ANTINOCICEPTIVE ACTIONS OF DESCENDING CATECHOLAMINERGIC TRACTS ON DORSAL HORN SOMATOSENSORY NEURONES

by

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ABSTRACT

Ionophoretically-applied dopamine and noradrenaline selectively inhibited the nociceptive responses of multireceptive somatosensory dorsal horn neurones, whilst non-nociceptive responses, spontaneous activity and activity evoked by an ionophoretically-applied excitatory amino acid, DL-homocysteic acid were unaffected. Many of the neurones tested had long ascending projections, capable of transmitting nociceptive information to supraspinal sites; in the rat (spinothalamic tract neurones) and in the cat (spinocervical tract neurones).

The use of ionophoretically-applied receptor-specific agonists and antagonists demonstrated that the actions of noradrenaline and dopamine were pharmacologically distinct. The selective antinociceptive action produced by noradrenaline was mediated by an α_2-adrenoreceptor, whilst the selective antinociceptive effect of dopamine was mediated by a D_2 dopamine receptor.

A glyoxylic-acid histofluorescence study was undertaken to ascertain the optimal stereotaxic placement of stimulating electrodes, in the regions of those dopamine cell groups (A9 and A11) that have been considered to provide a spinal projection.

Focal electrical stimulation in the region of the A11 dopamine cell group evoked a selective antinociceptive effect on multireceptive dorsal horn neurones in the rat. This stimulus-evoked effect was rapidly and consistently reversed by ionophoresis of the D_2 dopamine-receptor antagonist, sulpiride, in the vicinity of the dorsal horn neurone being tested, whilst an opiate antagonist (naloxone) and an α_2-antagonist (RX781094) had no effect. Using the same parameters, focal electrical stimulation in the region of the A9 dopamine cell group did not affect the evoked responses of any multireceptive neurones tested.

The results of this study present data supporting selective antinociceptive roles for dopamine and noradrenaline at the spinal level. The A11 dopamine cell group was demonstrated as a supraspinal source of a selective antinociceptive effect, mediated by dopamine at the level of the dorsal horn.

INTRODUCTION

A. General Introduction.

The nature of pain has been the source of philosophical and scientific argument for many years. Pain has been defined as an unpleasant sensory and emotional experience associated with actual, or potential damage (IASP, 1979). Before the advent of electrophysiological techniques, two opposing theories were proposed to explain how noxious cutaneous stimuli were transmitted through the peripheral and central nervous systems. Goldscheider (1894) formulated the 'intensity theory', in which weak stimulation was presumed to excite cutaneous sensory nerve endings to a level of neuronal activity which would be interpreted as non-painful, whereas an increased strength of stimulation would eventually evoke pain. This concept presumed no special neuronal apparatus for detecting noxious stimuli and did not call for a specific central pathway. This view was challenged by advocates of the 'specificity theory'. One of the earliest references to pain as a specific sensation, separate from that of touch, is attributed to Müller (1842). Müller proposed that activity in a particular nerve gave rise to a specific sensation, regardless of the stimulus used. This view embodied the concept that specific neural substrates mediated specific cutaneous sensations, including pain. Von Frey (1897) extended Müller's concept of 'specific nerve energies'

and proposed the existence of four distinct classes of cutaneous sensory receptors, subserving the modalities of touch, warmth, cold and pain, based on psychophysical studies. Although, a strict segregation of somatosensory traffic into four channels, as implied by Von Frey's theories, has proved erroneous, such views firmly established pain as a specific sensation mediated by nociceptors.

The 'intensity theory' was restated by Weddell (1955) and Sinclair (1955) and received some support from recordings of single visceral afferent fibres (Iggo, 1959). However, it cannot account for many facts now known about the somatic system, for example, the different characteristics of cutaneous receptors.

B. Cutaneous receptors.

The development of sensitive electrophysiological techniques enabled discharges from single primary afferent fibres to be recorded in order to intercept the signals from individual cutaneous receptors (e.g. Adrian , 1926). The consensus from a variety of these techniques, describes three general classes of cutaneous receptors (Burgess and Perl, 1973; Hensel, 1973; Iggo, 1974):

Mechanoreceptors, which are highly sensitive to mechanical displacement of the skin and relatively insensitive to temperature changes.

Themoreceptors, which are highly sensitive to either a

rise or fall in skin temperature, and almost completely insensitive to cutaneous mechanical stimulation. Nociceptors, which are distinguished by their ability to respond to potentially damaging stimulation of the skin. Three main classes of nociceptors have been recognised. Mechanical nociceptors, in the non-primate innervated by C- and $A\delta$ -fibres (Iggo, 1960; Burgess and Perl, 1967). Thermal nociceptors which respond to high (>42^{o}C) and very low (<10^{o}C) temperatures surrounding the skin and may also respond to noxious mechanical stimulation (Iggo, 1959). In addition, some nociceptors may be excited by locally-released chemical agents (Besson and Perl, 1969), which may be produced during inflammatory reactions (Chahl and Iggo, 1977) as well as mechanical and thermal stimuli.

The separation of a distinct class of nociceptors and the finding that mechanoreceptors can be activated maximally by innocuous cutaneous stimulation (Iggo and Muir, 1969), is difficult to reconcile with the 'intensity theory'. Damage to tissues in pathological conditions may result in changes in the environment of the receptor, such as the production of algesic substances like bradykinins and serotonin (Chahl and Iggo, 1977). In the absence of pathology, however, a primary stage of effective control of afferent inputs is thought to operate at the level of the dorsal horn (e.g. Hentall and Fields, 1979). Anatomical and physiological studies have been undertaken to determine the principles of organisation and function of this part of the central

nervous system. In recent years detailed and systematic analyses of the various neural elements of the dorsal horn and their interactions have been carried out.

C. Laminar organisation of cutaneous afferent input to the dorsal horn.

Rexed (1952; 1954) systematically described the laminar organisation of the neuronal perikarya of the dorsal horn, as visualized by cytoarchitectonic stains. The marginal layer previously described by Lissauer (1886) and Waldeyer (1888) and the substantia gelatinosa (SG), (Rolando, 1824) were termed laminae I and II, respectively. A classification for a series of deeper dorsal horn laminae was also introduced (Figure 1). These laminae are a useful guide to an understanding of the basic anatomy of the dorsal horn, but cytoarchitectonic studies do not provide any information about dendritic and axonal interconnections. Such considerations are essential for elucidation of the relationships between the complex anatomy and function of the spinal cord.

Morphological studies suggested that fine afferent fibres terminate in the superficial dorsal horn, in laminae I and II (Ranson, 1913; Ralston and Ralston, 1979; Rethelyi, 1977; La Motte, 1977; Gobel, Falls and Humphrey, 1981), whereas larger afferent fibres terminate in the deeper dorsal horn, laminae II to V (Szentagothai, 1964; Scheibel and Scheibel, 1968).

Since fine myelinated (i.e. Aδ) and unmyelinated (i.e. C) dorsal root afferent fibres may respond to innocuous, as well as noxious cutaneous stimulation (Iggo, 1974; Hensel and Iggo, 1973), it is not sufficient, in the context of nociception, simply to trace the terminations of such fibres. Intracellular labelling of individual, identified, afferent fibres with horseradish peroxidase (HRP) (Snow, Rose and Brown, 1976; Jankowska, Rastad and Westman, 1976) has allowed definitive statements to be made about the local organisation of afferent fibre terminations within the dorsal horn, with respect to cutaneous sensory modalities. Such experiments have indicated a functional segregation of cutaneous afferent termination within the dorsal horn. Collaterals of mechanical nociceptors, with myelinated (Aδ) axons, were described by Light and Perl (1979a) to terminate in lamina I, around the lamina I/II border and also more deeply in lamina V. In contrast, Type D hair follicle, non-nociceptive (Aδ) afferents (Brown and Iggo, 1967) terminated in the deeper dorsal horn laminae, II and IV, with no apparent terminations in lamina I, or outer lamina II (Rethelyi, Light and Perl, 1982). Unmyelinated afferent (C) fibres have not been so readily amenable to single unit analysis, although Perl (1984) reported incompletely characterised HRP-labelled unmyelinated fibres (in the primate and guinea pig) terminating mainly in the SG, with some indication of projections ventral to the SG. From these studies it is

apparent that cutaneous afferent termination within the dorsal horn is related to the sensory function, not simply to the axon diameter of the fibre (Figure 1).

Within the dorsal horn, a segregation of cutaneous afferent innervation has functional implications for somatosensory neurones. Electrophysiological and anatomical techniques have enabled investigators to establish many characteristics of dorsal horn neurones excited by cutaneous stimulation.

Dorsal horn neurones may be classified according to several parameters, in a manner proposed by the IUPS Somatosensory and Pain Commission (Brown and Rethelyi, 1981), on the basis of:-

(i) their responses to electrical and natural cutaneous stimuli,

(ii) their location in the dorsal horn and

(iii) the destination of their axons.

These parameters are discussed in the following sections, along with their implications for the processing of somatosensory information.

D. The organisation of dorsal horn neurones with respect to processing of nociceptive information.

D(i) Responses of dorsal horn neurones to cutaneous stimuli:

Several attempts have been made to classify those dorsal horn neurones that have been recorded in

electrophysiological experiments, in terms of their responses to electrical and/or cutaneous stimuli (reviewed by Willis and Coggeshall, 1978). Some of these systems are described below, within a broad outline of the general categorisation of dorsal horn neurones suggested by Iggo (1974; 1977) and Handwerker, Iggo and Zimmermann (1975).

Class 1 neurones are excited by non-noxious cutaneous stimuli, via sensitive mechanoreceptors innervated by A- but not C-fibres. Class 1 neurones do not receive a noxious cutaneous input and may therefore be described as non-nociceptive.

Class 2 neurones are excited by inputs from sensitive mechanoreceptors and thermal and mechanical nociceptors, supplied by both A- and C-fibres.

