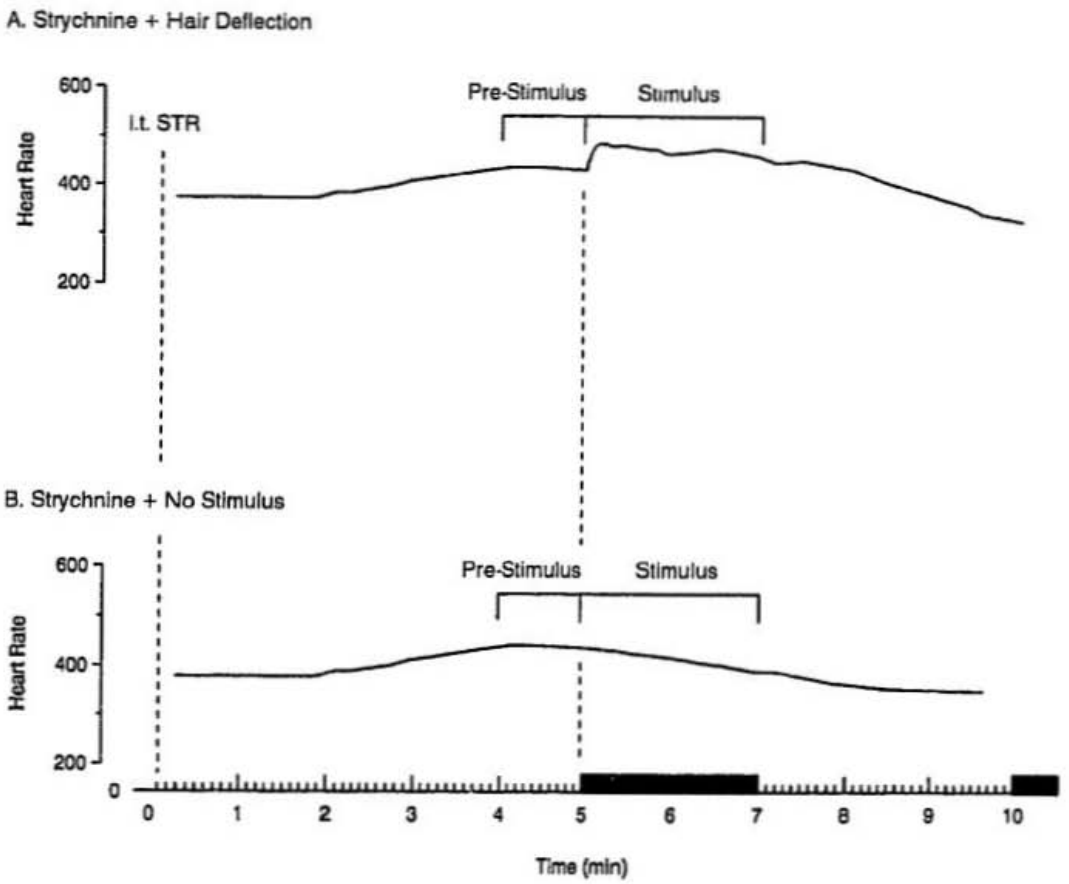


Fig. 3.1. Method of calculating changes in mean arterial pressure and heart rate (HR) evoked by cutaneous stimuli. These examples use simulated HR tracings on a compressed time axis. The *evoked* change in HR is the difference between the maximum response observed during the 2-min hair deflection stimulus (horizontal bar on time axis) minus the maximum response observed in the 1-min pre-stimulus interval (*not* T = 0).

In experiments where several conditions were tested in the same group of animals, statistically significant differences ($p < 0.05$) were detected by either a one-way (Fig. 3.6) or a 2-way (Fig. 3.2 to 3.5) repeated measures ANOVA. Significant differences were identified using a Neuman-Keuls test. Variability associated with single measurements is indicated by S.E.M., while variability associated with blocks of data is indicated by pooled 95% C.I. values. Dose-response data (Fig. 3.7; 3.8) were analyzed by regression ANOVA and a modified t-test was used to determine if regression lines had different slopes. Methods of data analysis were based on general statistics texts (Box, 1978; Winer, 1991).

3.3 Results

3.3.1 Effects of Neonatal Capsaicin and Acute Intrathecal STR on Responses Evoked by Hair Deflection

The cardiovascular and motor responses evoked by HD were significantly enhanced after i.t. STR as compared to those following i.t. saline or i.t. STR without HD (*no stimulus*; Fig. 3.2), consistent with the results described in Chapter 2.0. For each of three stimulus conditions (saline+HD, STR+NS and STR+HD), there was no significant difference

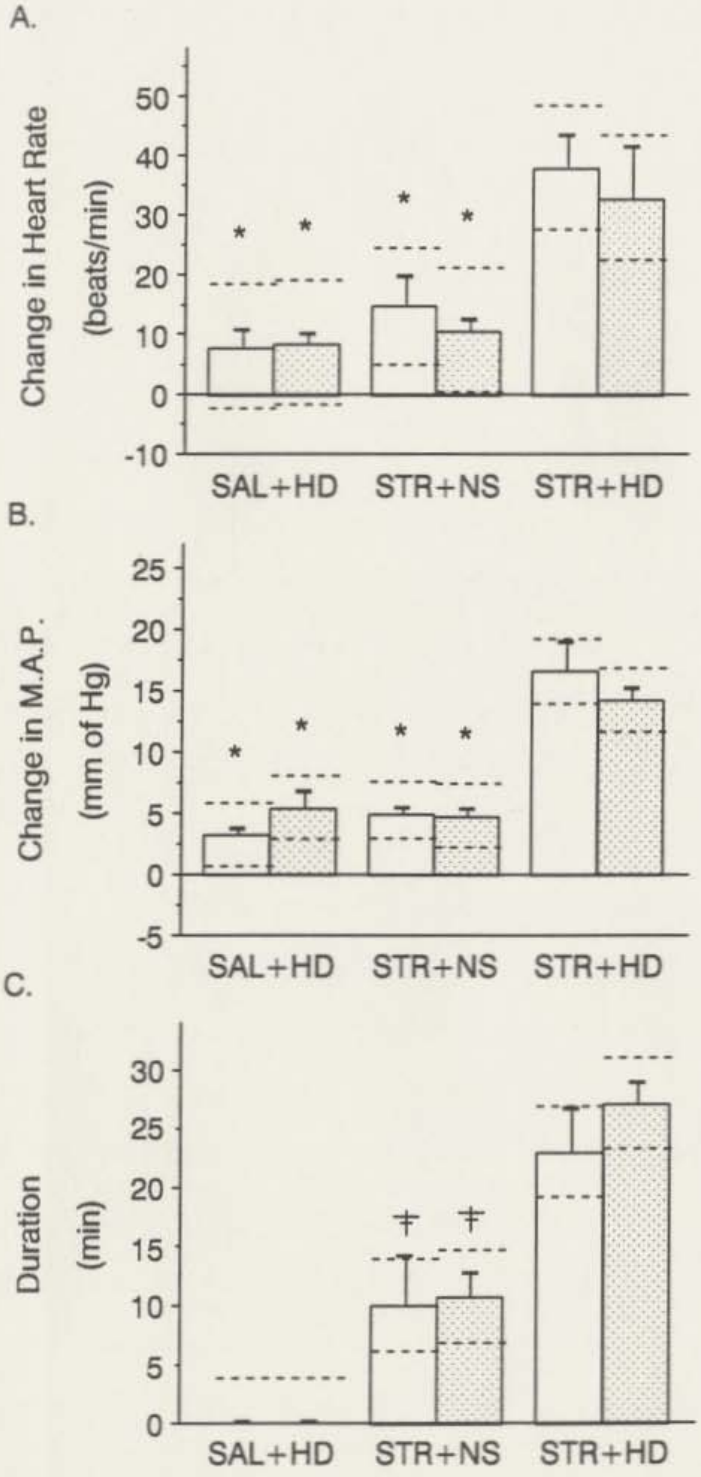
between neonatal capsaicin- and vehicle-treated rats (Fig. 3.2A, B, and C).

Immunohistochemical staining for substance P and CGRP was markedly reduced in the outer lamina of the spinal dorsal horn of adult animals treated neonatally with capsaicin as compared with vehicle-treated controls (data not shown). CGRP staining of motor neurons was unaffected by neonatal capsaicin. These data are characteristic of a selective neurotoxic effect of capsaicin on C-fibers.

The results of this experiment suggest that primary afferent C-fibers are not involved in STR-dependent allodynia since the depletion of these fibers had no effect on the abnormal response to HD in the presence of i.t. STR.

It should be noted that the motor responses observed after STR administration in the absence of stimulation (i.e. STR+NS; Fig. 3.2C) were spontaneous movements and not *evoked* responses. As noted in Chapter 2, these responses differ from the convulsive tonic extensions seen with high doses of STR (Al-Muhandis *et al.*, 1991). Rather, they resemble responses evoked by HD and may be responses evoked by contact with the bench top during respiratory movements. These responses occurred with relatively low frequency and could easily be distinguished from responses evoked by HD (STR+HD) which were time-linked to the stimulus.

Fig. 3.2. Effects of neonatal capsaicin and acute intrathecal (i.t.) strychnine (STR) on responses evoked by hair deflection (HD). Rats receiving neonatal capsaicin (shaded bars; N = 7) or vehicle (open bars; N = 5) were used in acute anesthetized preparations as adults (see Methods). Using HD as a stimulus, the maximum evoked increase in heart rate (A), the maximum evoked increase in mean arterial pressure (M.A.P.; B) and the duration for which a motor withdrawal response could be evoked (C) were determined following i.t. saline (SAL; 15 μL) or i.t. STR (40 μg). The effects of i.t. STR (40 μg) with no stimulus (NS) were also determined. Error bars represent S.E.M. and dotted lines delineate pooled 95 % confidence intervals. An asterisk indicates a significant reduction relative to both STR+HD groups. A dagger ($\dagger$) indicates a significant difference from both the SAL+HD and the STR+HD groups. While not shown, the % synchrony in EEG was within acceptable limits and did not vary significantly among the treatment groups.


A.
Change in Heart Rate
(beats/min)
50
40
30
20
10
0
-10
SAL+HD
STR+NS
STR+HD
B.
Change in M.A.P.
(mm of Hg)
25
20
15
10
5
0
-5
SAL+HD
STR+NS
STR+HD
C.
Duration
(min)
30
25
20
15
10
5
0
SAL+HD
STR+NS
STR+HD

3.3.2 Effects of Neonatal Capsaicin on Responses to Noxious Stimuli

As shown in Fig. 3.3 and 3.4, PP or TI evoked marked increases in HR and M.A.P. following i.t. saline administration to rats that had been treated neonatally with vehicle. Comparable average increases in each of the HR (≈34-40 beats/minute) and M.A.P. (≈25 mm of Hg) responses were observed with both PP and TI. Topical application of xylene to the hindpaw elicited an even greater cardiovascular response, with average increases in HR and M.A.P of 67 beats/minute and 42 mm of Hg, respectively (Fig. 3.5). TI and PP also yielded reproducible motor withdrawal responses whereas those evoked by xylene were inconsistent (data not shown).

All cardiovascular responses to noxious stimuli, except for the HR increase evoked by PP (Fig. 3.3), were significantly attenuated in capsaicin-treated animals (Fig. 3.3; 3.4; 3.5). This effect was most pronounced for the chemical nociceptive agent, xylene. The effects of capsaicin on responses to noxious stimuli were neither reduced nor enhanced by acute i.t. STR. Motor withdrawal responses to PP and TI were unaffected by either capsaicin or STR as compared to their respective controls (data not shown).

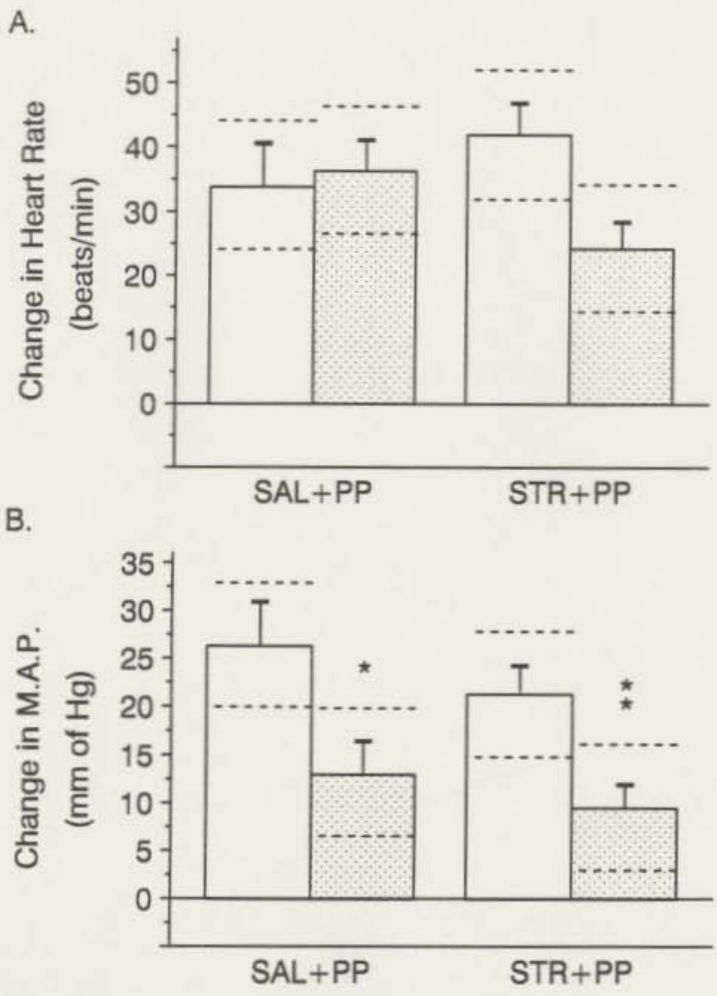


Fig. 3.3. Effects of neonatal capsaicin and acute intrathecal strychnine (i.t. STR) on responses evoked by paw pinch (PP). Peak increases in heart rate (A) and mean arterial blood pressure (M.A.P.; B) evoked by noxious PP were determined following i.t. saline (SAL, 15 μL) or i.t. STR (40 μg) in rats treated with neonatal capsaicin (shaded bars; N = 7) or vehicle (open bars; N = 5). Error bars represent S.E.M. and dotted lines delineate pooled 95% confidence intervals. One asterisk indicates a significant difference from the SAL+PP vehicle control, while 2 asterisks indicate a significant difference from both open bars. While not shown, PP evoked a motor withdrawal response in all animals regardless of their treatment.