Wall (1967) reported that neurones located in laminae IV, V and VI tended to have different properties. Many neurones in laminae IV were reported to respond exclusively to non-noxious, cutaneous stimulation (Class 1 neurones). Cells in lamina V were reported to typically respond to both noxious and non-noxious, cutaneous stimuli (Class 2 neurones), whilst neurones in lamina VI, additionally received propriospinal inputs. From these observations an informal nomenclature developed in which, for example, a non-nociceptive neurone was called a 'lamina IV-type cell' whilst a neurone receiving noxious and non-noxious inputs was called a 'lamina V-type cell' (e.g., Hillman and Wall,

1969; Besson, Guilband and Le Bars, 1975). As Wall (1967) pointed out, however, neurones located in lamina IV may also receive nociceptive inputs, so this nomenclature did not prove adequate. Mendell (1966) had previously described a system of classification which took into account the intensity of stimulation required to evoke a response from a dorsal horn neurone. Neurones that could be activated by both light tactile and more intense stimuli (Class 2 neurones) were called 'wide dynamic range' neurones. Class 2 neurones receive both noxious and innocuous inputs and may therefore also be described as multireceptive, in terms of their cutaneous afferent input. In a number of cases, very detailed investigations have been carried out to study the nature of the different stimuli that evoke responses from dorsal horn neurones. It has become clear that within the general classification systems proposed by Iggo (1974; 1977) and others, there exists a variety of subdivisions of responsiveness to different noxious and innocuous stimuli (e.g. Heavner and De Jong, 1973; Tapper, 1973). For example, Menetrey, Giesler and Besson (1977) subdivided non-nociceptive and multireceptive dorsal horn neurones, in the rat. Class 1A neurones were activated only by hair movement and/or touch, whilst class 1B neurones additionally responded to innocuous pressure. As well as responding to noxious, cutaneous stimuli (pinch or pinprick), multireceptive neurones were subdivided into 2 classes, on the basis of their

responses to innocuous stimuli. Class 2A neurones responded to hair movement and/or touch, whilst class 2B neurones were excited only by innocuous pressure.

Class 3 neurones respond only to nociceptive stimulation and are generally encountered more rarely than class 2 neurones (Christensen and Perl, 1970; Menetrey et al. 1977; Cervero, Iggo and Ogawa, 1976). Cervero et al. (1976) described two subclasses, class 3a and 3b. Class 3a neurones are excited by Aδ fibres and respond to noxious mechanical stimuli, whereas class 3b neurones, in addition to an Aδ- input, receive input from C-fibres and respond to both noxious, mechanical and thermal stimuli. Some class 3 neurones can be excited by group III and IV muscle afferents (Cervero et al. 1976). Class 3 neurones do not receive innocuous cutaneous inputs and may therefore be described as nocispecific.

This terminology should not, however, imply that only class 3 neurones have a nociceptive function. Many class 2, multireceptive neurones have ascending axonal projections and may thereby, also contribute to the perception of pain. The anatomical and physiological characteristics of ascending tracts, formed by such axonal projections, are described in section D(iii).

D(ii) Distribution and properties of nociceptive neurones in the dorsal horn:

It has become clear that the laminar boundaries apparent between dorsal horn neurones (Rexed, 1952; 1954)

are difficult to define, either in terms of morphology or function. This is particularly true of neurones located in the deeper dorsal horn laminae, III to V. Characteristic differences have, however, been demonstrated between neurones in laminae I, II and those in the deeper dorsal horn.

Lamina I: A number of groups have reported the presence of nociceptive neurones in lamina I, many of which were described in morphological-functional studies as large marginal Waldeyer cells (e.g. Woolf and Fitzgerald, 1983; Perl, 1984). Some of these neurones were divided into classes 3a and 3b (as described in the previous section D(i), by Cervero et al. 1976). However, not all Waldeyer cells are nocispecific. Rethelyi, Light and Perl (1983) described HRP-stained Waldeyer cells, some of which were nocispecific, whilst others were multireceptive. Some studies have reported that the majority of lamina I cells are multireceptive (McMahon and Wall, 1983a; Woolf and Fitzgerald, 1983). However, it is clear that a subpopulation of nocispecific neurones are present in lamina I, some having long ascending projections, for example, to the thalamus (Dubner and Bennett, 1983; Craig and Kniffiki, 1985), although the relative proportions of multireceptive versus nocispecific neurones is unclear. Caution must be exercised in accepting the properties of the larger (Waldeyer) lamina I neurones, reported in electrophysiological studies (Rethelyi et al. 1983; Woolf and Fitzgerald, 1983), as characteristic of all lamina I

neurones since they constitute only a small proportion (5%) of the total population of lamina I neurones (Lima and Coimbra, 1983).

In electrophysiological studies, it has been reported that as many as 60% of nocispecific lamina I neurones may be segmental or local interneurones, without long ascending projections (Kumazawa, Perl, Burgess and Whitehorn, 1975; Handwerker, Iggo, Ogawa and Ramsey, 1975; Cervero, Iggo and Molony, 1979). McMahon and Wall (1983a) however, reported that many multireceptive lamina I cells were antidromically activated from the contralateral dorsolateral funiculus (DLF).

It is clear that cells of lamina I exhibit a marked heterogeneity, in both morphology (Cajal, 1909; Perl, 1984) and function (Molony, Steedman, Cervero and Iggo, 1981; Woolf and Fitzgerald, 1983; Perl, 1984), which is not consistent with a single functional role.

Lamina II: Only continued improvements in electrode and related recording techniques have allowed investigation of the small neurones located in lamina I and II. Considering the estimate of Cervero and Iggo (1980), that there are 75,000 - 90,000 lamina II cells per millimetre of spinal cord in the cat, on one side, it is clear that only a tiny minority of these neurones have been investigated. Extracellular recordings, attributed to SG neurones on the basis of electrode tip position by Hentall (1977) and intracellular HRP studies by Cervero, Molony, Iggo and Steedman (1980), described cells which

would be inhibited by noxious and/or innocuous cutaneous stimuli in the lumbar spinal cord of the cat. Kumazawa and Perl (1976; 1978) also attributed extracellular recordings to SG neurones on the basis of electrode tip position, in the coccygeal region of the spinal cord of the monkey and reported an excitatory C-fibre input to these neurones. Other studies, as well as recording from neurones located in the SG, may well have included lamina I neurones (Light and Perl, 1979a; 1979b; Light, Trevino and Perl, 1979) or lamina III neurones (Wall, 1978; Wall, Merrill and Yaksh, 1979; Dubuisson, Fitzgerald and Wall, 1979). The emphasis, however, on SG neurones being powerfully affected by inputs from C-fibre afferents and on showing marked habituation to iterative skin stimuli has been agreed upon by other groups (Cervero et al. 1980; Kumazawa and Perl, 1976; 1978).

Intracellular staining of identified neurones in the SG has confirmed the presence of neurones with inhibitory (Molony, Steedman, Cervero and Iggo, 1981) and excitatory receptive fields (Bennett, Abdelmoumene, Hayashi and Dubner, 1980), responding to noxious and/or innocuous cutaneous stimulation. Neurones in the SG have been classified into two main groups (Cajal, 1909; Gobel, 1975; 1978) and these classes of cells have been reported to receive differential inputs (Bennett et al. 1980). Stalked and islet cells located in the outer SG were reported to receive both nociceptive and non-nociceptive inputs, whilst the islet cells located more ventrally in

the inner SG were reported to receive predominantly non-nociceptive inputs. However, it is thought that these two groups stalked and islet cells, may represent the extremes of a spectrum of cells in the SG (Molony et al. 1981; Light et al. 1979b; Beal and Cooper, 1978; Steedman, Molony and Iggo, 1985). Immunohistochemical studies have demonstrated enkephalin containing interneurones in lamina I and II, as well as in the deeper dorsal horn (Dubner, Ruda, Miletic, Hoffert, Bennett, Nishikawa and Coffield, 1984).

Gobel, Falls, Bennett, Abdelmoumene, Hayashi and Humphrey (1980) used intracellular HRP staining in combination with electron microscopy, to provide pictures of synaptic contacts, judged to be axo-axonal, made by nociceptor afferents with dendrites in lamina I. As the majority of dendrites in lamina I arise from neurones with cell bodies in lamina I, it may be concluded that some of these neurones receive a monosynaptic nociceptive input. This conclusion may be extended to include some SG neurones. Electrical stimulation of peripheral nerves and dorsal roots above C-fibre threshold caused SG neurones, recorded intracellularly, to discharge impulses with long duration and remarkably constant latencies of about 250 ± 2 msecs (Steedman et al. 1985). Although this is incomplete evidence, it suggests that a monosynaptic, C-fibre input may be received by some SG neurones. Fitzgerald and Wall (1980) came to a similar conclusion on the basis of extracellular studies.

A number of studies have reported lamina I neurones with axons projecting to supraspinal sites (section D(iii)). Some SG neurones may also provide ascending axons, being retrogradely labelled with HRP, injected into the cervical cord and lower brain stem, in the rat (Giesler, Cannon, Urca and Liebeskind, 1978) and the contralateral thalamus of the monkey (Willis and Leonard, 1978). Szentagothai (1964), however, observed large numbers of small neurones in laminae I and II, whose axons had only limited projections (2 or 3 segments) and terminated in laminae I and II. Thus a major proportion of SG neurones may form a propriospinal system, with ample opportunity to make contacts with neurones in laminae I and III and the dendrites of laminae IV and V neurones, which project extensively into lamina III (Brown, Rose and Snow, 1976; 1977), as well as with afferent terminations (Figure 1).

Laminae II-V: A considerable number of larger nociceptive neurones are located in the deeper dorsal horn. Both neurones identifiable by the projection of their axons to supraspinal sites (section D(iii)) and unidentified neurones (some of which may be propriospinal) are present. As in laminae I and II, this region also contains a variety of morphologically and functionally different neurones.

Many neurones recorded in electrophysiological experiments are multireceptive. This finding, combined with clinical observations, prompted Melzack and Wall

(1965) to propose the 'Gate Control Theory'. A gating mechanism in the SG was proposed to be able to modulate afferent input to cells whose axons projected directly to supraspinal sites ('T-cells'). The activity of the SG gating mechanism was dependent on the balance of activity in larger (non-nociceptive, A) and fine (nociceptive, C and Aδ) fibres. A detailed description of the reflex pathways proposed, is given in Figure 2.

It has been consistently demonstrated, as predicted by the 'Gate Control Theory', that the activation of larger (non-nociceptive, A) fibres can inhibit the nociceptive responses of dorsal horn neurones (Gregor and Zimmermann, 1972; Brown, Hamann and Martin, 1973; Cervero et al. 1976). This effect seems to be somatotopically organised, being evoked from areas surrounding the cutaneous excitatory receptive fields (Hillman and Wall, 1969; Besson, Catchlove, Feltz and Le Bars, 1974) and also seems to involve both presynaptic and postsynaptic mechanisms (Whitehorn and Burgess, 1973; Zieglgansberger and Herz, 1971; Besson et al. 1974). It has been proposed that these electrophysiological results could represent the basis for analgesia obtained in humans using transcutaneous and dorsal root stimulation techniques (Wall and Sweet, 1967; Nathan and Wall, 1974).