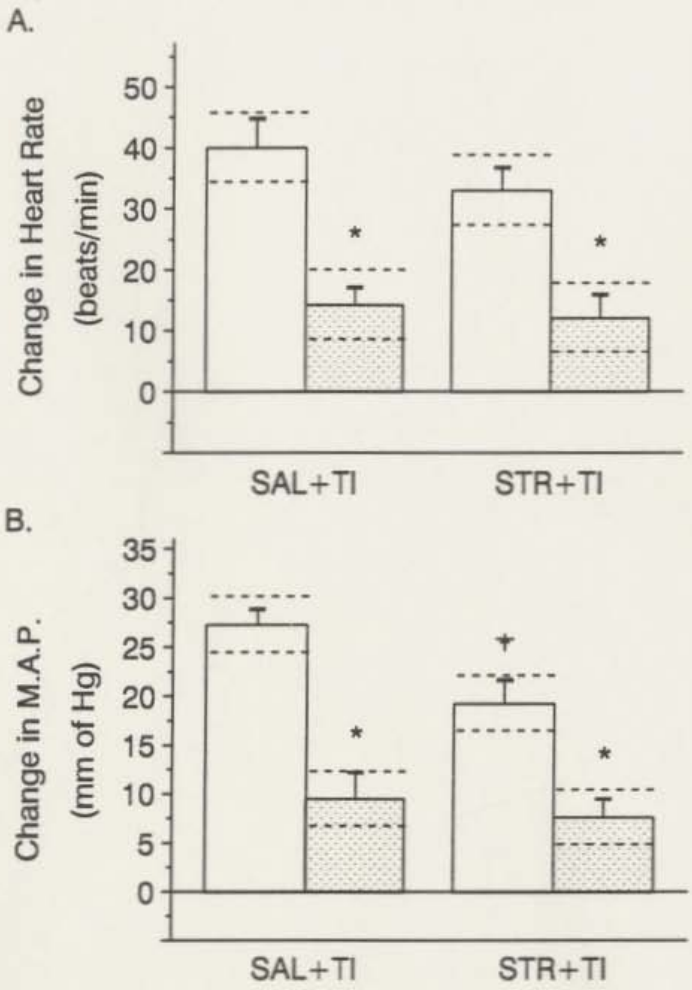


Fig. 3.4. Effects of neonatal capsaicin and acute intrathecal strychnine (i.t. STR) on responses evoked by tail immersion (TI). Peak increases in heart rate (A) and mean arterial blood pressure (M.A.P.; B) evoked by noxious TI were determined following i.t. saline (SAL, 15 μL) or i.t. STR (40 μg) in rats treated with neonatal capsaicin (shaded bars; N = 7) or vehicle (open bars; N = 5). Error bars represent S.E.M. and dotted lines delineate pooled 95% confidence intervals. An asterisk indicates a significant reduction relative to both open bars. A dagger (†) indicates a significant difference from the other 3 treatment groups. While not shown, TI evoked a motor withdrawal response in all animals regardless of their treatment.

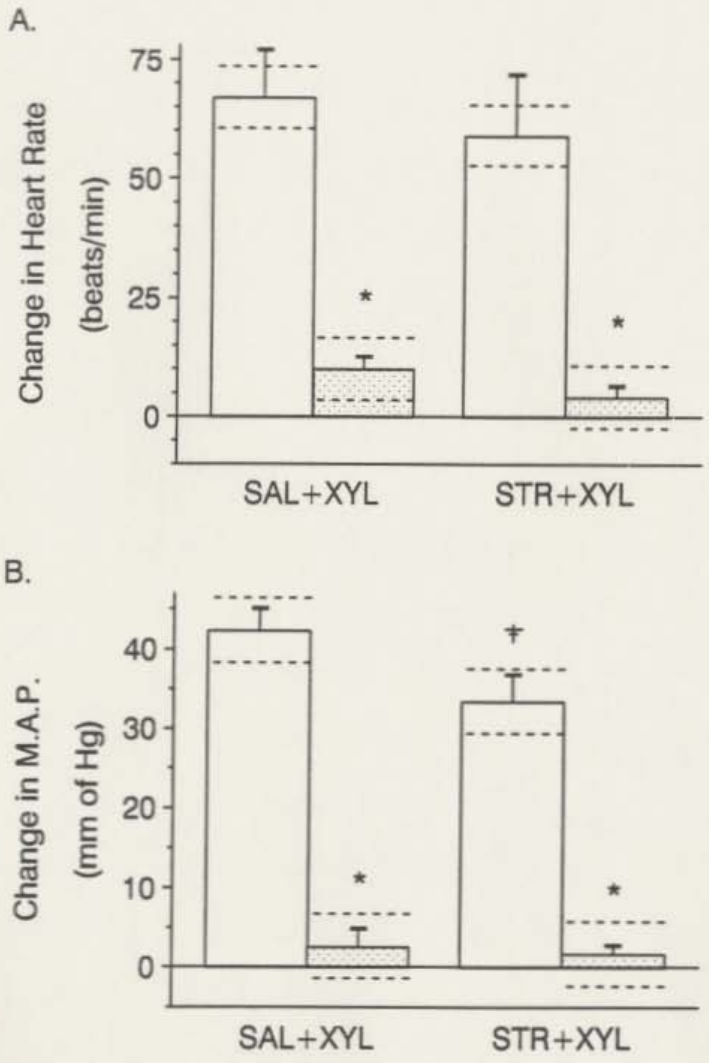


Fig. 3.5. Effects of neonatal capsaicin and acute intrathecal strychnine (i.t. STR) on responses evoked by topical xylene (XYL). Peak increases in heart rate (A) and mean arterial blood pressure (M.A.P.; B) evoked by topical XYL were determined following i.t. SAL (15 μL) or i.t. STR (40 μg) in rats treated with neonatal capsaicin (shaded bars; N = 7) or vehicle (open bars; N = 5). Error bars represent S.E.M. and dotted lines delineate pooled 95% confidence intervals. An asterisk indicates a significant reduction relative to both open bars. A dagger (ǂ) indicates a significant difference from the other 3 treatment groups.

3.3.3 Effects of Acute Intrathecal Strychnine on Responses to Noxious Stimuli

In rats treated neonatally with vehicle alone (not capsaicin, see methods), the increases in M.A.P. evoked by TI and topical xylene were significantly *reduced* after i.t. STR as compared to i.t. saline (indicated by daggers in Fig. 3.4; 3.5). A similar downward trend was observed with the HR response, but this was not statistically significant. In capsaicin-treated rats, the effects of i.t. STR were less evident. It is possible that responses to noxious stimuli were already maximally suppressed by neonatal capsaicin, and that a further reduction by i.t. STR could not be detected. Acute i.t. STR had no significant effect on responses to PP.

3.3.4 Effects of Intrathecal Morphine

Fig. 3.6 contrasts the effects of i.t. morphine on STR-dependent, HD-evoked responses with its effects on responses to standard noxious stimuli. In the absence of i.t. morphine, both TI and PP elicited pronounced elevations in HR and M.A.P. (Fig. 3.6, left panel) which were reproducible throughout the 4-h experiment. Under these conditions, the maximum HD-evoked increase in HR and M.A.P. following 40 μg of i.t. STR was similar in magnitude to the increase in HR and M.A.P. evoked by noxious stimuli (no STR). When i.t.

morphine (50 μg) was administered prior to the second series of stimuli (Fig. 3.6, right panel) cardiovascular responses to TI and PP were significantly suppressed. However, STR-dependent, HD-evoked responses remained unchanged. The %synchrony in the EEG (not shown) did not change significantly throughout this experiment, indicating that the effects of morphine were not the result of a non-specific CNS depression, but rather appear to be the result of a local spinal action. Also the duration for which withdrawal responses were evoked by HD after i.t. STR was not significantly affected by i.t. morphine (data not shown).

3.3.5 Effects of Intrathecal DGG and Intrathecal NBQX

All of the STR-dependent responses were dose-dependently inhibited by the non-selective excitatory amino acid antagonist, DGG (Fig. 3.7) and by the AMPA receptor selective antagonist, NBQX, (Fig. 3.8). The ED_{50}'s and 95% C.I. of DGG were 8.1 (5.2-12.5) μg for withdrawal duration, 16.9 (11.7-24.3) μg for changes in M.A.P. and 15.6 (11.3-21.6) for changes in HR. For NBQX, the ED_{50}'s and 95% C.I. were 10.4 (5.5-19.6) for withdrawal duration, 14.4 (8.6-24.0) for changes in M.A.P. and 12.2 (6.8-21.8) for changes in HR. EEG synchrony was not significantly affected by i.t. DGG or i.t. NBQX.

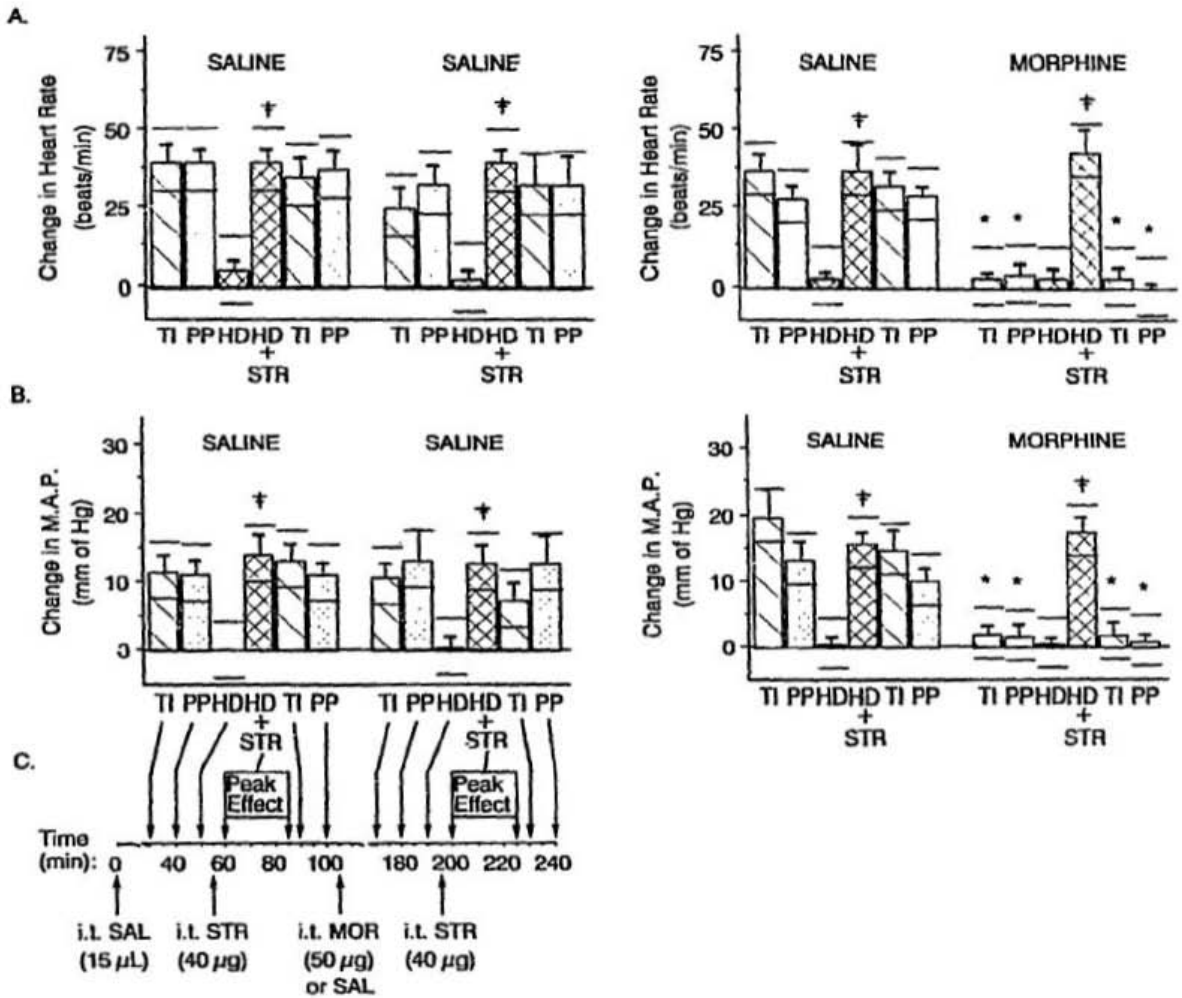

A.
Change in Heart Rate (beats/min)
75
50
25
0
SALINE
SALINE
SALINE
MORPHINE
TI PP HD HD + STR TI PP
B.
Change in M.A.P. (mm of Hg)
30
20
10
0
C.
Peak Effect
Time (min): 0 40 60 80 100 180 200 220 240
i.t. SAL (15 μL)
i.t. STR (40 μg)
i.t. MOR (50 μg) or SAL
i.t. STR (40 μg)

Fig. 3.6. Comparison of the effects of intrathecal (i.t) morphine on responses evoked by either non-noxious hair deflection (HD) in the presence of i.t. strychnine (STR), or by noxious stimuli in the absence of STR. Non-noxious HD was applied to the legs, flanks and back of urethane-anesthetized rats 5 minutes before and at 5-minute intervals for 30 minutes after each injection of i.t. STR (40 μg). HD-evoked responses were bracketed by noxious tail immersion (TI) and paw pinch (PP) stimuli administered before, and 40 minutes after i.t. STR (when the effect of STR on HD-evoked responses was no longer evident). Maximum stimulus-evoked increases in heart rate (A) and mean arterial pressure (M.A.P.;B) are shown. The graphs on the left (Saline-Saline) illustrate experiments where i.t. saline (15 μL) was administered prior to each of the 2 blocks of stimuli (N = 4). On the right (Saline-Morphine), an identical paradigm was used except that i.t. morphine (50 μg) was administered prior to the second block of stimuli (N = 10). Asterisks indicate significant reductions in the responses relative to the saline control. A dagger (ǂ) indicates a significant increase in heart rate or M.A.P. relative to the response to HD in the absence of STR. Error bars are S.E.M. and solid horizontal lines delineate pooled 95% confidence intervals.

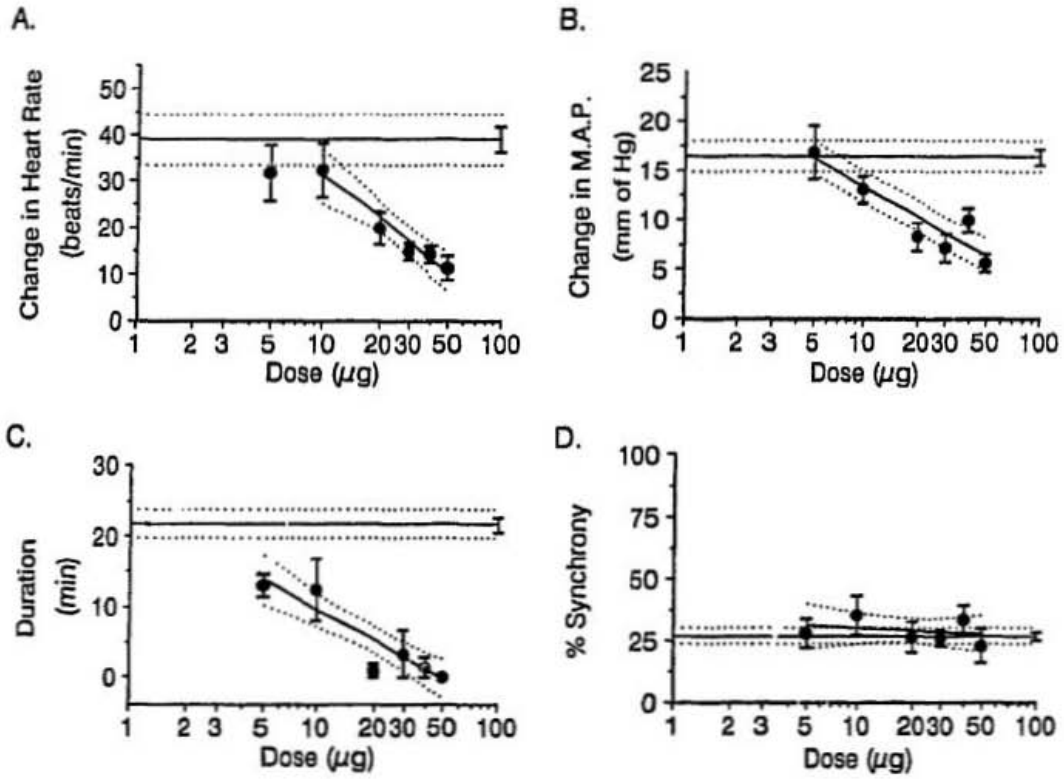


Fig. 3.7. Log dose-response relationship of intrathecal (i.t.) gamma-D-glutamylglycine (DGG) inhibition of responses to hair deflection (HD) in the presence of i.t. strychnine (STR). Following i.t. STR (40 μg), the maximum HD-evoked increase in heart rate (A), the maximum evoked increase in M.A.P. (B), the duration for which a withdrawal response could be evoked (C) and the % synchrony in the EEG (D) were determined in the presence of saline or one of several doses of i.t. DGG. Each point represents the mean ± S.E.M. of 5-8 animals. Least squares regression lines and corresponding 95% confidence intervals (C.I.; dotted lines) are shown. The horizontal solid lines and adjacent dotted lines indicate the mean ± 95% C.I. of all saline treated animals (N = 42).

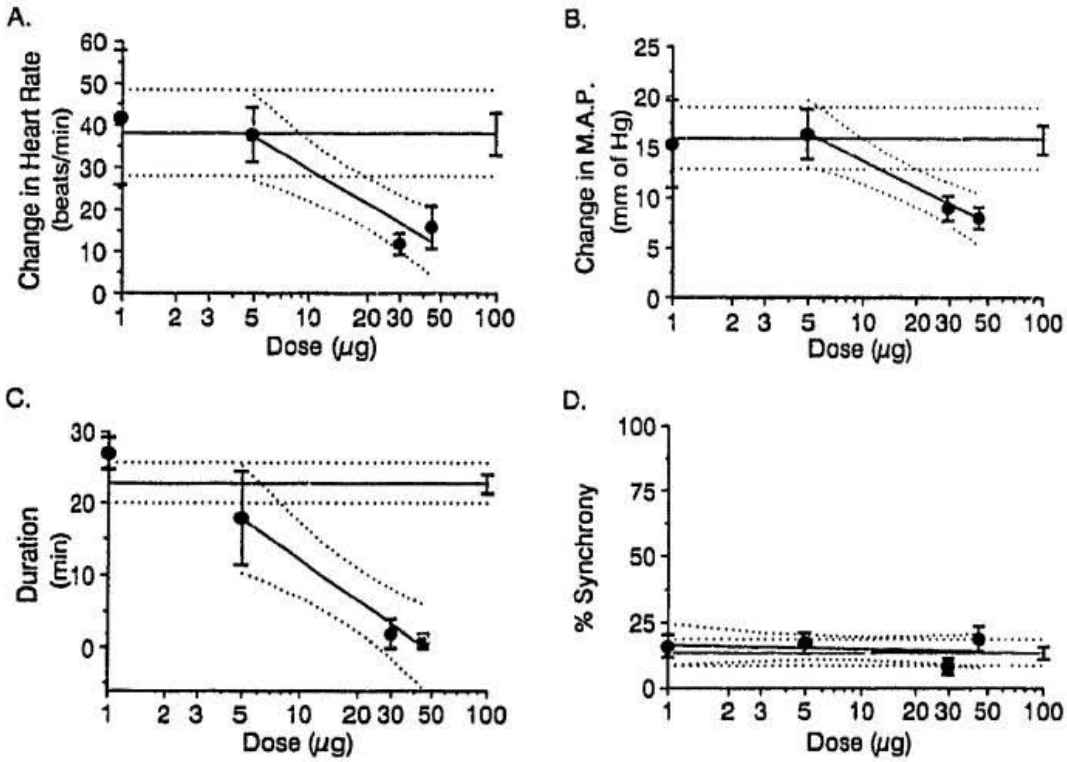


Fig. 3.8. Log dose-response relationship for intrathecal (i.t.) 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) inhibition of responses to hair deflection (HD) after i.t. strychnine (STR). Following i.t. STR (40 μg) the maximum HD-evoked increase in heart rate (A), the maximum evoked increase in M.A.P. (B), the duration for which a withdrawal response could be evoked (C) and the % synchrony in the EEG (D) were determined in the presence of saline or one of several doses of i.t. NBQX. Each point represents the mean ± S.E.M. of 5 animals. Least squares regression lines and corresponding 95 % confidence intervals (C.I.; dotted lines) are shown. The horizontal solid lines and adjacent dotted lines indicate the mean ± 95% C.I. of all saline treatments (N = 20).

3.4 Discussion

3.4.1 Neonatal Capsaicin and Intrathecal Morphine did not Affect STR-Dependent Allodynia

Neonatal capsaicin, which decreased substance P-IR in the dorsal horn of adult rats and attenuated the responses of these same animals to noxious stimuli (see 3.4.3), had no significant effect on i.t. STR-dependent allodynia. The data indicate that capsaicin-sensitive primary afferent neurons do not mediate the abnormal sensation evoked by HD following i.t. STR.

The present results are in general agreement with a previous report that behavioral allodynia scores in STR-treated rats were not significantly affected by i.t. capsaicin at a dose eliciting thermal antinociception (70 μg; Yaksh, 1989). Capsaicin-insensitive allodynia has also been reported following *partial* tight ligation of the rat sciatic nerve (Shir and Seltzer, 1990). In contrast, thermal hyperalgesia in the same ligation model was sensitive to neonatal capsaicin treatment, suggesting that allodynia and hyperalgesia are mediated by different neural substrates. The independence of the neural pathways affected by neonatal capsaicin versus i.t. STR in the present model is supported by the observation that i.t. STR neither reversed nor enhanced the effects of neonatal capsaicin on responses to

mechanical, thermal or chemical noxious stimuli. These observations suggest that STR-dependent allodynia is mediated by neural pathways not normally involved in nociception; possibly involving Aß primary afferent neurons.

Further support for the involvement of a non-nociceptive pathway arises from the observation that HD-evoked cardiovascular and motor responses following i.t. STR were not significantly reduced by pretreatment with high dose i.t. morphine (50 μg); a dose which completely blocked the pronounced autonomic responses to noxious thermal (TI) and mechanical (PP) stimuli. Allodynia to light tactile stimuli with concurrent analgesia to noxious stimuli has been reported with high dose (90-150 μg) i.t. morphine in conscious rats (Yaksh and Harty, 1988). The authors noted that morphine is known to antagonize glycine and GABA, and that comparable behaviours could be produced with i.t. STR or bicuculline. Touch-evoked allodynia was believed to result from the removal of tonic inhibition from pathways relaying information about light tactile stimuli (Yaksh and Harty, 1988). In our hands, i.t. morphine, at doses as low as 50 μg, produced allodynia in conscious rats (unpublished observations). These effects appeared within 15 minutes of morphine administration and lasted approximately 20 minutes. In the present study, only one anesthetized animal exhibited

evidence of morphine-dependent allodynia (50 μg, i.t.). This effect was short-lived, and there was no evidence of morphine allodynia in any animal at the time of the pre-STR HD stimulus (e.g. 80 minutes after i.t. morphine; Fig. 3.6). The failure of morphine to prevent allodynia at this high antinociceptive dose indicates that the STR-dependent, HD-evoked responses are opioid-insensitive. These results are consistent with the selective inhibitory effects of opioids on Aδ/C-fiber activity evoked by high-threshold electrical stimulation (Yaksh, 1987; Willer, 1988) and underscore the independence of C fiber-mediated nociception and allodynia.

Animal models of neural injury pain differ in: 1) the types of abnormal sensation exhibited; 2) the classes of primary afferent neurons which initiate these sensations; and 3) their sensitivity to opioid analgesics. The focal spinal ischemia model of Hao and associates is relatively insensitive to the analgesic action of morphine (Hao *et al.*, 1991c). Within 24 h of photochemically-induced ischemic spinal injury using Erythrosin B, morphine (2 mg/kg i.p.) was ineffective in preventing tactile-evoked agitation and did not alter allodynia as determined by the vocalization threshold to stimulation with von Frey hairs (Hao *et al.*, 1991c). A deficit in spinal glycinergic interneurons has been reported following spinal ischemia (Davidoff *et al.*,

1967). If a loss of spinal glycinergic function is a prerequisite for the development of opioid-resistant allodynia, the pharmacological removal of glycinergic inhibition with i.t. STR would be expected to mimic this condition. The results of the present study support this contention, since both cardiovascular and motor responses to tactile stimuli after i.t. STR were resistant to blockade with a high dose of i.t. morphine. There is some evidence to suggest that allodynia associated with the focal spinal ischemia model of Hao and associates is initiated by Aß primary afferent fibers (Hao *et al.*, 1992a). Extracellular recordings of spinal WDR neurons following focal spinal ischemia indicated hyperexcitability expressed as an augmented A-fiber response to electrical stimulation and hypersensitivity to low-intensity mechanical stimuli. The authors also noted a lack of separation between A- and C-fiber responses in allodynic rats which they attributed to the augmented A-fiber response. Because spinal ischemia-induced sensory abnormalities are mainly expressed as mechanical allodynia, with no behavioral hypersensitivity to noxious thermal stimuli, and because there was no difference in the response to thermal stimulation between normal and allodynic rats, the authors concluded that the abnormally prolonged discharge to electrical stimulation in this model

involved low-threshold myelinated afferent fibers (Hao *et al.*, 1992a).

Allodynia associated with *loose ligation* of the sciatic nerve appears to be mediated mainly by C-fiber primary afferent neurons (Kajander and Bennett, 1992) and is sensitive to morphine treatment (Yamamoto and Yaksh, 1992). After the development of maximal hyperalgesia in the rat hind paw following unilateral loose ligation of the sciatic nerve, dose-dependent antinociception was produced in the hyperesthetic hind paw with only moderately higher doses of i.t. morphine than were required for the normal paw (Yamamoto and Yaksh, 1992). Dose-dependent (0.1-1.0 mg/kg), naloxone-reversible antinociception has also been reported following i.v. morphine in rats with sciatic mononeuropathy (Attal *et al.*, 1991). The antinociceptive effect of i.v. morphine on the lesioned paw was significantly greater than its effects on both the (sham-operated) contralateral paw (Neil *et al.*, 1990; Attal *et al.*, 1991) and on the paws of normal (unlesioned) rats (Neil *et al.*, 1990). These data indicate that the sciatic nerve ligation model of Bennett and Xie (1988) is not only opioid sensitive, but may be more opioid sensitive than animal models of nociceptive pain.

The opioid sensitivity of the Bennett and Xie (1988) model may be attributable to the absence of Aß-fiber

involvement. Very light tactile stimuli, which would be expected to selectively activate Aß afferent fibers, do not elicit allodynia following loose ligation of the sciatic nerve of the rat. No evidence of pain-related behaviour was seen following light brushing of the nerve-injured hind paw, in spite of the presence of mechanical hyperalgesia (Attal *et al.*, 1991). Animals could be handled without evoking squealing or biting, and the affected hind paw appeared to be groomed normally since lipstick applied to both hind paws was cleaned within 24 h without any indication of reluctance to clean the nerve-lesioned paw (Bennett and Xie, 1988). Moreover, 3 days after ligation, 89% of Aß axons did not conduct through the injury site, while the majority of C-fibers remained capable of action potential propagation (Kajander and Bennett, 1992). These results indicate that the loose ligation model mimics a different aspect of N.I.P. than either the i.t. STR model or the focal ischemia model and suggest that the effectiveness of opioids in treating allodynia is related to the class of primary afferent neuron initiating the allodynia.

The question of opioid sensitivity is important since the clinical effectiveness of opioid analgesics in N.I.P. is controversial (see Chapter 1.0). While patients with N.I.P. should not be denied the potential benefits of opioid drugs,

the study of both opioid sensitive and opioid insensitive animal models of N.I.P. should provide valuable information that will aid in differentiating the mechanisms underlying these conditions.

3.4.2 Strychnine-Dependent Allodynia is Dose-Dependently Suppressed by Excitatory Amino Acid Receptor Antagonists

In the present study, all of the i.t. STR-dependent responses to HD were dose-dependently inhibited by DGG and NBQX, indicating that non-NMDA receptors are involved in STR-dependent allodynia. Non-NMDA receptor antagonists are known to block input from large diameter myelinated afferent neurons (Hill and Salt, 1982; Morris *et al.*, 1989), so the effects of NBQX agree with the proposed role of Aß-fibers in initiating the allodynia. The present results are also consistent with reports that NBQX dose-dependently inhibited allodynia in the rat focal spinal cord ischemia model (7.5-30 mg/kg, i.p.; Xu *et al.*, 1993) and prevented STR-dependent, sensory-evoked convulsions in mice (NBQX ED_{50} ≈ 68 mg/kg, i.p.; McAllister, 1993). These data support the proposal that impulses initiated by normally innocuous stimuli and carried by large diameter myelinated afferent fibers could be miscoded as nociceptive input following i.t. STR.

Evidence concerning the role of NMDA receptors in

allodynia is more complex. While the effects of selective NMDA receptor antagonists were not investigated in the present study, MK-801, AP5, kynurenic acid, SKF10047 and ketamine have all been reported to dose-dependently block i.t. STR-dependent allodynia as determined by behavioral responses in conscious rats (Yaksh, 1989). On the surface, these observations seem inconsistent with the hypothesized involvement of non-nociceptive primary afferent neurons in initiating STR-dependent allodynia, since NMDA receptor antagonists are known to selectively prevent "wind-up" and hyperalgesia without altering responses to innocuous stimuli (Dougherty *et al.*, 1992; Mao *et al.*, 1992). In contrast, non-NMDA receptor antagonists inhibit the effects of both noxious and innocuous sensation (Dickenson and Sullivan, 1990) but are ineffective in reversing hyperalgesia (Mao *et al.*, 1992, Coderre and Melzack, 1991). In urethane-anesthetized rats, extracellular recordings from caudal trigeminal neurons indicated that the responses of these cells to non-noxious sensory stimulation (air jet, camel hair brush) were not blocked by the NMDA antagonist, *D*-α-aminoadipate, iontophoretically applied to the medulla, but were antagonized by DGG (Hill and Salt, 1982). In addition, allodynia following focal spinal ischemia was only partially blocked by the NMDA antagonist MK-801, even at doses that

produced severe motor deficits (0.5 mg/kg, i.p.; Xu *et al.*, 1993). Thus, the ability of NMDA receptor antagonists to block STR-dependent allodynia in conscious rats appears to contradict the evidence for involvement of non-nociceptive afferent neurons in STR-dependent allodynia.