Several objections, however, to the 'Gate Control Theory' have arisen (e.g. Nathan, 1976). Firstly, it did not account for the presence of non-nociceptive C-fibres innervating the dorsal horn (Iggo, 1959; 1965).

Secondly, the prediction was made by this theory that stimulation of fine (nociceptive, C and Aδ) fibres would inhibit the activity of neurones in the SG and result in reduced presynaptic inhibition (hyperpolarization) of afferent fibres. The positive dorsal root potential expected from this action has not been generally confirmed (Zimmermann, 1968; Franz and Iggo, 1968; Janig and Zimmermann, 1971; Gregor and Zimmermann, 1973), although some positive dorsal root potentials have been reported (Dawson, Merrill and Wall, 1970; Hodge, 1971). Anatomical and physiological studies generally provide little evidence for any significant monosynaptic nociceptive input to multireceptive neurones located in the deeper dorsal horn laminae III to V (introduction section C).

Furthermore, other components of the dorsal horn, such as a population of nociceptive lamina I neurones, described since the publication, by Melzack and Wall (1967), have made the 'Gate Control Theory' (in the form originally proposed) an unsatisfactory experimental model. Some elements do remain useful, however, particularly those aspects stressing the presence of both descending and propriospinal modality-selective controls that operate predominantly in superficial dorsal horn.

D(iii) Ascending nociceptive somatosensory tracts originating from the dorsal horn:

General acceptance of the 'specificity theory'

promoted clinical and physiological searches for a spinal 'pain pathway'. Whereas it was once concluded that a major pain pathway ascended in the ventrolateral quadrant of the spinal cord (Head and Holmes, 1911), it has become clear that a number of ascending tracts may contribute, perhaps to different aspects, of pain (e.g. Price and Dubner, 1975). The following sections briefly describe the physiological characteristics of the major classes of dorsal horn neurones, whose axons project to identified supraspinal sites and which may contribute to the perception of nociceptive information.

The Spinothalamic Tract:

The well developed spinothalamic tract (STT) in the rat and the monkey has been divided into three components, in electrophysiological and anatomical studies (Giesler, Yezierski, Gerhart and Willis, 1981; Kevetter and Willis, 1984). The paleospinothalamic tract is said to be equivalent to the medial spinothalamic tract (M-STT), which innervates the medial and intralaminar nuclei of the thalamus (Kevetter and Willis, 1984). Dorsal horn neurones, antidromically activated from the contralateral medial thalamus, in general have large receptive fields, often bilateral, covering most of the body surface and respond predominantly to noxious cutaneous stimulation (Giesler et al. 1981). These very large excitatory receptive fields of M-STT neurones depend on a neural pathway that involves a supraspinal

relay, because transection of the rostral spinal cord abolishes the excitatory effects from stimulation everywhere except on the ipsilateral hindlimb (Giesler et al. 1981). In contrast, dorsal horn neurones antidromically activated from the contralateral, lateral thalamus (L-STT, neospinothalamic tract) have restricted, unilateral receptive fields responding to noxious and/or innocuous stimuli. Most electrophysiological studies have described L-STT neurones, identified by antidromic activation from the ventrolateral posterior nucleus (VLP) of the rat and monkey. Geisler et al. (1981) also reported a population of dorsal horn neurones which were antidromically activated from sites in both the contralateral, lateral and medial thalamus. These, LM-STT neurones were reported to have unilateral receptive fields and respond to noxious and/or innocuous cutaneous stimulation, like L-STT neurones. The idea of a population of STT neurones with collaterals projecting to both the contralateral, medial and lateral thalamus, was supported in retrograde labelling studies, by Kevetter and Willis (1984).

The distribution of STT neurones in the rat and monkey are similar, whether described in electrophysiological or anatomical studies, located mainly in laminae I and IV to VI of the dorsal horn with some neurones in laminae VII to VIII (Trevino, Coutler and Willis, 1973; Albe-Fessard, Boivie, Grant and Levante, 1974). This distribution seems to be similar for L-STT,

LM-STT and M-STT neurones, although fewer STT neurones were reported retrogradely labelled, from the medial thalamus, in lamina I of the dorsal horn of the rat (Kevetter and Willis, 1984). Some nocispecific neurones in lamina I have been reported to belong to the L-STT tract (Willis, Trevino, Coulter and Maunz, 1974; Giesler et al. 1981). However, many STT neurones in the deeper laminae of the rat and monkey are multireceptive (Giesler, Menetrey, Guilbaud and Besson, 1976; Price, Hayes, Ruda and Dubner, 1978).

Even within the medial and lateral thalamus, different nuclei are reported to receive differential spinal projections. Within medial thalamus, for example, only the submedius nucleus has been reported to receive a dense projection, arising from lamina I dorsal horn neurones, which may potentially subserve a specific nociceptive function (Craig and Burton, 1981). Other medial nuclei, such as the central median, parafasiculus, central and lateral and paracentral nuclei receive inputs from more ventrally located spinal neurones in the rat, cat and monkey (Carstens and Trevino, 1978; Giesler, Menetrey and Bausbaum, 1979; Craig and Burton, 1981).

The distribution of STT neurones in the spinal cord of the cat differs from that described above, for the monkey and rat. Electrophysiological experiments, using signal averaging techniques (Trevino, Maunz, Bryan and Willis, 1972; McCreery and Bloedel, 1975; Meyers and Snow, 1982), have supported anatomical findings (Trevino

and Carstens, 1975; Carstens and Trevino, 1978), that the majority of STT neurones, in the cat, are located in laminae VII and VIII, with only a few neurones located in lamina I, IV and V. Neurones responding to noxious and/or innocuous cutaneous stimulation were reported (Trevino et al. 1972; McCreery and Bloedel, 1975) as well as some also responding to joint or visceral stimulation (Meyers and Snow, 1982).

The termination of the STT neurones in the lateral thalamus (VLP and posterior nucleus) is limited in the cat, compared to the rat and monkey (Boivie, 1971; Kerr, 1975; Berkely and Mash, 1978). Holloway, Fox and Iggo (1978) in an electrophysiological study and Boivie (1971) using anatomical techniques, reported that the STT of the cat did not terminate in the VLP. Recent orthograde labelling studies demonstrated some spinal projections to the VLP of the cat (Mantyn, 1983; Burton and Craig, 1983). The orthograde labels used in these studies (lectin-conjugated HRP) may label neurones with collaterals in the spinal cord and thalamus, therefore the presence of a small spinal projection to the VPL of the cat remains open to question. It may be possible that the spinoreticular tract which is well developed in the cat (Fields, Clanton and Anderson, 1977; Maunz, Pitts and Peterson, 1978), undertakes part of the role of the STT in this species.

The STT has been regarded as a 'pain pathway' for some years. This view was supported by clinical

observations, in which lesions of the ventrolateral quadrant (VLQ) of spinal cord were reported to relieve otherwise intractable pain states in some people (Spiller and Martin, 1912). However, reports that as many as 50% of these patients may experience a return of painful sensations has limited the use of chordotomy to patients with short life expectancies (White and Sweet, 1969). Since it is not only the STT that ascends in the VLQ, lesions of propriospinal projections or the SRT may also contribute to this temporary relief of pain (Vierck, Greenspan, Ritz and Yeomans, 1985). Furthermore, extensive lesions of the dorsal columns do not result in complete loss of tactile sensation (Wall and Noordenbos, 1977) suggesting that pathways in VLQ are involved in the transmission of innocuous, as well as noxious cutaneous, stimuli and thus do not have a solely nociceptive role.

The Spinoreticular Tract:

Spinoreticular tract (SRT) neurones have been retrogradely labelled from the contralateral and ipsilateral medullary pontine reticular formation (MPRF) of the cat (Abols and Basbaum, 1979; Kevetter and Willis, 1982). These studies found SRT neurones located in lamina V of the dorsal horn and laminae VII and VIII of the ventral horn. Maunz et al. (1978) reported extracellularly recorded SRT neurones located in the ventral horn, which were antidromically activated from the medial MPRF. Fields et al. (1977) electrically

stimulated both medial and lateral MPRF sites and identified SRT neurones in both the dorsal and ventral horn of the spinal cord. Both groups of workers found that a major proportion of SRT neurones had complex bilaterally located, excitatory and inhibitory receptive fields (unlike the ipsilateral, excitatory receptive fields of L-STT or SCT neurones, described by Price et al. 1978; Brown and Franz, 1969). Neurones located in the reticular formation receive an input from the nociceptive SRT neurones and also provide a major projection to the medial (intralaminar) thalamus, in the cat (McGuiness and Krauthamer, 1980) and rat (Peckanski and Besson, 1984) Another major input to the thalamus, in the cat, originates from the lateral cervical nucleus (Craig and Burton, 1979). This nucleus is a terminus for dorsal horn neurones forming the spinocervical tract.

The Spinocervical Tract:

A variety of methods have shown that the neurones of the spinocervical tract (SCT) are situated mainly in laminae III, IV and V of the spinal dorsal horn, in the cat, using intracellular recording techniques (Hongo, Jankowska and Lundberg, 1968), extracellular recording techniques (Bryan, Trevino, Coulter and Willis, 1973; Cervero, Iggo and Molony, 1977 ; Brown, Rose and Snow, 1980) and intracellular recording combined with intracellular staining with HRP (Brown, House, Rose and Snow, 1976; Brown, Rose and Snow, 1980). Some SCT neurones appeared from

extracellular studies to be more dorsally located, in laminae I and II (Cervero et al. 1977). This was confirmed by injections of HRP into the lateral cervical nucleus (LCN). Approximately 5% of the total population of cells stained with HRP were located in laminae I, II and IV of the dorsal horn (Brown et al. 1980). It is worth noting, however, that with intracellular recording electrodes it is easier to obtain recordings from larger neurones. It was found that SCT neurones with axons conducting at less than 33 m/sec were rarely stained with HRP, which gives a biased view of the population sampled (Brown et al. 1980). Uptake by damaged axons of passage or spread outside the LCN may distort results from HRP transport studies. However, extracellular recording of superficially located (lamina I and II) SCT neurones confirms their presence but not their numbers (Cervero et al. 1977 ; Brown et al. 1980).

The majority (70%) of SCT neurones are multireceptive (Brown, 1971; Bryan et al. 1973; Cervero et al. 1977), responding to both noxious and innocuous stimuli with receptive fields on the hairy, but not glabrous skin. The remaining population of SCT neurones are either non-nociceptive or more rarely, nocispecific cells. Nocispecific SCT neurones may be divided into class 3a and 3b cells, according to Cervero et al. (1977) (introduction section D(i)).

The presence of a LCN to which SCT neurones project ipsilaterally, is generally regarded as acceptable

evidence for the existence of the SCT. This nucleus has been identified in a number of species: in the cat (Rexed and Strom, 1952), dog (Rexed, 1958), monkey (Ha and Morin, 1964) and man (Treux, Taylor, Smythe and Gildenburg, 1970). There were some initial doubts as to the presence and morphology of a discretely organised LCN in the rabbit and rodents: rabbit (Mizuno, 1966), rat (Gywn and Waldron, 1968) and guinea pig (Gywn and Waldron, 1969). Recent studies indicate that these doubts were unfounded, for the rat at least (Giesler et al. 1978; 1979).

As described previously, the STT has been regarded as a classical 'pain pathway', whilst, in contrast the SCT has been considered as essentially being concerned with the transmission of responses to light tactile stimuli. The finding that a majority of SCT and STT neurones respond to both noxious and innocuous cutaneous stimuli implies that both ascending tracts may be involved in the transmission of nociceptive (and non-nociceptive) information to supraspinal sites. Whilst the STT projects directly to the thalamus the SCT terminates at the level of the LCN , which in turn provides a major thalamic input (Craig and Burton, 1979). Thus somatosensory information form SCT may well undergo further processing before reaching the thalamus. The manner in which derivatives of the afferent information from specific nociceptors are resolved is crucially dependent on its processing at relay and end stations

which transmit and receive this information. Decoding of these inputs may involve analysis of relative spatial or temporal patterns, as yet little understood.

The Post Synaptic Dorsal Column System:

Neurones of the post synaptic dorsal column (PSDC) system can be excited by innocuous mechanical, noxious mechanical and thermal stimuli, applied ipsilaterally to both the hairy and glabrous skin of the cat (Angaut-Petit, 1975; Noble and Riddell, 1985). The neurones of the PSDC system are also located in laminae II to V of the dorsal horn. PSDC neurones, however, have a more complicated receptive field organisation than that of SCT or STT neurones. Intracellular studies have revealed that the excitatory receptive fields of PSDC neurones often had subliminal fringes and that within the perimeter of the excitatory field, inhibitory receptive fields also occurred (Brown and Fyffe, 1981). Intracellular HRP staining studies have demonstrated characteristic morphological differences between PSDC and SCT neurones (Brown et al. 1977; Brown and Fyffe, 1981). This finding supports the proposal that the nomenclature which arose in response to the observations of Wall (1967) (introduction section D(i)), i.e. 'lamina IV-type cells', is inadequate for describing the great variety of neuronal types present in the dorsal horn.

The Spinomesencephalic Tract:

Retrograde HRP studies have demonstrated a projection to the midbrain, originating largely from neurones located in lamina I, with a smaller fraction originating in lamina IV and V of the dorsal horn, in the cat (Wikberg and Blomqvist, 1984) and rat (Menetrey, Chaouch, Binder and Besson, 1982).

Separate roles have been proposed for nociceptive neurones located in lamina I and those in the deeper dorsal horn laminae. The population of nocispecific lamina I dorsal horn neurones (Christensen and Perl, 1970), some of which receive a monosynaptic nociceptive inputs (Gobel et al. 1980) have been proposed to be capable of transmitting a quantitatively valid account of noxious stimuli, from the periperal tissues to various supraspinal sites (Christensen and Perl, 1970). Not all lamina I neurones are nocispecific (section C(ii)) and therefore may not all subserve the same role. For example, the mesencephalic periaquaductal grey (PAG), to which a major population of lamina I neurones project, as described by Wikberg and Blomqvist, (1984), is also a potential source of both spinal antinociception (Gebhart, Sankuhler, Thalhammer and Zimmermann, 1984) and behavioural analgesia which may be evoked by electrical stimulation which may be evoked by electrical stimulation, first described by Reynolds (1969) .

The ascending tracts, described in this section, have at least two major characteristics in common; (a)

they have been shown to transmit both non-nociceptive and nociceptive information to supraspinal levels and (b) each tract appears to be under profound, mainly inhibitory influences, which originate from supraspinal sites. It may be envisaged that some lamina I neurones (perhaps belonging to the SMT) could be involved in reflexly - evoking these influences. Descending influences, which seem to be exerted particularly on nociceptive dorsal horn neurones, are discussed in the following section.

E. Descending influences, exerted on nociceptive dorsal horn neurones.

The principle of descending modulation of spinal responses was appreciated by a number of early workers (Sherrington and Sowton, 1905; Head and Holmes, 1911; Fulton, 1926). Electrical stimulation of brain sites was observed to evoke spinal effects, indicating, in general terms, that descending influences could modulate transmission in the spinal cord (Magoun and Rhines, 1946; Hagbarth and Kerr, 1954).

Tonic descending inhibition exerted on dorsal horn neurones, has been investigated in spinal cord reversible cold-blocking studies (Wall, 1967;Brown,1971;Handwerker, Iggo and Zimmerman, 1975; Besson, Guilbaud and Le Bars, 1975; Cervero et al. 1976; Noble and Riddell, 1985). Wall (1967) reported that cold-block of the spinal cord resulted in changes in the types of cutaneous sensory

responses of some dorsal horn neurones. Neurones, which initially responded only to innocuous cutaneous stimuli, in the spinal state, responded to both noxious and innocuous cutaneous stimuli. Handwerker, Iggo and Zimmermann (1975) reported that descending inhibition preferentially acted on the nociceptive responses of multireceptive dorsal horn neurone. Reversible cold-block of the spinal cord revealed that tonic descending inhibition was also exerted on SCT neurones (Brown, 1970; 1971). It was found that tonic descending inhibition attenuated neuronal responses to heavy pinch or noxious heating of the skin. This action, however, was not completely selective, as neuronal responses to light tactile stimuli were also affected, but the degree of tonic descending inhibition seemed more pronounced on the nociceptive responses of SCT neurones (Brown, 1970; 1971).

Parallels to these observations have been reported in response to electrical stimulation of medial brain stem structures, which reliably and selectively inhibits nociceptive behavioural responses, in the rat (Reynolds, 1969), cat (Mayer and Liebeskind, 1974) and monkey (Goodman and Holocombe, 1975). The postulated presence of specific endogenous modulatory systems, which would contribute significantly to antinociception was supported by parallels in clinical studies (Adams, 1976; Boethius, Carlsson and Meyerson, 1978). This phenomenon has been termed 'stimulus-produced analgesia' (SPA).

The evaluation of behavioural analgesia must demonstrate that a specific antinociceptive effect has occurred, whether evoked by electrical stimulation, the administration of pharmacological agents or by stressful manipulations. A generalized sensorimotor or motivational deficit indicates lack of specificity. The hot-plate and tail-pinch tests involve behavioural responses, mediated via supraspinal pathways, which a spinal animal could not perform, i.e. licking the paws or trying to remove a clip. The possibility of a general motor deficit, may be tested by assessing other sensory modalities. For example, the animal should still exhibit normal exploratory behaviour in response to light touch or sound (Mayer, Wolfle, Akil, Carder and Liebeskind, 1971; Soper, 1976). The tail-flick test evokes a spinal reflex, present in spinal animals and animals with intact neuraxes and may only be regarded as a test of antinociception. In this case an effect on the motor, rather than the sensory component of the response, cannot be assessed unless further behavioural tests are undertaken.

Evidence has accumulated that brain stem SPA results from the inhibition of nociceptive transmission, at the level of the dorsal horn and spinal trigeminal nucleus caudalis. Spinal nociceptive reflexes are inhibited by SPA (Mayer et al. 1971; Mayer and Liebeskind, 1974) and a similar effect was demonstrated on the jaw opening reflex (Oliveras, Besson, Guilbaud and Liebeskind, 1974).

Although it cannot be argued that the inhibition of nociceptive reflexes means nociceptive transmission to higher levels is also blocked, this may suggest that these pathways share some common neural mechanism.

Electrical stimulation, in the region of the nucleus raphe magnus (NRM) is reported to cause analgesia in man (Young, Feldman Kroening, Fulton and Morris, 1984) and also inhibits the nociceptive responses of dorsal horn neurones. In general, electrical stimulation in the region of the NRM preferentially inhibits the nociceptive, compared to non-nociceptive, responses of STT (and other unidentified dorsal horn) neurones (Oliveras et al. 1974; Beall, Martin, Applebaum and Willis, 1976; Gerhart, Wilcox, Chung and Willis, 1981; Willis, 1984). NRM stimulation produces inhibitory postsynaptic potentials in STT neurones (Giesler, Gerhart, Yezierski, Wilcox and Willis, 1981). However, electrical thresholds of the spinal terminals of C-fibre nociceptors were also increased, therefore both presynaptic and postsynaptic actions may be evoked at the spinal level.

Electrical stimulation studies, such as those described above, have mostly tested neurones located in the deeper dorsal horn. Few studies have investigated effects on the smaller neurones in laminae I and II. Nociceptive neurones in lamina I, both projecting and unidentified, were found to be relatively or completely unaffected by reversible cold block of the spinal cord

(Cervero et al. 1976). Neurones in laminae I, II and III have been reported to be excited by stimulation of the DLF (Dubuisson and Wall, 1980). Hypothetically, such effects may provide a mechanism for mediating either descending inhibitory actions or even segmental inhibition, evoked by afferent input (Taub, 1964; Brown, 1981), by controlling the excitability of inhibitory SG interneurones.