Results from human psycho-physical studies may explain these seemingly incongruous results. In normal subjects, the perceptual phenomenon of summation has an exact parallel in the electrophysiological phenomenon of wind-up. When trains of *noxious stimuli* were applied to cutaneous regions of neuropathic pain, evoked summation was similar to that of normal subjects, indicating that summation (wind-up) of noxious input was unchanged after nerve injury (Price *et al.*, 1989). However, in neuropathic pain patients (unlike normal subjects), repetitive activation of Aß afferent fibers at 0.3 Hz (or more) with either natural (stroking the skin with a gauze pad) or electrical stimuli, resulted in summation. The summated Aß-mediated pain had the same burning quality and tendency to radiate as summated noxious heat-evoked pain. The authors of this study suggested that, in neuropathic pain, "Aß input acquires access to the summation mechanism which is normally activated only by C nociceptors" (Price *et al.*, 1989). Thus, in the rat, if STR-dependent allodynia resulted from temporal summation of Aß afferent input to

spinal WDR neurons, blockade with either NMDA or non-NMDA receptor antagonists would be expected. Temporal summation would also explain the relative effectiveness of repetitive, oscillating stimuli, as opposed to static stimuli, in evoking STR-dependent cardiovascular and motor responses. Further experiments, using electrophysiological techniques, will be required to directly ascertain the specific classes of primary afferent neuron which mediate STR-dependent allodynia, as well as to determine if summation of Aß afferent input is involved in this phenomenon.

3.4.3 Neonatal Capsaicin Reduced Responses to Noxious Stimuli

In the present study, neonatal capsaicin substantially reduced responses to noxious heat and topical xylene. Cardiovascular responses (but not motor responses) to noxious mechanical stimuli were marginally reduced by neonatal capsaicin. These data are consistent with the immuno-histochemical results, and confirm the functional depletion of C-nociceptive fibers in these animals. There are contradictory reports about the effects of capsaicin on responses evoked by noxious pressure and noxious heat. A moderate increase in mechanical nociceptive threshold has been described by some authors (Hayes *et al.*, 1980; Faulkner

and Growcott, 1980; Hara *et al.*, 1984) while others indicate no change in mechanical nociception (Jancsó *et al.*, 1977). In the case of thermal nociception, neonatal capsaicin has been reported to produce a small increase in the threshold to noxious heat (Holzer *et al.*, 1979; Nagy *et al.*, 1980; Gamse, 1982). Other authors observed a modest *decrease* in thermal nociceptive threshold following neonatal capsaicin (Hayes and Tyers, 1980), while still others found virtually no change in responses to noxious heat (Hayes *et al.*, 1980; Meller *et al.*, 1992).

There are a number of plausible explanations for the disparate reports concerning the effects of neonatal capsaicin on thermal and mechanical nociception. The age of the animal at the time of testing is critical since thermal and mechanical thresholds are maximally increased at 6 weeks of age, after which thresholds decline to vehicle-treated values (Hammond and Ruda, 1991). (The multiple capsaicin treatments in the present study eliminate the influence of the animal's age on nociceptive threshold.) Other important variables include the rate of stimulus application and the chosen endpoint. Abrupt, sharp stimuli, such as pinch or high temperature, are more resistant to neonatal capsaicin than more gradually applied mechanical or thermal stimuli; presumably due to the involvement of different cutaneous

receptors (see Fitzgerald, 1983). For example, somato-visceral reflexes (including changes in blood pressure) evoked by heating the skin were abolished by neonatal capsaicin, while similar responses to abrupt pinching of the same cutaneous region were unaffected (Cervero and McRitchie, 1981). The endpoint was apparently important in the present study since only cardiovascular responses were affected while motor withdrawal reflexes were resistant to capsaicin treatment. The differing effects on cardiovascular and motor responses do not imply that capsaicin acted on cardiovascular efferents rather than nociceptive afferent neurons. Cardiovascular responses evoked by the combination of i.t. STR and HD were unaffected by neonatal capsaicin, indicating that cardiovascular efferents remained functional. Instead, the rapidity of the response was more likely a factor, with the more gradual endpoint exhibiting the greatest sensitivity to capsaicin (Faulkner and Growcott, 1980; Fitzgerald, 1983; Hammond and Ruda, 1991).

The results with topical xylene are in agreement with a broad literature demonstrating that capsaicin elicits insensitivity to chemical noxious stimuli. Neonatal capsaicin prevents the neurogenic inflammation normally produced by topical xylene or mustard oil (Jancsó *et al.*, 1977). It has also been reported to reduce responses to a

number of chemical noxious stimuli including: wiping responses to zingerone applied to the eye (Jancsó *et al.*, 1977), writhing after i.p. acetylcholine (Hayes *et al.*, 1980) and behavioral responses to intradermal formalin (Hara *et al.*, 1984), i.v. bradykinin or i.v. HCl (Faulkner and Growcott, 1980). The dramatic reduction in responses to topical xylene observed in the present study thus provides strong evidence, albeit indirect evidence, that the neonatal capsaicin treatments were effective in destroying chemosensitive nociceptive primary afferent neurons.

3.4.4 Intrathecal Strychnine Reduced Some Responses to Noxious Stimuli

Intrathecal STR did not significantly affect cardiovascular or motor responses evoked by mechanical noxious stimuli, but did cause a small, though statistically significant, reduction in the pressor responses to TI and topical xylene. A similar downward trend was observed with the HR response. These modest antinociceptive actions of STR are in sharp contrast to the hyperalgesic effects anticipated prior to initiation of the study. Hyperalgesia to tail shock has previously been reported after i.t. STR (Beyer *et al.*, 1985). Interestingly, the same study found a minor trend

toward antinociception after i.t. STR in the tail flick test. While those authors dismissed this effect as equivocal, it is consistent with the present effects of i.t. STR on responses to noxious heat. Since the present results indicate that i.t. STR produces allodynia through disinhibition of myelinated primary afferent neurons, the reduction in tail shock vocalization threshold reported by Beyer and associates (1985) might be explained by an enhanced response to the vibration associated with the electric current.

The mechanism underlying the modest antinociceptive effect of STR is unknown. It is possible that STR-induced disinhibition of large diameter primary afferent neurons might lead to an inhibitory modulation of small diameter afferent neurons. It has long been known that large diameter afferent fibers exert an inhibitory action on slowly conducting nociceptive afferent neurons (see Nathan, 1976 for review). Since, in the STR model, input from both large and small diameter afferent fibers is believed to result in the perception of pain, the relatively small changes observed in the cardiovascular responses would be consistent with such a mechanism. These observations are in agreement with the postulated actions of i.t. STR on non-nociceptive spinal pathways.

3.4.5 Conclusions

Cardiovascular and motor responses to HD following i.t. STR were unaffected by neonatal capsaicin or i.t. morphine, indicating that STR-dependent allodynia is initiated by primary afferent neurons not normally involved in nociception--presumably Aß-fibers. Furthermore, i.t. STR did not produce hyperalgesia to mechanical, thermal or chemical noxious stimuli, confirming the independence of capsaicin- and STR-sensitive primary afferent neurons in this model. The sensitivity of STR-dependent allodynia to both NMDA and non-NMDA receptor antagonists distinguishes the pharmacology of this model from that of experimental models of hyperalgesia.

4.0 GENERAL DISCUSSION

4.1 Summary of Results

The present study characterized the effects of i.t. STR on responses to low threshold tactile stimuli in urethane-anesthetized rats. Under light anesthesia, i.t. STR, but not i.t. saline, altered responses to tactile stimulation such that (normally innocuous) HD evoked a brisk motor withdrawal response, tachycardia and hypertension. In naive animals, this combination of nocifensive and autonomic responses was evoked only by noxious stimuli, and thus responses seen after i.t. STR are consistent with the IASP definition of allodynia. All manifestations of this STR-dependent allodynia were reversible with time (over a period of 15-30 minutes), observed in the absence of convulsions, and had a segmental distribution determined by the i.t. STR injection site. The magnitude of the HD-evoked responses was dependent on the dose of i.t. STR (10-50 μg).

An important objective of the present research was to evaluate the effects of the anesthesia on STR-dependent allodynia. Cortical EEG was used to monitor the degree of CNS depression produced by the urethane anesthetic. The results indicate that cardiovascular and motor responses of relatively constant magnitude could be evoked by HD, provided

that the EEG was synchronous for less than 60% of the test period. Significant suppression of HD-evoked responses was observed when greater than 60% synchrony was present in the EEG. These data demonstrate that an important symptom of N.I.P., allodynia, can be produced reliably in an anesthetized animal preparation, as long as a suitably light and stable plane of anesthesia is maintained.

Glycine produced dose-dependent inhibition of all indices of STR-dependent allodynia. The ED_{50} values and 95% C.I. for i.t. glycine were 609 (429-865) μg for HR responses, 694 (548-878) μg for elevation of M.A.P. and 549 (458-658) μg for motor withdrawal responses. These data suggest that i.t. STR produces allodynia through antagonism of spinal glycine receptors, consistent with the hypothesis that a disruption of endogenous glycinergic modulation systems underlies the allodynia associated with N.I.P. In addition, i.t. betaine inhibited STR-dependent allodynia, possibly through metabolism to glycine. The ED_{50} and 95% C.I. values of i.t. betaine were 981 (509-1889), 1045 (740-1476) and 1083 (843-1391) μg for inhibition of heart rate, M.A.P. and motor responses, respectively. EEG synchrony was not significantly affected by i.t. glycine or i.t. betaine.

Neonatal capsaicin, which significantly attenuated noxious stimuli-evoked responses, had no effect on STR-

dependent allodynia. This indicates that capsaicin-sensitive primary afferent neurons do not play a critical role in mediating abnormal sensation in this model. The independence of the neural pathways affected by neonatal capsaicin versus i.t. STR in the present model is further supported by the observation that i.t. STR neither reversed nor enhanced the effects of neonatal capsaicin on responses to mechanical, thermal or chemical noxious stimuli. These results indicate that STR-dependent allodynia is mediated via neural pathways not normally involved in nociception.

Further support for the involvement of a non-nociceptive pathway arises from the observation that STR-dependent allodynia was not significantly reduced by pretreatment with high dose (50 μg, i.t.) morphine; this dose of morphine completely blocked the pronounced autonomic responses to noxious thermal (TI) and mechanical (PP) stimuli. These results are consistent with the selective inhibitory effects of opioids on Aδ/C-fiber activity evoked by high-threshold electrical stimulation (Yaksh, 1987; Willer, 1988) and underscore the independence of C fiber-mediated nociception and allodynia.

All of the i.t. STR-dependent responses to HD were dose-dependently inhibited by i.t. DGG and i.t. NBQX, indicating that non-NMDA receptors are involved in STR-dependent

allodynia. The ED_{50}'s and 95% C.I. of DGG were 8.1 (5.2-12.5) μg for withdrawal duration, 16.9 (11.7-24.3) μg for changes in M.A.P. and 15.6 (11.3-21.6) for changes in heart rate. For NBQX, the ED_{50}'s and 95% C.I. were 10.4 (5.5-19.6) μg for withdrawal duration, 14.4 (8.6-24.0) μg for changes in M.A.P. and 12.2 (6.8-21.8) μg for changes in heart rate. EEG synchrony was not significantly affected by i.t. DGG or i.t. NBQX.

4.2 Proposals for Future Experiments

4.2.1 Further Characterization of the Role of Glycine in Somatosensation and Neural Injury Pain

Experimental spinal ischemia results in hypersensitivity of WDR neurons, the loss of interneurons and cutaneous mechanical allodynia (Tureen, 1936; Davidoff *et al.*, 1967; Cameron *et al.*, 1990; Hao *et al.*, 1991c; Hao *et al.*, 1992b,c,d; Marsala and Yaksh, 1994; Marsala *et al.*, 1994). Indirect evidence, including a reduction in spinal glycine content, suggests that glycinergic neurons are vulnerable to ischemic and excitotoxic damage (Davidoff *et al.*, 1967). If this is correct, then glycine-IR within lamina II-V of the dorsal horn should decline in those segments affected by occlusion of spinal cord blood flow.

Reversible occlusion of the rat thoracic aorta can be achieved, with minimal surgical intervention, using a Fogarty catheter inserted through the femoral artery (Marsala and Yaksh, 1992; 1994). The duration of spinal ischemia is controlled by the time of inflation of the balloon at the catheter tip. Rats exposed to 20 minutes of spinal ischemia exhibited paralysis of the hind (not fore) limbs and prominent tactile-evoked allodynia. The allodynia appeared as early as 45 minutes after the onset of re-perfusion, became fully developed within 4-8 h, and persisted for at least 8 h (Marsala and Yaksh, 1992; 1994).

Immunohistochemical staining of spinal cords from animals exposed to ischemia could be used to determine whether a temporal relationship exists between the loss of spinal glycinergic interneurons and the onset of allodynia. It should be noted that allodynia would be expected to preceed any immunohistochemical changes, since dysfunction (as opposed to complete destruction) of interneurons is the postulated cause of allodynia. By comparing changes in glycine-IR with GABA-IR, at time points ranging from hours to days after spinal ischemia, the relative importance of these substances to allodynia could be ascertained.

The effectiveness of i.t. glycine in reversing STR-dependent allodynia raises the critical question of whether

glycine receptor agonists are effective in other experimental models of N.I.P. At the time of writing this thesis, the question was unanswered. An investigation of the effects of glycine in other models of N.I.P. would provide a useful basis for comparison of these models. Of particular interest would be the effects of i.t. glycine on models of spinal ischemia (Hao *et al.*, 1991c; Marsala and Yaksh, 1994) since there is already evidence that this type of insult may destroy spinal glycinergic interneurons (Davidoff *et al.*, 1967).

GABA receptor agonists have received considerably more attention than glycine receptor agonists in current models of N.I.P. The $GABA_B$ receptor agonist, baclofen has been tested in the behavioral model of STR-dependent allodynia (Yaksh, 1989), as well as in the acute (Hao *et al.*, 1992b) and chronic (Xu *et al.*, 1992) focal ischemia models. Baclofen was effective in reversing tactile-evoked allodynia following acute focal ischemia, but was inactive in the other two models. The $GABA_A$ agonist, muscimol was ineffective against allodynia in both the acute and chronic focal ischemia models (Hao *et al.*, 1992b; Xu *et al.*, 1992) but has not been tested in the i.t. STR model. These data suggest that alterations in the function of GABAergic systems cannot completely account for allodynia observed after focal spinal ischemia.