Behavioural analgesia may be electrically evoked from supraspinal sites, including the reticular formation, thalamic and hypothalamic nuclei, as well as the mesencephalic central grey area (Mayer, 1979). Not all stimulus-evoked descending influences are inhibitory, however. For example, electrical stimulation of wide areas of the cerebral cortex may produce excitation as well as inhibition of dorsal horn neurones (Wall, 1967), SCT neurones (Brown and Short, 1974; Brown Coulter, Rose, Short and Snow, 1977) and STT neurones (Coulter, Maunz and Willis, 1974). Electrical stimulation is not selective; it evokes activity in both neurones and fibres of passage in the region of current spread. Furthermore, activation of pathways in this manner cannot give any indication as to whether they have similar physiological actions in the normal animal. It may be that electrical stimulation can cause an antinociceptive effect, from a region which does not have an endogenous antinociceptive role. Other approaches, which may help to clarify this problem have investigated the pharmacological basis of

stimulus-evoked effects and the activation of intrinsic analgesic mechanisms using environmental stimuli (see below). SPA, evoked from the dorsal raphe region, can be blocked by the opiate antagonist, naloxone, whilst SPA evoked from the PAG was not affected (Cannon, Prieto, Lee and Liebeskind, 1982). Apparently, therefore opioid (naloxone-sensitive) and non-opioid (naloxone-insensitive) substrates for SPA exist in close proximity in the mesencephalon and both can exert spinal antinociceptive actions.

It has been suggested that the awareness of pain normally impels adaptive reactions essential for survival, therefore analgesic systems should not be activated trivially (Terman, Shavit, Lewis, Cannon and Liebeskind, 1984). Under emergency conditions however, such as attack by a predator, when pain perception could disrupt effective action, analgesia might be a suitable adaptive response. The pathways reported to subserve the effects of SPA may be involved in this adaptive response. This view may be supported by studies of the analgesic effects of certain environmental stressors (Bodnar, 1984). Stress-induced analgesia may, for example, be evoked by inescapable electric footshock which may produce either opioid or non-opioid-dependent analgesia, depending on slight differences in the protocol used (Terman et al. 1984). Both opioid and non-opioid dependent footshock-induced analgesia can be disrupted by spinal DLF lesions (Watkins, Cobelli and Mayer, 1982;

Lewis, Terman, Watkins, Mayer and Liebeskind, 1983). Spinal pathways, particularly in the DLF, have also been implicated in stimulus-produced and opiate analgesia (Basbaum, Marley, O´Keefe and Clanton, 1977; Barton, Basbaum, Clanton and Fields, 1980).

Interest in the non-opioid systems demonstrated in both stimulus-produced and stress-induced analgesia, has developed because activation of these systems may result in analgesia without unwanted opiate side-effects such as tolerance and dependence. The monoamines are possible candidates for the mediation of both stress-induced analgesia (Hutson, Curzon and Tricklebank, 1984; Lewis, Terman, Nelson and Liebeskind, 1984) and SPA (Satoh, Akaike, Nakazawa and Takagi, 1980; Satoh, Oku and Akaike, 1983; Jensen and Yaksh, 1984b). There have been a variety of investigations into the mechanisms by which 5-HT may mediate antinociceptive effects at the spinal level (e.g. Belcher, Ryall and Schaffner, 1978; Headley, Duggan and Griersmith, 1978) with generally some lack of agreement as to the selectivity of action of 5-HT. However, the lack of receptor-specific agonists and antagonists may have hindered these studies. Investigation of the precise action of NA at the level of the spinal cord have so far been inconclusive (section G). However, the development of a number of receptor-specific adrenergic antagonists and agonists has enabled the detailed examination of the action of noradrenaline (NA) on identified dorsal horn neurones in the present study.

Since the commencement of the present study, dopaminergic systems have been shown to also have a distinct selective analgesic action (Jensen and Smith, 1983a; 1983b; Jensen and Yaksh, 1984a) at the spinal level, which promoted the investigation of the receptor specific dopaminergic actions on identified dorsal horn neurones here.

The following sections discuss firstly the evidence for NA and dopamine (DA) as spinal neurotransmitters and then go on to consider their possible role in the modulation of spinal somatosensory transmission and the putative origins for NA and DA innervation of the spinal cord.

F. Noradrenaline and Dopamine: separate neurotransmitters at the spinal level.

For a number of years there was no reliable evidence as to whether DA was a neurotransmitter, independent of NA at the spinal level. This was mainly due to the inability of assays available at that time, to quantitatively separate DA from the higher concentration of NA present in the mammalian spinal cord. Such problems have led DA to be regarded by some (now clearly mistakenly) merely as a precursor of NA at the spinal level.

Magnusson (1973) investigated the effect of chronic mid-thoracic spinal transection on DA and NA levels below the lesion using a biochemical assay, in the rat. If DA

were only a precursor of NA, it would be expected that the levels of the 2 catecholamines (CAs) would decline in parallel, below the transection. On the contrary, it was found that DA levels increased over the first three days and then disappeared within nine days of the operation, whereas NA levels decreased steadily and disappeared completely after fifteen days (also reported by Haggendal and Dahlstrom, 1973). The report of Magnusson (1973) was the first firm evidence that DA existed independently from NA, at the spinal level. Commissiong and Sedgwick (1974) used fluorimetric techniques to measure the concentration of DA in the rat spinal cord and found the levels of DA present to be higher than those considered to be expected for precursor. The ratio of NA to DA was calculated for various parts of the central nervous system (Commissiong et al. 1979). In the cerebellum, where DA is still regarded as only a precursor of NA, the ratio was calculated to be 47:1 (NA:DA), i.e. very low levels of DA compared to NA. In the spinal cord, the relative levels of DA to NA were much higher and not in accord with DA being present only a precursor, the ratio being in the range 19:1 to 11:1.

The levels of NA in the spinal cord may be manipulated, from supraspinal sites, separately from those of DA. The nucleus locus coeruleus, has been shown to provide an extensive noradrenergic innervation to the spinal cord of the rat (Nygren and Olsen, 1977; Westlund, 1983). Commissiong, Galli and Neff (1978) found that

bilateral lesions of the locus coeruleus reduced NA levels in the spinal cord of the rat by 50% and that this reduction was not paralleled by DA. This confirmed that a predominently noradrenergic innervation to the spinal cord had been eliminated by lesions of the locus coeruleus and implied that DA in the spinal cord may originate from a different supraspinal site.

DA and NA have been shown to have different distributions within the spinal grey matter. NA is found in both the dorsal and ventral horns of the spinal cord (CA-histofluorescence: Nygren and Olsen, 1977; radioenzymic assay: Fleetwood-Walker and Coote, 1981; immunocytochemical study: Westlund et al. 1983). DA, however, has a more restricted distribution within the spinal grey matter than NA (Figure 3). Commissiong et al. (1979) and Fleetwood-Walker and Coote (1981) found significantly higher levels of DA in the dorsal horn, than in the ventral horn, measured using gas-liquid chromatography and a radioenzymic assay, respectively. Further strong evidence, supporting the restricted distribution of DA in the spinal cord, was reported by Skagerberg et al. (1982). In this study the dopaminergic innervation of the spinal cord was dissociated from other monoamines by the use of selective neurotoxins; 6-hydroxydopamine was administered subcutaneously to neonatal rats to destroy noradrenergic innervation. Serotonergic innervation was destroyed by the intra-ventricular administration of 5, 7-dihydroxytryptamine

two to three months later. 6-Hydroxydopamine, when administered intraventricularly or intracerebrally, is known to disrupt DA as well as NA neurones. This effect can be minimised by administration of benztropine, to prevent uptake of 6-hydroxydopamine by dopaminergic terminals. Neonatal subcutaneous administration of 6-hydroxydopamine, however, as undertaken by Skagerberg et al. (1982) has been reported not to affect the dopaminergic innervation of a number of regions of the brain of the rat (septum, neostriatum, hypothalamus, mesencephalon and cerebral cortex: Jonsson and Sachs, 1976; Schmidt and Bhathager, 1979). Thus 6-hydroxydopamine was not used in conjunction with benztropine, in the study of Skagerberg et al. (1982), although it cannot be absolutely certain that the dopaminergic innervation of the spinal cord was completely unaffected by this procedure. Skagerberg et al. (1982) confirmed the findings of Commissiong et al. (1979) and Fleetwood-Walker and Coote (1981), that there were significantly higher levels of DA in the dorsal horns, than in the ventral horns of both control and neurotoxin-treated rats, using a radio-enzymic assay. Spinal cord tissue, from neurotoxin treated rats, was also processed for CA-histofluorescence. This method is not specific for DA. However, the 5-HT and NA content of the spinal cord after neurotoxin treatment was negligible. Furthermore, it is unlikely that the histo-fluorescence method used (aluminium/formaldehyde-induced

histofluorescence, ALFA) would result in the fluorescence of the extremely small population of adrenergic terminals that are present predominantly in other regions of the spinal cord (Reid, Zivin and Foppen, 1975; Zivin, Reid, Saavedra and Kopin, 1975; Fleetwood-Walker and Coote, 1981). Therefore the histofluorescent fibres visualized in this study were presumed to be dopaminergic. Skagerberg et al. (1982) emphasised the restricted distribution of "presumed" DA fibres in the spinal grey matter. "Presumed" DA fibres were distributed throughout the dorsal horn, with some concentration of fibres in the intermediolateral cell column, at thoracic levels of the spinal cord. The ventral horn was reported to be virtually free of fluorescent fibres in neurotoxin treated rats. A review of the different methods used to assay CA levels in the spinal cord indicates that the levels and distribution of DA detected may depend on the sensitivity of the assay being used. Zivin et al. (1975) stated that the levels of DA measured, in the spinal cord of the rabbit, were on the limits of the sensitivity of the method employed and a difference between DA levels in the dorsal and ventral horns was not distinguished. This implies that this assay was not sensitive enough to validly attempt any conclusions on the distribution of DA within the spinal grey matter. As techniques have been refined to separate DA from NA, it has become increasingly clear that these two CAs are separate neurotransmitters at the spinal level. Their possible physiological roles in the dorsal

horn, however, have been relatively little investigated.

The involvement of monoamines (especially the NA and DA) in the spinal integration and transmission of nociceptive information and the supraspinal origins of descending monoaminergic pathways are the main theme of this thesis. A number of investigations have already been made as to the nature of serotonergic actions on dorsal horn neurones (Engberg and Ryall, 1966; Randic and Yu, 1976; Headley et al. 1978; Belcher et al. 1978). The degree of selectively of such actions has sometimes been in doubt (Headley et al. 1978; Belcher et al. 1978), although it may now be possible to clarify this situation with the help of recent advances in serotonergic pharmacology (Bradley and Costa, 1984). Adrenaline is found in only very low levels in the spinal cords, is not concentrated in the dorsal horn and has not been proposed as a modulator of somatosensory transmission (Reid et al. 1975; Zivin et al. 1975; Fleetwood-Walker and Coote, 1981). NA and DA have both been firmly demonstrated to exert selective antinociceptive actions at the spinal level. Studies investigating the action of NA and DA at the level of the dorsal horn and the putative supraspinal origins of such innervation are discussed in the following sections.

G(i) Noradrenaline: Antinociceptive actions at the level of the dorsal horn:

Evidence suggesting a modulation of nociceptive

transmission through the dorsal horn of the spinal cord by NA has appeared from a variety of both behavioural and electrophysiological studies.

Intrathecally-administered NA has been demonstrated to have an analgesic action, as assessed in behavioural tests, in the rat and cat (Kuraishi et al. 1979 ; Reddy and Yaksh, 1980). Doses of 3 to 30 nmoles intrathecally-administered in the rat and 600 nmoles in the cat resulted in profound antinociceptive or analgesic effects, tested using the tail-flick or hot-plate tests. Analgesic actions of intrathecally-administered NA and adrenergic agonists were also demonstrated in the monkey (Yaksh and Reddy, 1981). The cutaneous area of analgesia that resulted form intrathecally-administered NA, at lumbar levels of the spinal cord, was found to be restricted to the caudal regions of the animal. In the rat, this effect was shown to be dose-dependent and to be mimicked by α-adrenoreceptor agonists, phenylephrine (Reddy and Yaksh, 1980) and clonidine (Yaksh and Reddy, 1980; Howe, Wang and Yaksh, 1983). Pretreatment with intrathecally-administered α-antagonists significantly reduced the analgesic action of NA: phenoxybenzamine, used in the rat (Kuraishi et al. 1979) and phenotolamine, in both the rat and cat (Reddy and Yaksh, 1980), whilst pretreatments of the rat with a monoamine oxidase inhibitor potentiated the analgesic action of NA: (Reddy and Yaksh, 1980). In contrast, pretreatment with an intrathecally-administered β-antagonist did not alter the

nociceptive thresholds tested (Reddy and Yaksh, 1980). In these behavioural studies, the analgesic effects of intrathecally-administered NA were reported to be selective and mediated spinally. The animals did not show any signs of motor weakness or sedation and continued normal exploratory behaviour during periods of profound analgesia, and intracerebral injection of NA failed to reproduce the spinal analgesic effects.

These behavioural studies demonstrated that intrathecally-administered NA and α-agonists selectively inhibit responses to high-threshold cutaneous stimuli. It is possible that intrathecally-administered NA may be exerting its effect either on dorsal or ventral horn neurones, or both. White and Neumann (1980) reported that ionophoretically applied NA facilitated spinal motor neurone excitability, therefore it is unlikely that intrathecally-administered NA acts to inhibit motor activity. It is probable that the diffusion of intrathecally-administered NA from the cannula inserted on the dorsal surface of the spinal cord will be unable to raise concentrations in the ventral horn to levels as high as those in the dorsal horn. Intravenous administration of an agent however might be expected to deliver the agent equally to both the dorsal and ventral horns. Intravenous administration of clonidine (an α_2 -agonist) has been reported to cause antinociception in spinal mice (Spaulding, Venafro and Mani, 1978). Therefore, there seems little reason to suggest that the

primary site mediating the antinociceptive action of intrathecally-administered NA is other than in the dorsal horn. Although some attempts (Belcher et al. 1978; Headley et al. 1978) have been made to investigate the action of NA on single dorsal horn neurones, no clear conclusions can be drawn from the results.

Belcher et al. (1978) reported that NA ionophoretically-applied in the vicinity of the dorsal horn neurones being tested, caused a general inhibition of nociceptive responses, activity evoked by dl-homeocysteic acid (DLH, a direct excitant) and spontaneous activity of the majority of multireceptive cells tested (90%). The responses of multireceptive neurones to innocuous cutaneous stimuli were not tested. The activity of neurones, which responded only to low threshold stimuli, was unaffected by NA. These authors concluded that the antinociceptive action of NA was exerted directly on the multireceptive neurones located in the deeper laminae of the dorsal horn of the cat (laminae IV and V). Headley et al. (1978) compared the effects of ionophoresing NA either in the vicinity of the dorsal horn neurone being tested or more dorsally, in the region of the SG. In the study of Headley et al. (1978), responses to either noxious or innocuous cutaneous stimuli of multireceptive neurones were tested. Ionophoretic ejection of NA into the superficial laminae of the dorsal horn resulted in a selective reduction of the nociceptive responses of 50% of the neurones tested in laminae IV-V

(the responses to innocuous cutaneous stimuli were unaffected). Ionophoretic application of NA in the vicinty of the dorsal horn neurones being tested resulted in a less selective effect, the responses to innocuous stimuli were often reduced, but to a lesser degree than the inhibition of the nociceptive responses. Therefore, Headley et al. (1978) concluded that the primary site of action for the expression of the selective antinociceptive effect of NA was the SG of the cat and not, as proposed by Belcher et al. (1978), at the level of multireceptive neurones located in laminae IV and V of the dorsal horn. Other studies have failed to report a selective antinociceptive action of ionophoretically-applied NA, in the rabbit (Satoh, Kawajiri, Ukai and Yamamoto, 1979) and rat (Cahusac and Hill, 1983).

It is possible that NA may have action at more than one site in the dorsal horn. Early autoradiographic binding studies (Young and Kuhar, 1979) gave only scant consideration of spinal adrenoreceptors, with the mention of some α_2- but little α_1- binding in cervical regions. Recently, more detailed studies on lumbar and thoracic dorsal horn have demonstrated α_2-receptor sites present throughout the dorsal horn (Unnerstall, Kopajtic and Kuhar (1984); Dashwood, Fleetwood-Walker, Gilbey, Mitchell and Spyer, 1985). Binding (1984) of α_1-receptor ligands was also demonstrated, located sparsely in the deeper laminae of the dorsal horn (Dashwood et al. 1985). This is in accordance with the earlier biochemical

demonstration of α_1 binding sites in dorsal horn by Coote, Fleetwood-Walker and Mitchell, (1980).

Although the behavioural effect of intrathecally-administered NA have been reported to be markedly selective on nociceptive responses, the mechanism by which NA may evoke this effect, at the neuronal level, was not clarified by the ionophoretic studies cited above. This thesis describes in detail the effects and possible mechanisms of action of ionophoretically-applied NA and DA on defined populations of dorsal horn neurones (in the rat and cat).

G(ii) Supraspinal origin(s) of the noradrenergic innervation of the spinal cord.

The overwhelming majority of NA present in the spinal cord has long been known to originate from localized groups in the brainstem (Dahlstrom and Fuxe, 1964). Stevens, Hodge and Apkarian (1983), however, demonstrated apparent CA-histofluorescent fibres in lumbar dorsal root ganglia and the ventral root of the cat. Proximal and distal lesions of the dorsal root ganglia and total transection of the spinal cord did not alter the level of CA-histofluorescence observed and implied that this CA innervation of dorsal root ganglia does not project either to or from peripheral or central sites, but may well originate from transdural innervation of blood vessels supplying the dorsal root ganglia. The CA containing cell groups of the brain do not correspond

strictly to morphologically-defined nuclei, (Figure 4). The terminology and descriptions of Dahlstrom and Fuxe (1964) are the conventional basis for their nomenclature. A1 to A7 cell groups are considered as primarily noradrenergic and A8 to A14 cell groups are considered primarily as dopaminergic. Some further CA groups not originally described by Dahlstrom and Fuxe (1964) have been demonstrated in the cat; for example, in the region of the parabrachial and Kolliker-Fuse nuclei (Stevens, Hodge and Apkarian, 1982). A number of noradrenergic cell groups have been proposed to project to the spinal cord. The evidence that suggests projections from particular noradrenergic cell groups to the spinal cord is discussed in the following subsections.

Locus coeruleus and sub-coeruleus groups:

Bilateral lesions of the A6 cell group (the locus coeruleus nucleus, Dahlstrom and Fuxe, 1964), have been reported to result in significant reductions in NA levels in the spinal cord, using a variety of assay methods, in the rat (Nygren and Olsen, 1977; Commissiong et al. 1979) and the cat (Fleetwood-Walker and Coote, 1981). Westlund et al. (1980; 1983) employed two methods to investigate the origins of the noradrenergic innervation of the spinal cord of the rat and monkey. (a) Immuno-cytochemical localization of an antibody to dopamine-β-hydroxylase (DβH), which had been retrogradely transported from the spinal cord to supraspinal CA cell groups. (b) Dopamine-β-hydroxylase antibody labelling of

catecholaminergic cells, retrogradely stained with HRP that had been injected into the spinal cord. The majority of catecholaminergic cells (>80%) demonstrated to project to the spinal cord were located in the A6 and A7 (locus coeruleus and sub-coeruleus) cell groups. Bilateral lesions of the regions of the A6 cell group have been reported to reduce NA levels, primarily in the ventral horn of the spinal cord of the rat (Nygren and Olsen, 1977) and cat (Fleetwood-Walker and Coote, 1981).

It has recently been suggested that the A6 cell group is not a primary source of noradrenergic innervation of the spinal cord of the cat, unlike the rat and monkey (Westlund et al. 1980 ; 1983). Stevens et al. (1982) reported only a relatively few A6 CA-fluorescent cells retrogradely labelled with Evans Blue, injected into the spinal cord of the cat. The most dense group of double labelled cells were visualized lateral to the locus coeruleus, in the region of the Kolliker-Fuse nucleus. Without detailed information on the extent of lesions of the locus coeruleus region, undertaken in previous studies, it is not possible to assess whether such lesions encroached into the Kolliker-Fuse nucleus and contributed to the results reported (Nygren and Olsen, 1977; Commissiong et al. 1979; Fleetwood-Walker and Coote, 1981). Lesions of this region, however, primarily reduce NA levels in the ventral, not the dorsal, horn of the spinal cord.

A5:

Orthograde transport of [^{3}H]-amino acids, injected into the region of the A5 cell group revealed a projection to the intermediolateral cell column of the thoracic spinal cord of the rat. This projection was proposed to be noradrenergic as CA-histofluorescent cells of the A5 cell group were retrogradely labelled with HRP, injected into the spinal cord, of the rat (Loewy, McKeller and Saper 1979). Westlund et al. (1980; 1983), using the methods described in the previous section, found evidence of only a minor spinal projection from the A5 cell group (containing only 8% of proposed noradrenergic neurones projecting to the spinal cord). Fleetwood-Walker and Coote (1981) found no evidence for a projection to the spinal cord of the cat from the A5 cell group, in a lesion study.