Several factors may explain the lack of attention given to glycine receptor agonists, relative to GABA agonists, as possible treatments for allodynia. One reason for this might be the paucity of spinal interneurons using glycine as the sole neurotransmitter. Glycine-IR was found in 64% of rat dorsal horn cells exhibiting GABA-IR, but virtually none of these cells exhibited glycine-IR in the absence of GABA (Todd and Sullivan, 1990). Other factors might include the demonstrated role of GABAergic systems in the modulation of nociceptive pathways (Barber *et al.*, 1978; Désarmenien *et al.*, 1984) and the ability of GABA receptor agonists to produce analgesia in models of nociceptive pain (Hill *et al.*, 1981). In contrast, i.t. glycine, at doses from 5 to 100 μg, produced hyperalgesia in the tail-shock vocalization paradigm, and doses up to 400 μg were inactive in the tail-flick test (Beyer *et al.*, 1985). These observations suggest that glycine is not an important inhibitory modulator of nociceptive pathways. Since many investigators view allodynia as a variation of nociceptive pain, this may explain the relative lack of interest concerning the role of glycine in other models of N.I.P.

A final, but important, limiting factor has been a shortage of selective glycine receptor agonists (Drummond *et al.*, 1989). Early characterization of [^{3}H]-STR binding and

displacement using rat spinal cord synaptic membranes, indicated that several amino acids were able to compete for STR binding sites. The most potent displacers of [^{3}H]-STR were glycine and ß-alanine, with *L*-α-alanine, *DL*-ß-aminoisobutyric acid, *L*-serine and taurine being moderately less potent (Young and Snyder, 1973). This potency order was consistent with the relative potencies of these amino acids in eliciting STR-sensitive depression of spinal dorsal horn neurons (Curtis *et al.*, 1968), and has since been confirmed using whole cell voltage clamp on cultured rat embryonic neurons (Lewis *et al.*, 1991). Gamma and omega amino acids had negligible glycine-like physiological effects and negligible potency in displacing [^{3}H]-STR specific binding (Young and Snyder, 1973; Curtis *et al.*, 1968). A systematic study of a series of aminohydroxyisoxazoles and pyrazoles, with structural similarities to glycine, failed to produce a single ligand with significant glycine receptor binding activity (Drummond *et al.*, 1989). Thus, there is a very limited number of glycine receptor agonists available for use as pharmacological tools.

An important limitation of most currently available glycine receptor agonists is their poor selectivity for the glycine receptor, and their diverse actions at other receptor classes. For example, a study using whole-cell patch-clamp

demonstrated that ß-alanine was both a glycine receptor agonist and a partial agonist at GABA receptors (Choquet and Korn, 1988). Similarly, taurine, another of the few amino acids exhibiting potent glycine receptor agonism, has also been found to modulate the actions of GABA and to mimic GABA agonists in behavioral pharmacological studies (Beyer *et al.*, 1988). Voltage clamp studies in cultured rat spinal dorsal horn neurons demonstrated that *L*-proline is a weak agonist at NMDA and non-NMDA glutamate receptors, as well as at glycine receptors (Henzi *et al.*, 1992). Other glycine receptor agonists have exhibited a similar lack of selectivity or low potency.

In view of the limited choices of glycine receptor agonists, the report by Beyer *et al.* (1988) that i.t. betaine selectively blocked i.t. STR-induced convulsions but not skin hyperalgesia was intriguing, and led to the investigation of the effects of i.t. betaine in the current project. Unlike, glycine, betaine readily crosses the blood-brain barrier and was found to prevent STR-induced seizures following i.p. administration in mice (Freed *et al.*, 1985). Using a complete dose-response analysis, the present study found that betaine blocked all indices of STR-dependent allodynia, which is at odds with Beyer *et al.*, (1988). While, the reason for these differing results is unclear, the Beyer study used only

a single dose of betaine (800 μg, i.t.) and monitored behavioral endpoints. It is possible that these differences in methodology may have contributed to the incongruous outcomes. The high doses required to block both motor and sensory effects of i.t. STR in the current study also raise questions as to whether betaine is acting as a glycine agonist, or a prodrug that is metabolized to glycine.

Development of glycine prodrugs is another approach which might overcome some limitations of glycine receptor agonists described above. At present, the best-characterized glycine prodrug is milacemide, which has been reported to significantly elevate the glycine concentration in the CSF following i.p. (200-400 mg/kg) administration to rats (Semba and Patsalos, 1993). However, milacemide has also been reported to elevate the concentrations of serine and taurine and to increase dopamine turnover in the CNS. A comparison of the actions of milacemide and betaine might be helpful in identifying the optimal structural requirements for glycine prodrugs. Despite numerous attempts to produce new glycine receptor agonists, glycine itself remains the most potent agent available. Thus, glycine prodrugs may represent the most effective way to compensate for the loss of glycinergic modulation in the CNS without the use of i.t. injections. The i.t. STR model of allodynia could be used to characterize

the actions of these agents against N.I.P.

Alternative strategies for the investigation of the role of glycine in somatosensation and N.I.P. include the use of glycine facilitators and reuptake inhibitors. MDL 27,531 (4-methyl-3-methylsulphonyl-5-phenyl-4*H*-1,2,4-triazole) selectively reversed STR-induced extensor seizures in mice with little or no activity against audiogenic, electrical or convulsant-induced seizures (e.g. bicuculline, quinolinic acid, mercaptopropionic acid). Prevention of seizures occurred at doses that did not cause sedation, motor dysfunction or general CNS depression. MDL 27,531 also reduced spontaneous hindlimb contractions in rats with chronic spinal cord transection suggesting that it may enhance spinal glycinergic function by an unknown mechanism. This drug does not appear to bind to glycine, GABA, benzodiazepine or picrotoxin binding sites, since concentrations up to 100 μM failed to displace [^{3}H]-strychnine, [^{3}H]-muscimol, [^{3}H]-flunitrazepam or [^{35}S]-t-butylbicyclo-phosphorthionate from rat or mouse neural tissues *in vitro* (Kehne *et al.*, 1992a,b). Instead, these authors proposed that MDL 27,531 may act at an allosteric site on the glycine receptor to facilitate agonist activity. Considering the effectiveness of GABA-facilitating drugs (e.g. benzodiazepines), MDL 27,531 warrants further

investigation in animal models of N.I.P. involving a glycinergic dysfunction.

Another method of facilitating glycine action would be the use of glycine uptake inhibitors. For example, ρ-chloromercuriphenylsulphonate strongly inhibited high affinity glycine uptake in rat spinal cord slices. However, the same concentration of ρ-chloromercuriphenylsulphonate (10^{-4} M), also partially blocked uptake of GABA, *L*-aspartate and *L*-glutamate (Balcar and Johnston, 1973). This inhibition of EAA uptake would be counterproductive to the treatment of allodynia. In addition, the efficacy of these agents would depend on the presence of residual spinal glycine stores after neural trauma or ischemia. Alternatively, glycine uptake inhibitors could be used as adjuncts to therapy with exogenous glycine or glycine prodrugs. Regardless, the problem of poor selectivity will need to be overcome before these agents can be considered as treatments for allodynia.

The outcome of the present study indicates that glycine receptor agonists may play a useful role in the management of allodynia and an important objective of future studies should be to further characterize this class of drugs as potential treatments for N.I.P. Before this can be achieved, however, a number of limitations of the currently available glycine receptor agonists need to be overcome.

4.2.2 Investigation of the Physiology and Pharmacology of Aß-Mediated Allodynia

A number of experiments could be conducted as a follow-up to the demonstration that i.t. morphine did not prevent STR-dependent allodynia. Morphine was chosen for the present study because it is a commonly used and well-characterized opioid. However, it has the disadvantage of eliciting allodynia at very high doses (90-150 μg, i.t.; Yaksh and Harty, 1988). While appropriate precautions were taken to prevent morphine-dependent allodynia in the current study, it would be useful to investigate the effects of selective μ, δ and κ-opioid receptor agonists to determine if there are any exceptions to the apparent opioid resistance of the model.

The results with neonatal capsaicin, i.t. morphine and EAA antagonists are consistent with the hypothesis that STR-dependent allodynia is mediated by Aß primary afferent fibers. Further investigation of this problem is required using electrophysiological techniques. To verify the involvement of Aß afferent fibers in the i.t. STR model, peripheral nerves could be stimulated at intensities which activate Aß primary afferent fibers, in a manner similar to that used by Kajander and Bennett (1992) in their investigations of the neural substrates eliciting hyperalgesia after loose ligation of the sciatic nerve. The

firing rate of spinal WDR neurons could be monitored in the presence and absence of STR using extracellular recordings. Hao and associates (1991d) employed this approach to identify the abnormal responsiveness of spinal WDR neurons to tactile stimuli in their model of focal spinal ischemia. This preparation could also be used to determine if abnormal temporal summation of tactile stimuli occurred in the STR model, as is suggested by the sensitivity of the model to both NMDA and non-NMDA receptor antagonists (see section 3.4.2). Price and colleagues (1989) have suggested that temporal summation of Aß input, which resembles C-fiber-evoked wind-up, may occur in patients with N.I.P. and might explain the inappropriate perception of innocuous stimuli as pain.

4.2.3 Variations of the Anesthetized Model of Strychnine-Dependent Allodynia

In the interest of maintaining consistency, all experiments in the present thesis were performed under the same general conditions. However, several variations of the model might offer advantages for some research questions. For example, allodynia in the tail was relatively rare in the

current model, but some forms of stimuli (such as innocuous warmth or cold) might be more easily applied to an extremity. The use of a longer i.t. catheter would be expected to result in a higher incidence of allodynia in the tail region.

Continuous i.t. infusion of strychnine would provide a stable baseline for time course studies as well as cumulative dose-response curves. Preliminary studies indicate that STR is rapidly cleared from the spinal cord, thus requiring a high infusion rate to produce a stable allodynic state. Brief interruption of the STR infusion to allow i.t. drug injection was sufficient to perturb steady-state allodynia (unpublished observations). Thus, at present, this approach could only be used for testing systemically (i.v./i.p./s.c.) administered agents against i.t. STR-dependent allodynia. It is possible that the use of a double lumen i.t. catheter, allowing simultaneous infusion of STR and acute injection of test drugs, might overcome this limitation. The possibility of using continuous i.t. STR infusion warrants further investigation, since it would permit more data to be collected from each animal in the same amount of time.

Another variation of the model would be to test alternative glycine receptor antagonists in a effort to find one which is more slowly cleared from the CSF than STR. This might improve the results with continuous infusion since the

basal level of allodynia would be more stable over time. A more slowly cleared drug would probably need to be less potent than STR, to avoid the possibility of convulsions if the CSF concentration became too high.

The steroid derivative, 3-α-hydroxy-16-imino-5ß-17-aza-androstan-11-one (RU 5135) was reported to be approximately 10-fold more potent than STR as a competitive glycine antagonist in the cat spinal cord (Curtis and Malik, 1985). However, the selectivity of this compound for glycine receptors appears to be poor. Complete blockade of the depressant action of glycine on *DL*-homocysteate-induced firing of interneurons and Renshaw cells was accompanied by a partial (≈50%) blockade of the actions GABA in this preparation (Curtis and Malik, 1985).

A number of glycine receptor antagonists have been described which are considerably less potent than STR, including *N,N*-dimethylmuscimol, *N*-methyl-4,5,6,7-tetrahydro isoxazole[5,4-c]pyridin-3-ol (*N*-methyl-THIP), 5,6,7,8-tetra-hydro-4*H*-isoxazolo[5,4-*c*]azepin-3-ol (THIA), 5,6,7,8-tetra-hydro-4*H*-isoxazolo[4,5-*d*]azepin-3-ol (THAZ), 5,6,7,8-tetra-hydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol (iso-THAZ), 5-(3-pyrrolidinyl)-3-isozolol (3-PYOL) 2,5,6,7-tetrahydro-1*H*-azepine-4-carboxylic acid (3,4-TAZA) and 2,3,6,7-tetrahydro-1*H*-azepine-4-carboxylic acid (4,5-TAZA; Krogsgaard-Larsen *et*

al., 1982; Braestrup *et al.*, 1986). The following potency order was established based on the ability of these compounds to inhibit the depressant actions of glycine on interneurons and Renshaw cells in pentobarbitone-anesthetized cats: iso-THAZ > THAZ ≈ 4,5-TAZA > THIA ≈ 2,3-TAZA ≈ 3-PYOL. A similar order was obtained for displacement of [H^3]STR binding to rat pons and medulla membranes (Braestrup *et al.*, 1986). These authors cautioned, however, that the relationship between displacement of [H^3]STR binding and glycine antagonism is imperfect, since the $GABA_A$ antagonist, THIP, inhibits [H^3]STR binding with a K_i value similar to that of THAZ, but does not antagonize the actions of glycine *in vivo*. The most potent compound in this series, iso-THAZ, had a K_i value of 1,700 nM for inhibition of [H^3]STR binding, having considerably lower affinity for the glycine receptor than STR (K_i = 7.0 nM) and RU 5135 (K_i = 4.6 nM; Braestrup *et al.*, 1986).

With the exception of 3-PYOL, which blocked the effects of both GABA and glycine, all of the compounds listed in the preceding paragraph were selective glycine receptor antagonists. They were virtually inactive against GABA in the cat spinal cord and did not inhibit [H^3]GABA binding to rat brain synaptic membranes (Krogsgaard-Larsen *et al.*, 1982). In contrast to STR, *N,N*-dimethylmuscimol, *N*-methyl-THIP, iso-THAZ, THAZ, 4,5-TAZA, THIA and 2,3-TAZA are

zwitterions (Krogsgaard-Larsen *et al.*, 1982) and their polarity would be expected to substantially reduce their rate of clearance from the CSF. Characterization of the effects of continuous i.t. infusion of these agents might result in an improved version of the current model of allodynia.

4.3 Significance of the Results

4.3.1 Significance of Specific Findings

The demonstration that allodynia and nociception can be studied in an anesthetized preparation indicates that anesthetized preparations might be useful in other experimental pain studies.

The results demonstrating the relationship between the segmental distribution of allodynia and the spinal distribution of STR dispel the previous misconception that STR-dependent allodynia is confined to hairy skin (Beyer *et al.*, 1988). It follows that peripheral mechanoreceptors, other than rapidly-adapting hair receptors, could be involved in this sensory abnormality. (While "hair deflection" was the only innocuous stimulus used in the present study, it is believed to activate several other classes of peripheral mechanoreceptor.) The indiscriminant miscoding of all types

of low threshold tactile input as pain would be consistent with a *central* mechanism of STR-dependent allodynia, as proposed in Chapter 1.0. A detailed investigation of the effects of i.t STR on responses to innocuous punctate and vibratory stimuli would provide a further test of the hypothesized role of glycine in the spinal modulation of somatosensory pathways as well as in allodynia.

The failure of morphine and capsaicin to prevent STR-dependent allodynia suggests that the allodynia observed in this model is: 1) tactile-evoked, 2) Aß afferent fiber-mediated, and 3) opioid resistant. These features characterize the most prevalent form of clinical dysesthesia (Nurmikko and Hietaharju, 1992). I.t. STR-dependent allodynia may be a useful model for testing putative treatments for clinical N.I.P.