The findings of Westlund et al. (1980; 1983) and Fleetwood-Walker and Coote (1981) do not support a major spinal projection from the A5 cell group, as proposed by Loewy et al. (1979). None of these studies indicated a projection to either the dorsal or ventral horns from the A5 cell group.

A1 and A2:

These two cell groups were the first proposed to project to the spinal cord. Dahlstrom and Fuxe (1964) found that CA-histofluorescent cells in the region of these two groups underwent retrograde changes in response to total spinal transection in the rat. Nygren and Olsen

(1977) implicated a spinal projection from these 2 cell groups by comparing the total number of cells in the groups A1, A2, A3, A6 and A7 to axonal counts in the rat spinal cord, visualized by CA-histofluorescence. Including cells in groups A1 and A2, there was good agreement between cell number and the axon counts (assuming each cell gave rise to a single descending axon). More direct evidence has come fom lesion and electrophysiological studies. Fleetwood-Walker and Coote (1981) found that bilateral lesions of the region of the A1 cell group (lateral, ventral and medial to the nucleus reticularis lateralis) and the A2 cell group (mainly within the area of the nucleus commisuralis) resulted in a 70% and 20% reduction of NA levels respectively, in the intermediolateral cell column of the spinal cord of the cat. These lesions were also reported to reduce NA levels in the dorsal horn of the cat, to some extent, measured using a radioenzymic assay. Bragin and Durinyan (1983) reported that lesions of the region of the A1 cell group reduced NA levels in the spinal cord of the rat by 40%, assessed using a microspectrofluorimetry technique (a distinction between the dorsal and ventral horns of the spinal cord was not made in this study).

A number of studies in which HRP has been used to retrogradely label cells, from the spinal cord, in combination with a catecholamine marker, have failed to demonstrate any major spinal projection from either the A1 or A2 cell groups (Satoh, Tohyama, Yamamoto, Sakumoto and Shimizu, 1977; Loewy, et al. 1979;

Westlund et al. 1983). Nevertheless, HRP does not uniformly label all cells when injected into the central nervous system. Nauta (1974) found that HRP injections in the region of the caudoputamen conspicuously failed to label the neocortex, an area previously demonstrated to provide a major input to the caudoputamen using alternative histological techniques. It remains a possibility that the noradrenergic cells of the A1 (and A2) cell group are another exception to effective HRP labelling. Fluorescent dyes have been shown to label brain stem DA cells from the spinal cord (Skagerberg, Bjorklund, Lindvall and Schmidt, 1982), and may be useful in clarifying the supraspinal origins of NA innervation at the spinal level.

Electrophysiological studies have found that cells in the area of the A1 cell group, recorded extracellularly could be antidromically activated (with very slow conduction velocities) from the spinal cord of the rat (Fleetwood-Walker et al. 1983a). Pontamine sky blue dye was ejected from the recording electrode at the end of the test and subsequent histofluorescent processing of the tissue revealed that the tip of the electrode was regularly positioned close to a catecholaminergic cell body, in the lateral reticular nucleus of the rat.

Immediately rostral and to some extent overlapping with the noradrenergic cells of the A1 and A2 cell groups are adrenergic cells belonging to two groups named C1 and

C2 respectively (Hokfelt, Fuxe, Goldstein and Johnansson, 1974). There is no indication of any prominent adrenergic projection to the spinal cord, in studies in which NA and CA levels in the spinal cord have been compared (for example, Fleetwood-Walker and Coote, 1981). Therefore it is unlikely that the presence of these adrenergic cells has greatly interfered in the lesion and tracing studies cited.

A review of the studies cited above clearly shows that the origins of the noradrenergic innervation of specific laminae of the spinal cord have not been established. At the moment, the most likely candidates for providing a projection to the spinal dorsal horn included the A1 cell group and the region of the Kolliker-Fuse nucleus, although the terminal fields of the latter, in the cat, have not been fully investigated.

H(i) Dopamine: Antinociceptive actions at the level of the spinal cord.

Since the present study was started, a series of behavioural studies (Jensen and Smith, 1982a, 1983a, 1983b; Jensen and Yaksh, 1984a; Jensen, Schroder and Smith, 1984) have confirmed that DA can have a potent analgesic action, at the level of the spinal cord. Intrathecally-administered DA (30 to 300 nmoles) attenuated the tail-flick response, in a dose-dependent manner, in spinal rats. This effect appeared to be mediated by specific DA receptors, since it was blocked

stereospecifically by DA antagonists (+)-butaclamol and (cis)-flupenthixol, but not by their enantiomers (Jensen and Smith, 1982a, 1983a). Responses evoked using the hot-plate or acetic acid-writhing tests, both of which involved supraspinally mediated pathways, were inhibited by intrathecally-administered DA or apomorphine (a DA agonist), in rats with intact neuraxes. The acetic acid writhing test is considered to be a useful model for an assessment of the degree of visceral nociception.

The tail-flick response was not inhibited by intrathecally-administered apomorphine or DA in rats with intact neuraxes, in contrast to the potent antinociceptive effects reported in the spinal rat (Jensen and Smith, 1983a). Further investigations demonstrated that bilateral lesions of the DLF, in otherwise intact animals, unmasked a selective antinociceptive action by DA on the tail-flick reflex (Jensen et al. 1984a). Blockade of noradrenergic or serotonergic transmission in the spinal cord (by the use of either methysergide (a serotonin (5-HT) receptor antagonist) or FLA63 (a NA synthesis inhibitor) also revealed an inhibition of the tail-flick reflex by intrathecally-administered DA (Jensen and Smith, 1983b). These findings indicate that the action of DA on the spinal tail-flick reflex may be influenced by descending monoaminergic pathways. Ionophoretically-applied NA and 5-HT have been reported to have a facilitatory action on ventral horn neurones (Ahlman, 1977; Neumann and White,

1980). It may be envisaged that facilitatory actions of 5-HT or NA in the ventral horn may mask the antinociceptive action of DA on the tail-flick reflex (Jensen and Smith, 1983b).

It is unlikely that the analgesic effects of DA are mediated in the ventral horn, since ionophoretically-applied DA is reported to excite ventral horn neuronal activity (Barasi and Roberts, 1977). From such an action a facilitation, rather than an inhibition of the tail-flick reflex may be predicted. Thus the primary site of action of intrathecally-applied DA is most probably the dorsal horn.

DA receptor binding sites have been demonstrated in the dorsal horn of the rat, by stereospecific binding of [^{3}H]-haloperidol (Demenge, Feuerstein, Mouchet and Geurin, 1981) and [^{3}H]-N-propylnorapomorphine, (Scatton, Dubois and Cudennec, 1984). Intrathecally-administered DA may act at these receptor sites.

Ionophoretically-applied DA has been reported to inhibit DLH-evoked activity of dorsal horn neurones, although the specificity of DA was doubted (Biscoe, Curtis and Ryall, 1966) and to generally depress the responses of low-threshold, wide dynamic range and high-threshold neurones in the caudal trigeminal nucleus of the rat (Cahusac and Hill, 1983). Details as to the effects of ionophoreticallyapplied DA on specific cutaneous sensory modalities were not given in these studies, therefore any possible degree of selectivity on

nociceptive versus non-nociceptive responses cannot be assessed.

H(ii) Supraspinal origin(s) of the dopaminergic innervation fo the spinal cord.

DA is contained in localized cell groups in the brain, designated A8 to A14 (Dahlstrom and Fuxe, 1964; Ungerstedt, 1971; Bjorklund and Nobin, 1973). These cell groups have been mapped out, using CA-histofluorescence techniques, in the rat (Ungerstedt, 1971; Bjorklund and Nobin, 1973; Jacobwitz and Palkowitz, 1974) and the rabbit (Blessing et al. 1979). Subsequent immunocytochemical studies have confirmed that the cells of groups A8 to A14 are predominantly dopaminergic, by combining dopamine-B-hydroxylase (DβH) and tyrosine hydroxylase (TH) antibody labelling. The latter antibody will mark all CA-containing cells, by labelling the enzyme that converts DOPA into DA, whereas DβH converts DA to NA and thus is not present in dopaminergic cells (Hokfelt, Phillipson and Goldstein, 1979; Kolher and Goldstein, 1984).

Two dopaminergic cell groups have been proposed to project to the spinal cord of the rat, namely A9 and A11.

A9:

The A9 cell group is found in the region of the pars compacta of the substantia nigra (SN) (Ungerstedt, 1971). Lesions of the SN, by microinjections of 6-OHDA, have been reported to reduce DA levels in the ipsilateral spinal cord of the rat by 50%, measured using gas-

chromatography/mass spectrometry. On the other hand, electrical stimulation of the ipsilateral SN was reported to increase DA levels in the spinal cord (Commissiong et al. 1979). These results were presented as evidence for an uncrossed nigrostriatal pathway, projecting from the A9 cell group to the spinal cord.

Anatomical studies do not, however, support this proposal. Cells in the region of the A9 group, which have been retrogradely labelled with fluorescent dyes injected into the spinal cord have not been subsequently identified as catecholaminergic , using CA-histo-fluorescence (Skagerberg et al. 1982) or immunocytochemistry (Hokfelt et al. 1979).

Electrical stimulation of the region of the SN has been reported to cause antinociceptive effects on the tail-flick response of conscious rats (Segal and Sandberg, 1977) and the nociceptive responses of dorsal horn neurones, in decerebrate cats (Barnes, Fung and Adams, 1979). However, Barnes et al. (1979) reported that the inhibition of the nociceptive responses of dorsal horn neurones could be abolished by either DA or 5-HT antagonists, administered intravenously and therefore concluded that stimulus-evoked effects from the SN were not mediated directly by DA, at the spinal level. Barasi and Duggal (1985a) used appropriately lower and shorter stimulation parameters (up to 100 uA for up to 30 seconds) than Barnes et al. 1979)(up to 1mA for up to 5 minutes) and failed to evoke any antinociceptive effect

from the SN, tested by measuring the tail-flick latency of lightly anaesthetised rats. Barasi and Duggal (1985a) suggested the antinociceptive effects cited by Segal and Sandberg (1977) and Barnes et al. 1979) may have been due to current spread outside the region of the SN.