In the present study, all of the i.t. STR-dependent responses to HD were dose-dependently inhibited by i.t. DGG and i.t. NBQX, indicating that non-NMDA receptors are involved in STR-dependent allodynia. Non-NMDA receptor antagonists are known to block input from large diameter myelinated afferent neurons (Hill and Salt, 1982; Morris *et al.*, 1989), so the effects of NBQX agree with the proposed role of Aß-fibers in initiating the allodynia. A previous study found that the NMDA receptor antagonists MK-801, AP5,

kynurenic acid, SKF10047 and ketamine dose-dependently block the behavioral manifestations of i.t. STR-dependent allodynia in conscious rats (Yaksh, 1989), consistent with the effects of the non-selective EAA antagonist, DGG.

It is noteworthy that the 95% confidence limits associated with all dose-response curves were reasonably narrow indicating the utility and sensitivity of this anesthetized preparation for dose-response studies. These results are the first demonstration that reproducible, touch-evoked, STR-dependent cardiovascular and motor responses can be induced in lightly-anesthetized rats with sufficient magnitude and accuracy to permit quantitative dose-response analysis.

4.3.2 Significance of an Acute Model of Allodynia

An acute reversible model of sensory dysfunction, such as with i.t. STR, provides different but complimentary information from chronic animal models using experimental injury of peripheral nerves or the spinal cord (Wall *et al.*, 1979; Levitt and Levitt, 1981; Bennett and Xie, 1988; Yaksh, 1989; Seltzer *et al.*, 1990; Hao *et al.*, 1991c; Kim and Chung, 1991; DeLeo *et al.*, 1994; Marsala and Yaksh, 1994). Many of these models require a post-injury delay before hyperalgesia

or allodynia develop. Depending on the method of injury, this delay ranges from days to weeks. Furthermore, the somatosensory dysfunction resulting from nerve injury is the probable outcome of multiple changes in neural plasticity and function. These may include injury discharge, neural sprouting, ephaptic synapses, cell death, changes in excitatory or inhibitory neurotransmitter production, uptake and degradation, and receptor-effector coupling. Until the role of these individual changes in N.I.P. is better understood, it seems reasonable to use an acute pharmacological approach, like the i.c. STR model, to identify individual neurotransmitters involved in the somatosensory dysfunction of N.I.P.

An important advantage of the anesthetized STR model of allodynia is that it avoids some of the sensitive ethical issues arising from the use of conscious animals in the study of chronic pain. For example, animals have been reported to attack, bite and self-mutilate regions of partial or complete denervation in different experimental models of N.I.P. (Levitt and Levitt, 1981; Bennett and Xie, 1988; Attal *et al.*, 1990; Saadé *et al.*, 1990; Seltzer *et al.*, 1991; Xu *et al.*, 1992). Wall *et al.*, (1979), labelled this behaviour "autotomy", and claimed that it would be a useful model of anesthesia dolorosa (pain referred to an anesthetic region).

The use of autotomy as nociceptive index in models of N.I.P. is controversial for two fundamental reasons. First, there remains some question as to whether autotomy is a response to pain, paresthesia or anesthesia (Berman and Rodin, 1982; Rodin and Kruger, 1984; Blumenkopf and Lipman, 1991; Devor, 1991). Second, if autotomy is a response to spontaneous pain, its chronic, continuous and unavoidable nature would require serious reconsideration on ethical grounds (Rodin and Kruger, 1984). Clearly, the exposure of conscious animals to chronic pain cannot always be avoided if important problems of clinical pain are to be adequately investigated (Casey and Dubner, 1989). However, the i.t. STR model allows the investigation of a symptom of chronic pain without having to inflict injury or to expose a conscious animal to aversive painful conditions, and might provide a useful alternative for some types of experiments. At the very least, the i.t. STR model could serve as a preliminary screen for agents or manipulations that may eventually be tested in chronic, experimental nerve injury models.

5.0 REFERENCES

Aanonsen, L.M. and Wilcox, G.L., Phencyclidine selectively blocks a spinal action for N-methyl-D-aspartate in mice, Neurosci. Lett., 67 (1986) 191-197.

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

Al-Muhandis, W.M., Lauretti, G.R. and Pleuvry, B.J., Modification by drugs used in anaesthesia of CNS stimulation induced in mice by laudanosine and strychnine, Br. J. Anesth., 67 (1991) 608-613.

Alvord, E.C. and Fuortes, M.G.F., A comparison of generalized reflex myoclonic reactions elicitable in cats under chloralose anesthesia and under strychnine, Am. J. Physiol., 176 (1954) 253-61.

Angel, A., Animal models of myoclonus using 1,2-dihydroxybenzene (catechol) and chloralose, In: S. Fahn, C.D. Marsden and M.H. Van Woert (Eds). *Advances in Neurology*, Vol.43. Raven Press, New York, (1986) 589-609.

Angel, A., Dodd, J. and Gray, J.D., Fluctuating anaesthetic state in the rat anaesthetized with urethane, J. Physiol. (Lond.), 259 (1976) 11-12P.

Anis, N.A., Headley, P.M., Lodge, D. and West, D.C., Lack of involvement of N-methyl aspartate receptors in segmental synaptic excitation of cat lumbar dorsal horn neurones: studies with ketamine, J. Physiol.(Lond.), 328 (1982) 10P.

Aprison, M.H., Shank, R.P. and Davidoff, R.A., A comparison of the concentration of glycine, a transmitter suspect, in different areas of the brain and spinal cord in seven different vertebrates, Comp. Biochem. Physiol., 28 (1969) 1345-1355.

Arnér, S. and Meyerson, B.A., Genuine resistance to opioids--fact or fiction?, Pain, 47 (1991) 116-118.

Arnér, S. and Meyerson, B.A., Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain, Pain, 33 (1988) 11-23.

Attal, N., Jazat, F., Kayser, V. and Guilbaud, G., Further evidence of 'pain-related' behaviours in a model of unilateral peripheral mononeurotherapy, Pain, 41 (1990) 235-251.

Attal, N., Chen, Y.L., Kayser, V. and Guilbaud, G., Behavioral evidence that systemic morphine may modulate a phasic pain-related behaviour in a rat model of peripheral mononeuropathy, Pain, 47 (1991) 65-70.

Balcar, V.J. and Johnston, G.A.R., High affinity uptake of transmitters: studies on the uptake of L-aspartate, GABA, L-glutamate and glycine in cat spinal cord, J. Neurochem., 20 (1973) 529-539.

Barak, A.J. and Tuma, D.J., Betaine, metabolic by-product or vital methylating agent?, Life Sci., 32 (1983) 771-774.

Barber, R.P., Vaughn, J.E., Saito, K., McLaughlin, B.J. and Roberts, E., GABAergic terminals are presynaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord, Brain Res., 141 (1978) 35-55.

Baron, R. and Saguer, M., Postherpetic neuralgia. Are C-nociceptors involved in signalling and maintenance of tactile allodynia?, Brain, 116 (1993) 1477-1496.

Barron, S.E. and Guth, P.S., Uses and limitations of strychnine as a probe in neurotransmission, TIPS, 8 (1987) 204-206.

Becker, C.-M. and Betz, H., Strychnine: useful probe or not?, TIPS, 8 (1987) 379-380.

Bennett, G.J. and Xie, Y.-K., A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man, Pain, 33 (1988) 87-107.

Berman, D. and Rodin, B.E., The influence of housing condition on autonomy following dorsal rhizotomy in rats, Pain, 13 (1982) 307-311.

Bertolino, M. and Vicini, S., Voltage-dependent block by strychnine of N-methyl-D-aspartic acid-activated cationic channels in rat cortical neurons in culture, Molecular Pharmacol., 34 (1988) 98-103.

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

Beyer, C., Banas, C., Gomora, P. and Komisaruk, B.R., Prevention of the convulsant and hyperalgesic action of strychnine by intrathecal glycine and related amino acids, Pharmacol. Biochem. Behav., 29 (1988) 73-78.

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

Biscoe, T.J., Headley, P.M., Lodge, D., Martin, M.R. and Watkins, J.C., The sensitivity of rat spinal interneurones and Renshaw cells to L-glutamate and L-aspartate, Exp.Brain Res., 26 (1976) 547-551.

Blumenkopf, B. and Lipman, J.J., Studies in autotomy: its pathophysiology and usefulness as a model of chronic pain, Pain, 45 (1991) 203-209.

Boivie, J., Leijon, G. and Johansson, I., Central post-stroke pain - a study of the mechanisms through analyses of the sensory abnormalities, Pain, 37 (1989) 173-185.

Box, G.E.P., Hunter, W.G. and Hunter, J.S., Statistics for Experimenters, John Wiley & Sons Inc., New York, (1978).

Braestrup, C., Nielson, M. and Krogsgaard-Larsen, P., Glycine antagonists structurally related to 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol inhibit binding of [^{3}H]strychnine to rat brain membranes, J. Neurochem., 47 (1986) 691-696.

Cameron, T., Prado, R., Watson, B., Gonzalez-Carnajal, M. and Holets, V.R., Photochemically induced cystic lesion in the rat spinal cord, Exper. Neurol., 109 (1990) 214-223.

Campbell, J.N., Raja, S.N., Meyer, R.A. and Mackinnon S.E., Myelinated afferents signal the hyperalgesia associated with nerve injury, Pain, 32 (1988) 89-94.

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

Casey, K.L. and Dubner, R.D., Animal models of chronic pain: scientific and ethical issues, Pain, 38 (1989) 249-252.

Cervero, F. and McRitchie, H.A., Neonatal capsaicin and thermal nociception: a paradox, Brain Res., 215 (1981) 114-418.

Chapman, C.R. and Bonica, J.J., Chronic Pain, In: *Current Concepts*, Upjohn, (1985) 1-30.

Choquet, D. and Korn, H., Does ß-alanine activate more than one chloride channel associated receptor?, Neurosci. Lett., 84 (1988) 329-334.

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

Cousins, M.J., Mather, L.E., Glynn, C.J., Wilson P.R. and Graham, J.R., Selective spinal analgesia, Lancet, I (1979) 1141-1142.

Cousins, M.J. and Mather, L.E., Intrathecal and epidural administration of opioids, Anesthesiology, 61 (1984) 267-310.

Curtis, D.R. and Malik, K., Glycine antagonism by RU 5135, Eur. J. Pharmacol., 110 (1985) 383-384.

Curtis, D.R., Duggan, A.W. and Johnston, G.A.R., The specificity of strychnine as a glycine antagonist in the mammalian spinal cord, Exp. Brain Res., 12 (1971) 547-565.

Curtis, D.R., Hösli, L. and Johnston, G.A.R., A pharmacological study of the depression of spinal neurones by glycine and related amino acids, Exp. Brain Res., 6 (1968) 1-18.

Davidoff, R.A., Graham, L.T.Jr., Shank, R.P., Werman, R. and Aprison, M.H., Changes in amino acid concentrations associated with loss of spinal interneurons, J. Neurochem., 14 (1967) 1025-1031.

DeLeo, J.A., Coombs, D.W., Willenbring, S., Colburn, R.W., Fromm, C., Wagner, R., and Twitchell, B.B., Characterization of a neuropathic pain model: sciatic cryoneurolysis in the rat, Pain, 56 (1994) 9-16.

Désarmenien, M., Feltz, P., Occhipinti, G., Santangelo, F. and Schliter, R., Coexistence of GABA-A and GABA-B receptors on A and C primary afferents, Br. J. Pharmacol., 81 (1984) 327-333.

Devor, M., Sensory basis of autotomy in rats, Pain, 45 (1991) 109-110.

Dickenson, A.H., Recent advances in the physiology and pharmacology of pain: plasticity and its implications for clinical analgesia, J. Psychopharmacol., 5 (1991) 342-351.

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

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

Drummond, J., Johnson, G., Nickell, D.G., Ortwine, D.F., Bruns, R.F. and Welbaum, B., Evaluation and synthesis of aminohydroxyisoxazoles and pyrazoles as potential glycine agonists, J. Med. Chem., 32 (1989) 2116-2128.

Du, H.-J., Kitahata, L.M., Thalhammer, J.G. and Zimmerman, M., Inhibition of nociceptive neuronal responses in the cat's spinal dorsal horn by electrical stimulation and morphine microinjection in nucleus raphe magnus, Pain, 19 (1984) 249-257.

Du Pen, S.L. and Williams, A.R., Comments on 'The nature of opioid responsiveness and its implications for neuropathic pain: new hypotheses derived from studies of opioid infusions' by R.K. Portenoy, K.M. Foley and C.E. Inturrisi in Pain, 43 (1991)273-286, Pain, 47 (1991) 115.

Dubner, R. and Bennett, G.J., Spinal and trigeminal mechanisms of nociception, Ann. Rev. Neurosci., 6 (1983) 381-418.

Dubner, R., A call for more science, not more rhetoric, regarding opioids and neuropathic pain, Pain, 47 (1991) 1-2.

Duggan, A.W. and Foong, F.W., Bicuculline and spinal inhibition produced by dorsal column stimulation in the cat, Pain, 22 (1985) 249-259.

Edmondson, E.A., Simpson, R.K., Stubler, D.K. and Beric, A., Systemic lidocaine therapy for poststroke pain, South. Med. J., 86 (1993) 1093-1096.

Engberg, I., and Ryall, R.W., The inhibitory actions of noradrenaline and other monoamines on spinal neurons., J. Physiol. (Lond.), 185 (1966) 298-322.

Fagg, G.E. and Lane, J.D., The uptake and release of putative amino acid neurotransmitters, Neurosci., 4 (1979) 1015-1036.

Faulkner, D.C. and Growcott, J.W., Effects of neonatal capsaicin administration on the nociceptive response of the rat to mechanical and chemical stimuli, J. Pharm. Pharmacol., 32 (1980) 656-657.

Fitzgerald, M., Capsaicin and sensory neurones - a review, Pain, 15 (1983) 109-130.

Freed, W.J., Prevention of strychnine-induced seizures and death by the N-methylated glycine derivatives betaine, dimethylglycine and sarcosine, Pharmacol. Biochem. Behav., 22 (1985) 641-643.

Game, C.J.A. and Lodge, D., The pharmacology of the inhibition of dorsal horn neurones by impulses in myelinated cutaneous afferents in the cat, Exp. Brain Res., 23 (1975) 75-84.

Gamse, R., Capsaicin and nociception in rat and mouse, N.S. Arch. Pharmacol., 320 (1982) 205-216.

Gibson, S.J., Polak, J.M., Bloom, S.R. and Wall, P.D., 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 (1981) 65-79.

Gracely, R.H., Lynch, S.A. and Bennett, G.J., Painful neuropathy: altered central processing maintained dynamically by peripheral input, Pain, 51 (1992) 175-194.

Hammond, D.L. and Ruda, M.A., Developmental alterations in nociceptive threshold, immunoreactive calcitonin gene-related peptide and substance P, and fluoride-resistant acid phosphatase in neonatally capsaicin-treated rats, J. Comp. Neurol., 312 (1991) 436-450.

Hammond, D.L. and Yaksh, T.L., Antagonism of stimulation-produced antinociception by intrathecal administration of methysergide or phentolamine, Brain Res., 298 (1984) 329-337.

Hammond, D.L., Levy, R.A. and Proudfit, H.K., Hypoalgesia induced by microinjection of a norepinephrine antagonist in the raphe magnus: reversal by intrathecal administration of a serotonin antagonist, Brain Res., 201 (1980) 475-479.

Hammond, D.L., Tyce, G.M. and Yaksh, T.L., Efflux of 5-hydroxytryptamine and noradrenaline into spinal cord superfusates during stimulation of the rat medulla, J. Physiol. (Lond.), 359 (1985) 151-162.

Hao, J.-X., Xu, X.-J., Aldskogius, H., Seiger, Å. and Wiesenfeld-Hallin, Z., Beneficial effect of the opioid receptor antagonist naltrexone on the hypersensitivity induced by spinal cord ischemia in rats: disassociation with MK-801, J. Restorat. Neurol. Neurosci., 3 (1991a) 257-266.

Hao, J.-X., Xu, X.-J., Aldskogius, H., Seiger, Å. and Wiesenfeld-Hallin, Z., The excitatory amino acid receptor antagonist MK-801 prevents the hypersensitivity induced by spinal cord ischemia in the rat, Exp. Neurol., 113 (1991b) 182-191.

Hao, J.-X., Xu, X.-J., Aldskogius, H., Seiger, Å. and Wiesenfeld-Hallin, Z., Allodynia-like effects in rat after ischemic spinal cord injury photochemically induced by laser irradiation, Pain, 45 (1991c) 175-185.

Hao, J.-X., Xu, X.-J., Yu, Y.-X., Seiger, Å. and Wiesenfeld-Hallin, Z., Transient spinal cord ischemia induces temporary hypersensitivity of dorsal horn wide dynamic range neurons to myelinated, but not unmyelinated, fiber input, J. Neurophysiol., 68 (1992a) 384-391.

Hao, J.-X., Xu, X.-J., Yu, Y.-X., Seiger, Å. and Wiesenfeld-Hallin, Z., Baclofen reverses the hypersensitivity of dorsal horn wide dynamic range neurons to mechanical stimulation after transient spinal cord ischemia; implications for a tonic GABAergic inhibitory control of myelinated fiber input, J. Neurophysiol., 68 (1992b) 392-396.

Hao, J.-X., Xu, X.-J., Yu, Y.-X., Seiger, Å. and Wiesenfeld-Hallin, Z., Hypersensitivity of dorsal horn wide dynamic range neurons to cutaneous mechanical stimuli after transient spinal cord ischemia in the rat, Neurosci. Lett., 128 (1991d) 105-108.

Hara, A., Sakurada, T., Sakurada, S., Matsumura, H. and Kisara, K., Antinociceptive effects of neonatal capsaicin in rats with adjuvant arthritis, N.S. Arch. Pharmacol., 326 (1984) 248-253.

Hayes, A.G., Scadding, J., Skingle, M. and Tyers, M.B., Effects of neonatally applied capsaicin on nociceptive thresholds and dorsal horn substance P levels in conscious rats and mice, J. Physiol. (Lond.), 305 (1980) 99P.

Hayes, A.G. and Tyers, M.B., Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat, Brain Res., 189 (1980) 561-564.

Henry, J.L., Concepts of pain sensation and its modulation, J. Rheumatol., 16 Suppl. 19 (1989) 104-112.

Henzi, V., Reichling, D.B., Helm, S.W. and MacDermott, A.B., L-Proline activates glutamate and glycine receptors in cultured rat dorsal horn neurons, Mol. Pharmacol., 41 (1992) 793-801.

Hill, R.G. and Salt, T.E., An ionophoretic study of the response of rat caudal trigeminal nucleus neurones to non-noxious mechanical sensory stimuli, J.Physiol. (Lond.), 327 (1982) 65-78.

Hill, R.C., Maurer, H., Buesher, H. and Roemer, D., Analgesic properties of the GABA-mimetic THIP, Eur. J. Pharmacol., 69 (1981) 221-225.

Holzer, P., Jurna, I., Gamse, R. and Lembeck, F., Nociceptive threshold after neonatal capsaicin treatment, Eur. J. Pharmacol., 58 (1979) 511-514.

Hylden, J.L., and Wilcox, G.L., Pharmacological characterization of substance P-induced nociception in mice: modulation by opioid and noradrenergic agonists at the spinal level, J. Pharmacol. Exp. Ther., 226 (1983) 398-404.

Jancsó, G., Kiraly, E. and Jancsó-Gábor, A., Chemosensitive pain fibres and inflammation, Int. J. Tiss. Reac., 2 (1980) 57-66.

Jancsó, G., Kiraly, E. and Jancsó-Gábor, A., Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones, Nature, 270 (1977) 741-743.

Jancsó, G. and Kiraly, E., Sensory neurotoxins: chemically induced selective destruction of primary sensory neurons, Brain Res., 210 (1981) 83-89.

Jessell, T., Tsunoo, A., Kanazawa, I. and Otsuka, M., 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 (1979) 247-259.

Jessell, T.M., Yoshioka, K. and Jahr, C.E., Amino acid receptor-mediated transmission at primary afferent synapses in rat spinal cord, J. Exp. Biol., 124 (1986) 239-258.

Johnson, G., Drummond, J.T., Boxer, P.A. and Bruns, R.F., ß-proline analogues as agonists at the strychnine-sensitive glycine receptor, J. Med. Chem., 35 (1992) 233-241.

Kajander, K.C. and Bennett, G.J., Onset of a painful peripheral neuropathy in rat: a partial and differential deafferentation and spontaneous discharge in Aß and Aδ primary afferent neurons, J. Neurophysiol., 68 (1992) 734-744.

Kandel, E.R., Central representation of touch, In: *Principles of Neural Science*, 2nd Ed. (eds. E.R. Kandel and J.H. Schwartz), Elsevier Science Publishing Co. Inc., New York, (1985) 316-330.

Kangrga, I., Larew, J.S.A. and Randic, M., 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, Neurosci. Lett., 108 (1990) 155-160.

Kapadia, S.E. and LaMotte, C.C., Deafferentation-induced alterations in the rat dorsal horn: I. Comparison of peripheral nerve injury vs. rhizotomy effects on presynaptic, postsynaptic and glial processes, J. Comp. Neurol., 266 (1987) 183-197.

Kehne, J.H., Ketteler, J.M., Kane, T.C., McCloskey, T.M., Senyah, Y. and Palfreyman, M.G., MDL 27,531 reduces spontaneous hindlimb contractions in rats with chronic transections of the spinal cord, Neurosci. Lett., 147 (1992a) 101-105.

Kehne, J.H., Kane, J.M., Miller, F.P., Ketteler, H.J., Braun, D.L., Senyah, Y., Chaney, S.F., Abdallah, A., Dudley, M.W., Ogden, A.M. and Palfreyman, M.G., MDL 27,531 selectively reverses strychnine-induced seizures in mice, Br. J. Pharmacol., 106 (1992b) 910-916.

Kelly, D.D., Central representations of pain and analgesia, In: *Principles of Neural Science*, 2nd Ed., (eds. E.R. Kandel and J.H. Schwartz), Elsevier Science Publishing Co. Inc., New York, (1985) 331-343.

Khayyat, G.F., Yu, Y.J. and King R.B., Response patterns to noxious and non-noxious stimuli in rostral trigeminal relay nuclei, Brain Res., 97 (1975) 47-60.

Kim, S.H. and Chung, J.M., Sympathectomy alleviates mechanical allodynia in an experimental animal model for neuropathy in the rat, Neurosci. Lett., 134 (1991) 131-134.

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

Koltzenburg, M., Lundberg, L.E. and Torebjörk, H.E., Dynamic and static components of mechanical hyperalgesia in human hairy skin, Pain, 51 (1992) 207-19.

Krogsgaard-Larsen, P., Hjeds, H., Curtis, D.R., Leah, J.D. and Peet, M.J., Glycine antagonists structurally related to muscimol, THIP, or isoguvacine, J. Neurochem., 39 (1982) 1319-1324.

Kumazawa, T., Functions of the nociceptive primary neurons, Jap.J.Physiol., (1990) 1-14.

Kupers, R.C., Konings, H., Adriaensen, H. and Gybels, J.M., Morphine differentially affects the sensory and affective pain ratings in neurogenic and idiopathic forms of pain, Pain, 47 (1991) 5-12.

Lamotte, C., Pert, C.P. and Snyder, S.H., Opiate receptor binding in primate spinal cord: distribution and changes after root section, Brain Res., 112 (1976) 407-412.

Lawson, S.N. and Nickels, S.M., The use of morphometric techniques to analyze the effect of neonatal capsaicin treatment on rat dorsal root ganglia and dorsal roots, J. Physiol. (Lond.), 303 (1980) 12P.

Le Bars, D., Guilbaud, G., Jurna, I. and Besson J.M., Differential effects of morphine on responses of dorsal horn lamina V type cells elicited by A and C fibre stimulation in the spinal cat, Brain Res., 115 (1976) 518-524.

Lees, P., Pharmacology and toxicology of alpha chloralose: a review, Vet. Rec., 91 (1972) 330-333.

Lembeck, F. and Holzer, P., Substance P as a neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation, N.S. Arch. Pharmacol., 310 (1979) 175-183.

Levine, J., Pain and analgesia: the outlook for more rational treatment, Annals Int. Med., 100 (1984) 269-276.

Levitt, M. and Levitt, J.H., The deafferentation syndrome in monkeys: dysesthesias of spinal origin, Pain, 10 (1981) 129-147.

Light, A.R. and Perl, E.R., Spinal terminations of functionally identified primary afferent neurons with slowly conducting myelinated fibers, J. Comp. Neurol., 186 (1979) 133-150.

Lincoln, D.W., Hentzen K., Hin, T., Van der Schoot, P., Clarke, G. and Summerlee, A.J.S., Sleep; a prerequisite for reflex milk ejection in the rats, Exp. Brain Res., 38 (1980) 151-162.

Lindblom, U. and Verrillo, R.T., Sensory functions in chronic neuralgia, J. Neurol. Neurosurg. Psychiat., 42 (1979) 422-435.

Liu, D., Thangnipon, W. and McAdoo, D.J., Excitatory amino acids rise to toxic levels upon impact injury to the rat spinal cord, Brain Res., 547 (1991) 344-348.

Ljungdahl, A., Hokfelt, T. and Nilson, G., Distribution of substance P-like immunoreactivity in the central nervous system of the rat--I. Cell bodies and nerve terminals, Neuroscience, 3 (1978) 861-943.

Loeser, J.D., Definition, aetiology and neurological assessment of pain originating in the nervous system following deafferentation, Pain, 81 Suppl. 1, (1981) S81.

Logan, W.J. and Snyder, S.H., High affinity uptake systems for glycine, glutamic and aspartic acids in synaptosomes of rat central nervous tissues, Brain Res., 42 (1972) 413-431.

Loomis, C.W., Vyas, D., Sherman, S.E., Intrathecal oxymetazoline does not produce neurotoxicity in the spinal cord of the rat, Brain Res., 599 (1992) 73-82.

Mao, J., Price, D.D., Mayer, D.J., Lu, J. and Hayes, R.L., Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviors in rats with experimental peripheral mononeuropathy, Brain Res., 576 (1992) 254-262.

Marchettini, P., Lacerenza, M., Marangoni, C., Pellgata, G., Sotgiu, M.L. and Smirne, S., Lidocaine test in neuralgia, Pain, 48 (1992) 377-382.

Marsala, M. and Yaksh, T.L., Reversible aortic occlusion in rats: post-reflow hyperesthesia and motor effects blocked by spinal NMDA antagonism, Anesthesiology, 77 (1992) A664.

Marsala, M. and Yaksh, T.L., Transient spinal ischemia in the rat: characterization of behavioral and histo-pathological consequences as a function of the duration of aortic occlusion, J. Cereb. Blood Flow Metabol., 14 (1994) 526-535.

Marsala, M., Sorkin, L.S. and Yaksh, T.L., Transient spinal ischemia in the rat: characterization of spinal cord blood flow, extracellular amino acid release, and concurrent histopathological damage, J. Cereb. Blood Flow Metabol., 14 (1994) 604-614.

Marsala, M., Vanicky, I., Galik, J., Radonak, J., Kundrat, I. and Marsala, J., Panmyelic epidural cooling protects against ischemic spinal cord damage, J. Surg. Res., 55 (1993) 21-31.

Martin, J.H., Anatomical substrates for somatic sensation, In: *Principles of Neural Science*, (eds. E.R. Kandel and J.H. Schwartz), Elsevier Science Publishing Co. Inc., New York, (1985a) 301-315.

Martin, J.H., Receptor physiology and submodality coding in the somatic sensory system, In: *Principles of Neural Science*, (eds. E.R. Kandel and J.H. Schwartz), Elsevier Science Publishing Co. Inc., New York, (1985b) 287-300.

Maxwell, D.J. and Réthelyi, M., Ultrastructure and synaptic connections of cutaneous afferent fibres in the spinal cord, Trends Neurosci., 10 (1987) 117-123.

Mayer, D.J., Price, D.D. and Becker, D.P., Neurophysiological characterization of the anterolateral spinal cord neurons contributing to pain perception in man, Pain, 1 (1975) 51-58.

McAllister, K.H., Antagonism of non-NMDA receptors inhibits handling-induced, strychnine-potentiated convulsions, Eur. J. Pharmacol., 231 (1993) 309-312.

Meller, S.T., Gebhart, G.F. and Maves, T.J., Neonatal capsaicin treatment prevents the development of the thermal hyperalgesia produced in a model of neuropathic pain in the rat, Pain, 51 (1992) 317-321.

Merskey, H. (Ed.), Classification of chronic pain, Part II, Pain, Suppl. 3 (1986) S215-221.

Mokha, S.S., McMillan, J.A. and Iggo, A., Descending control of spinal nociceptive transmission. Actions produced on spinal multireceptive neurones from the nuclei locus coeruleus (LC) and raphe magnus (NRM), Exp. Brain Res., 58 (1985) 213-226.

Morris, R., 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. Lett., 105 (1989) 79-85.

Nagy, J.I., Emson, P.C. and Iversen, L.L., A re-evaluation of the neurochemical and antinociceptive effects of intrathecal capsaicin in the rat, Brain Res., 211 (1981) 497-502.

Nagy, J.I., Vincent, S.R., Staines, W.M.A., Fibiger, H.C., Reisine, T.D. and Yamamura, H.I., Neurotoxic action of capsaicin on spinal substance P neurons, Brain Res., 186 (1980) 435-444.

Näsström, J., Karlsson, U. and Post, C., Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice, Eur. J. Pharmacol., 212 (1992) 21-29.

Nathan, P.W., The gate-control theory of pain, a critical review, Brain, 99 (1976) 123-158.

Neil, A., Kayser, V., Chen, Y.L. and Guilbaud, G., Repeated low doses of morphine do not induce tolerance but increase the opioid antinociceptive effect in rats with a peripheral neuropathy, Brain Res., 522 (1990) 140-143.

Nistri, A., In: R.A. Davidoff (Ed.), *Handbook of the Spinal Cord*, Vol. 1., Marcel Dekker, New York (1983) 45-104.

Noordenbos, W. and Wall, P.D., Implications of the failure of nerve ressection and graft to cure chronic pain produced by nerve lesions, J. Neurol. Neurosurg. Psychiat., 44 (1981) 1068-1073.

Nurmikko, T. and Hietaharju, A., Effect of exposure to sauna heat on neuropathic and rheumatoid pain, Pain, 49 (1992) 43-51.

Ochoa, J.L., Essence, investigation, and management of "neuropathic" pains: hopes from acknowledgment of chaos, Muscle Nerve, 16 (1993) 997-1008.

Oguri, K., Tanimoto, Y., Mishima, M. and Yoshimura, H., Metabolic fate of strychnine in rats, Xenobiotica, 19 (1989) 171-178.

Olsen, U.B. and Lund, A., Inhibition of glutamate antagonists, MK-801 and NBQX, of cutaneo-cardiovascular pain reflex in rats, Eur. J. Pharmacol., 203 (1991) 133-135.

Palermo, N.N., Brown, H.K. and Smith, D.L., Selective neurotoxic action of capsaicin on glomerular C-type terminals in rat substantia gelatinosa, Brain Res., 208 (1981) 506-510.

Pang, I.-H. and Vasko, M.R., Morphine and norepinephrine but not 5-hydroxytryptamine and γ-aminobutyric acid inhibit the potassium-stimulated release of substance P from rat spinal cord slices, Brain Res., 376 (1986) 268-279.

Portenoy, R.K., Foley, K.M. and Inturrisi, C.E., Response to the letters to the editor by Dr. Du Pen and Ms. A Williams and by Drs. Arnér and Meyerson, Pain, 47 (1991) 119-121.

Portenoy, R.K., Foley, K.M. and Inturrisi, C.E., The nature of opioid responsiveness and its implications for neuropathic pain: new hypotheses derived from studies of opioid infusions, Pain, 43 (1990) 273-286.

Price, D.D., Bennett, G.J. and Rafii, A., Psychophysical observations on patients with neuropathic pain relieved by sympathetic block, Pain, 36 (1989) 273-288.

Price D.D., Long, S. and Huitt, C., Sensory testing of pathophysiological mechanisms of pain in patients with reflex sympathetic dystrophy, Pain, 49 (1992) 163-173.

Prichard, J.W., The effect of strychnine on the leech Retzius cell, Neuropharmacology, 10 (1971) 771-774.

Radhakrishnan, V. and Henry, J.L., Excitatory amino acid receptor mediation of sensory inputs to functionally identified dorsal horn neurons in cat spinal cord, Neuroscience, 55 (1993) 531-544.

Rexed, B., A cytoarchitectonic atlas of the spinal cord of the cat, J. Comp. Neurol., 100 (1954) 297-380.

Ribeiro-Da-Silva, A. and Coimbra, A., Neuronal uptake of [^{3}H] glycine in laminae I-III (substantia gelatinosa rolandi) of the rat spinal cord. An autoradiographic study, Brain Res., 188 (1980) 449-464.

Rodin, B.E. and Kruger, L., Deafferentation in animals as a model for the study of pain: an alternative hypothesis, Brain Res. Rev., 7 (1984) 213-228.

Rowbotham, M.C., Reisner-Keller, L.A. and Fields, H.L., Both intravenous lidocaine and morphine reduce the pain of postherpetic neuralgia, Neurology, 41 (1991) 1024-1028.

Ruda, M.A., Opiates and pain pathways: demonstration of enkephalin synapses on dorsal horn projection neurons, Science, 215 (1982) 1523-1525.

Saadé, N., Atweh, S.F., Jabbur, S.J. and Wall, P.D., Effects of lesions in the anterolateral columns and dorsolateral funiculi on self-mutilation behavior in rats, Pain, 42 (1990) 313-321.

Salt, T.E. and Hill, R.G., Neurotransmitter candidates of somatosensory primary afferent fibres, Neuroscience, 10 (1983) 1083-1103.

Salt, T.E., Crozier, C.S. and Hill, R.G., The effects of capsaicin pre-treatment on the response of single neurones to sensory stimuli in the trigeminal nucleus caudalis of the rat: evidence against a role for substance P as the neurotransmitter serving thermal nociception, Neuroscience, 7 (1982) 1141-1148.

Segal, M. and Sandberg, D., Analgesia produced by electrical stimulation of catecholamine nuclei in the rat brain, Brain Res., 123 (1977) 369-372.

Seltzer, Z., Dubner, R. and Shir, Y., A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury, Pain, 43 (1990) 205-18.

Semba, J. and Patsalos, P.N., Milacemide effects on the temporal inter-relationship of amino-acids and monoamine metabolites in rat cerebrospinal fluid, Eur. J. Pharmacol., 230 (1993) 321-326.

Sherman, S.E., Loomis, C.W., Milne, B. and Cervenko, F.W., Intrathecal oxymetazoline produces spinal analgesia via spinal α-adrenoceptors and potentiates spinal morphine, Eur. J. Pharmacol., 148 (1988) 371-380.

Sherrington, C.S., *Integrative Action of the Nervous System*, Yale University Press, New Haven, (1947).

Shibasaki, H. and Kuroiwa, Y., Painful tonic seizure in multiple sclerosis, Arch. Neurol., 30 (1974) 47-51.

Shir, Y., and Seltzer, Z., A-fibers mediate mechanical hyperesthesia and allodynia and C-fibers mediate thermal hyperalgesia in a new model of causalgiform pain disorders in rats, Neurosci. Lett., 115 (1990) 62-67.

Sjøgren, P., Jonsson, T., Jensen, N.-H., Drenck, N.-E. and Jensen, T.S., Hyperalgesia and myoclonus in terminal cancer patients treated with continuous intravenous morphine, Pain, 55 (1993) 93-97.

Skilling, S.R., Smullen, D.H. and Larsen, A.A., 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, J. Neuroscience, 10 (1990) 1309-1318.

Skilling, S.R., Smullin, D.H., Beitz, A.J. and Larson, A.A., Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation, J. Neurochem., 51 (1988) 127-132.

Sorkin, L.S., Nociceptive transmission within the spinal cord, Mount Sinai J. Med., 58 (1991) 208-216.

Sosnowski, M. and Yaksh, T.L., Role of spinal adenosine receptors in modulating the hyperesthesia produced by spinal glycine receptor antagonism, Anesth. Analg., 69 (1989) 587-592.

Steiner, T.J. and Turner, L.M., Cytoarchitecture of the rat spinal cord, J. Physiol.(Lond.), 222 Suppl. (1972) 123P-125P.

Sternberger, L.A., The unlabelled antibody peroxidase-anti-peroxidase (PAP) method. In: L. Sternberger (Ed.), *Immunocytochemistry*, Wiley, New York, (1979) 104-169.

Sugimoto, T., Bennett, G.J. and Kajander, K.C., Strychnine-enhanced transsynaptic degeneration of dorsal horn neurons in rats with an experimental painful peripheral neuropathy, Neurosci. Lett., 98 (1989) 139-143.

Sugimoto, T., Bennett, G.J. and Kajander, K.C., Transsynaptic degeneration in the superficial dorsal horn after sciatic nerve injury: effects of a chronic constriction injury, transection and strychnine, Pain, 42 (1990) 205-213.

Sugimoto, T., Takemura, M., Akira, S. and Ishimaru, M., Rapid transneuronal destruction following peripheral nerve transection in the medullary dorsal horn is enhanced by strychnine, picrotoxin and bicuculline, Pain, 30 (1987) 385-393.

Tallarida, R.J. and Murray, R.B., *Manual of Pharmacologic Calculations*, Springer-Verlag, New York, (1987) 10-30.

Tanelian, D.L. and Brose, W.G., Neuropathic pain can be relieved by drugs that are use-dependent sodium channel blockers: lidocaine, carbamazepine, and mexiletine, Anesthesiology, 74 (1991) 949-951.

Tasker, R., Pain resulting from central nervous system pathology (central pain). In: J. Bonica (Ed.), *The Management of Pain*, Vol. 1, Lea & Febiger, Philadelphia, (1990) 264-286.

Tasker, R.R., DeCarvalho, G.T.C. and Dolan, E.J., Intractable pain of spinal cord origin: clinical features and implications for surgery, J. Neurosurg., 77 (1992) 373-378.

Todd, A.J., An electron microscope study of glycine-like immunoreactivity in laminae I-III of the spinal dorsal horn of the rat, Neuroscience, 39 (1990) 387-394.

Todd, A.J. and Sullivan, A.C., Light microscopic study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat, J. Comp. Neurol., 296 (1990) 496-505.

Triggs. W.J. and Beric, A., Sensory abnormalities and dysaesthesias in the anterior spinal artery syndrome, Brain, 115 (1992) 189-198.

Tureen, L.L., Effect of experimental temporary vascular occlusion on the spinal cord, Arch. Neurol. Psychiat., 35 (1936) 789-807.

Verdugo, R. and Ochoa, J.L., High incidence of placebo responders among chronic neuropathic pain patients, Ann. Neurol., 30 (1991) P229.

Wall, P.D., Devor, M., Inbal, R. Scadding, D., Schonfeld, D., Selter, Z. and Tomkiewicz, M.M., Autotomy following peripheral nerve lesions: experimental anasthesia dolorosa, Pain, 7 (1979) 103-113.

Wall, P.D. and Gutnick, M., Ongoing activity in peripheral nerves: the physiology and pharmacology of impulses originating from a neuroma, Exper. Neurol., 43 (1974) 580-593.

Wall, P.D., Mechanisms of acute and chronic pain, In: L. Kruger and J.C. Liebeskind (Eds), *Advances in Pain Research and Therapy*, 6 (1984) 95-104.

Wanaka, A., Shiotani, Y., Kiyama, H., Matsuyama, T., Kamada, T., Shiosaka, S. and Tohyama, M., Glutamate-like immunoreactive structures in primary sensory neurons in the rat detected by a specific antiserum against glutamate, Exp. Brain Res., 65 (1987) 691-694.

Watson, C.P.N., Horshead, C., Van der Kooy, D., Deck, J. and Evans, R.J., Post-herpetic neuralgia: post-mortem analysis of a case, Pain, 34 (1988) 129-138.

Westlund, K.N., Bowker, R.M., Ziegler, M.G. and Coulter, J.D., Descending noradrenergic projections and their spinal terminations, Prog. Brain. Res., 57 (1982) 219-238.

Wiesenfeld-Hallin, Z., Substance P and somatostatin modulate spinal cord excitability via physiologically different sensory pathways, Brain Res., 372 (1986) 172-175.

Willcockson, W.S., Chung, J.M., Hori, Y.. Lee, K.H. and Willis, W.D., Effects of iontophoretically released amino acids and amines on primate spinothalamic tract cells, J. Neurosci., 4 (1984a) 732-740.

Willcockson, W.S., Chung, J.M., Hori, Y., Lee, K.H. and Willis, W.D., Effects of iontophoretically released peptides on primate spinothalamic tract cells, J. Neurosci., 4 (1984b) 741-750.

Willer, J.C., Bergeret, S., Broucker, T. and Gaudy, J.-H., Low dose epidural morphine does not affect non-nociceptive spinal reflexes in patients with postoperative pain, Pain, 32 (1988) 9-14.

Winer, B.J., Brown, D.R. and Michels, K.M., *Statistical Principles in Experimental Design*. 3rd. Ed., McGraw-Hill, Inc., New York, (1991).

Woolf, C.J., Evidence for a central component to post-injury pain hypersensitivity, Nature, 306 (1983) 686-688.

Woolf, C.J., Shortland, P. and Coggeshall, R.E., Peripheral nerve injury triggers central sprouting of myelinated afferents, Nature, 355 (1992) 75-78.

Xu, X.-J., Hao, J.-X., Aldoskogius, H., Seiger, Å., and Wiesenfeld-Hallin, Z., Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury, Pain, (1992) 279-290.

Xu, X.-J., Hao, J.-X., Seiger, Å. and Wiesenfeld-Hallin, Z., Systemic excitatory amino acid receptor antagonists of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and of the N-methyl-D-aspartate receptor relieve mechanical hypersensitivity after transient spinal cord ischemia in rats, J. Pharmacol. Exp. Ther., 267 (1993) 140-144.

Yaksh, T.L. and Harty, G.J., Pharmacology of the allodynia in rats evoked by high dose intrathecal morphine, J. Pharmacol. Exp. Ther., 244 (1988) 501-507.

Yaksh, T.L. and Rudy, T.A., Studies on the direct spinal action of narcotics in the production of analgesia in the rat, J. Pharmacol. Exp. Ther., 202 (1977) 411-428.

Yaksh, T.L., 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 (1989) 111-123.

Yaksh, T.L., Jessell, T.M., Gamse, R., Mudge, A.W. and Leeman, S.E., Intrathecal morphine inhibits substance P release from the mammalian spinal cord in vivo, Nature, 286 (1980) 155-157.

Yaksh, T.L., Pharmacology and physiology of spinal analgesia, *ISI Atlas of Science: Pharmacology*, 30 (1987) 173-176.

Yamamoto, T. and Yaksh, T.L., Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve. Excitatory amino acid antagonists, Pain, 49 (1992) 121-128.

Yokota, T., Nishikawa, N. and Nishikawa, Y., Effects of strychnine upon different classes of trigeminal subnucleus caudalis neurons, Brain Res., 168 (1979) 430-434.

Young, A.B. and Snyder, S.H., Strychnine binding associated with glycine receptors of the central nervous system, Proc. Nat. Acad. Sci., 70 (1973) 2832-2836.

Young, A.B. and Snyder, S.H., Strychnine binding in spinal cord membranes associated with the synaptic glycine receptor: cooperativity of glycine interactions, Mol. Pharmacol., 10 (1974) 790-809.

Zarbin, M.A., Wamsley, J.K. and Kuhar, M.J., Glycine receptor: light microscopic autoradiographic localization with [^{3}H] strychnine, J. Neurosci., 1 (1981) 532-547.

Zemlan, F.P., Leonard, C.M., Kow, L.-M. and Pfaff, D.W., Ascending tracts of the lateral columns of the rat spinal cord: a study using the silver impregnation and horseradish peroxidase techniques, Exper. Neurol., 62 (1978) 298-334.

Zieglgänsberger, W. and Hertz, A., Changes of cutaneous receptive fields of spino-cervical-tract neurones and other dorsal horn neurones by microelectrophoretically administered amino acids, Exp. Brain Res., 13 (1971) 111-126.