It appears that anatomical evidence supports a projection from the region of the SN to the spinal cord, but it is very unlikely that this projection is dopaminergic and may be polysynaptic.

A11:

The cells of the A11 group form an almost vertical band in the periventricular grey matter of the caudal thalamus and extend to some extent ventrally into the posterior and the dorsal hypothalamus (Bjorklund and Nobin, 1973). The cells of the A11 group are distinguished from those of the nearby A13 group on the basis of cellular morphology. A11 cells were described as larger and more angular, than A13 cells, with axons which were observed to bifurcate in a T-shaped manner, giving rise to one ascending and one descending branch in the rat (Bjorklund and Nobin, 1973). A number of studies have shown a projection to the spinal cord from the region of the A11 cell group by retrograde HRP labelling (Kuypers and Maisky, 1975; Hancock, 1976; Saper, Loewy, Swanson and Cowan, 1976) or orthograde [^{3}H]-amino acid labelling (Saper et al. 1976) or retrograde fluorescent dye labelling (Swanson and Kuypers, 1980).

Catecholaminergic cells were retrogradely labelled

from the spinal cord, using either flurorescent dyes in the rat (Bjorklund and Skagerberg, 1979; Skagerberg et al. 1982; ; Hokfelt et al. 1979; Skagerberg and Lindvall, 1985) or HRP in the rabbit (Blessing and Chalmers, 1979). In these studies, the only double-labelled cells in this part of the CNS were observed in the A11 cell group of the rat and the A13 cell group of the cat. There seems to be a species difference between the A11 and A13 groups of the rabbit and rat. In the rabbit a spinal projection seems to originate from the A13 group, whilst in the rat the cells of the A11 cell group provide an exclusive spinal projection.

Neurones belonging to the A11 cell group, retrogradely labelled from the spinal cord of the rat, were demonstrated as catecholaminergic, using a histofluoresence method (aluminium/formaldehdye-induced histofluorescence: Bjorklund and Skagerberg, 1979; Skagerberg et al. 1982; Skagerberg and Lindvall, 1985). It was reported by Bjorklund and Nobin (1973) that some cells located in the dorsal region of the A11 cell group were noradrenergic, using a technique of spectral analysis reported to be able to distinguish between NA and DA fluorophores, at the cellular level. Immunocytochemical studies failed to confirm the presence of any noradrenergic cells in this region. Cells of the A11 group were demonstrated to be labelled with tyrosine hydroxylase antibody but not with dopamine-β-hydroxylase antibody (Swanson and Hartman, 1975; Hokfelt et al.

1979). Hokfelt et al. (1979) used this technique to demonstrate that cells in the region of the A11 cell group, retrogradely labelled with fluorescent dyes from the spinal cord, were dopaminergic.

Mouchet, Guerin and Feuerstein (1982) lesioned the midbrain of the rat with an extensive knife cut which almost certainly separated the A11 cell group from the spinal cord and this resulted in a fall in spinal DA levels, measured using a radioenzymic assay. The caudal extent of the lesion may have included part of the A9 cell group, but as previously described, a direct dopaminergic projection from this region to the spinal cord is unlikely.

H(iii) Other possible inputs:

There is no evidence to support a spinal projection from any of the other dopaminergic cell groups in the rat diencephalon (A10, A12, A13 or A14: Bjorklund and Skagerberg, 1979; Hokfelt et al. 1979; Skagerberg et al. 1982).

Price and Mudge (1983) reported a small population (1%) of dorsal root ganglia cells as being labelled with tyrosine hydroxylase antibody, but not dopamine-β-hydroxylase antibody and therefore presumed to be dopaminergic, in the rat. Apart form this source of DA, possibly projecting to the spinal cord, the overwhelming majority of DA terminals innervating the spinal cord orginate from supraspinal sites.

Broad objectives of the present study.

In view of the functionally selective antinociceptive roles for DA and NA supported by observations in recent behavioural studies and evidence that NA and DA are involved in the modulation of nociceptive transmission through the dorsal horn of the spinal cord (introduction sections G(i) and H(i)), a detailed evaluation of the effects of ionophoretically-applied DA and NA on individual dorsal horn neurones would be important. A comparison of NA and DA actions on the nociceptive and non-nociceptive responses of dorsal horn neurones was made directly here to elucidate any selectivity of the effects of DA and NA on neuronal inputs which are hypothetically capable of being affected by the same modulatory influences. Neurones were also identified in terms of their ascending projections, thus any modulatory effects observed, may fulfil an active functional role in influencing the transmission of somatosensory information to supraspinal sites.

Focal electrical stimulation techniques in the regions of the A9 and A11 DA cell groups, in combination with ionophoresis of a DA antagonist at the spinal level, were employed to investigate the possible supraspinal origins of the dopaminergic innervation of the dorsal horn. Stimulus-evoked effects, from these supraspinal sites, were also assessed on the responses of multireceptive dorsal horn neurones, to directly compare

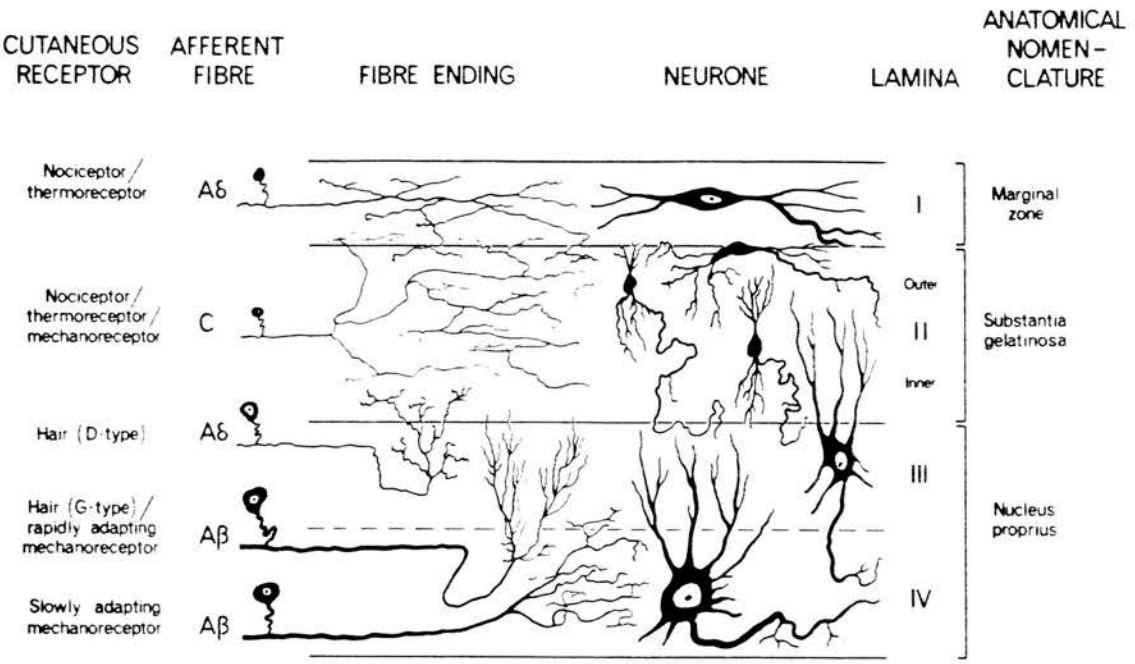
any evoked descending influence on non-nociceptive and nociceptive inputs and establish whether such a system has potential role as a physiologically important antinociceptive system.

Figure 1

Schematic diagram of the cutaneous afferent input to the dorsal horn and neuronal organisation

The relevant receptor groups and afferent innervation are shown. Standard neuronal types in the dorsal horn are represented, from top to bottom: a marginal cell, an SG limiting cell, two SG central cells and two neurones of the nucleus proprius. To the left of the diagram the types of afferent fibre and relevant receptor groups are listed and the distribution of fibres endings in the dorsal horn is shown, schematized from published morphological studies. Indicated on the right and on the diagram below are the laminar divisions of the lumbar dorsal horn (Rexed, 1952; 1954).

(From Cervero and Iggo, 1980)


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

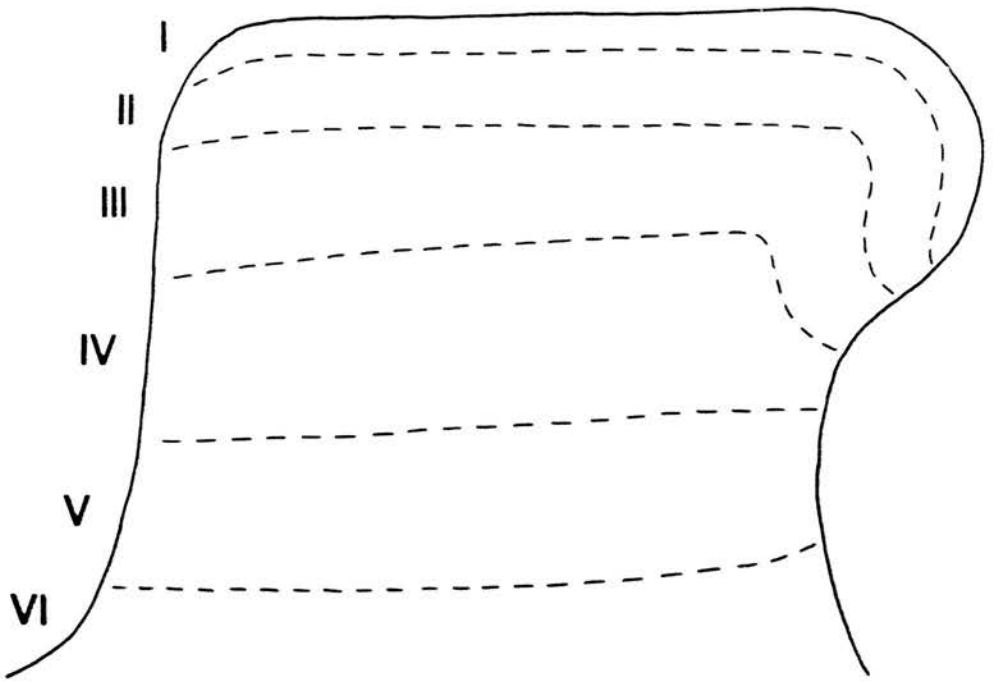

I
II
III
IV
V
VI

Figure 2

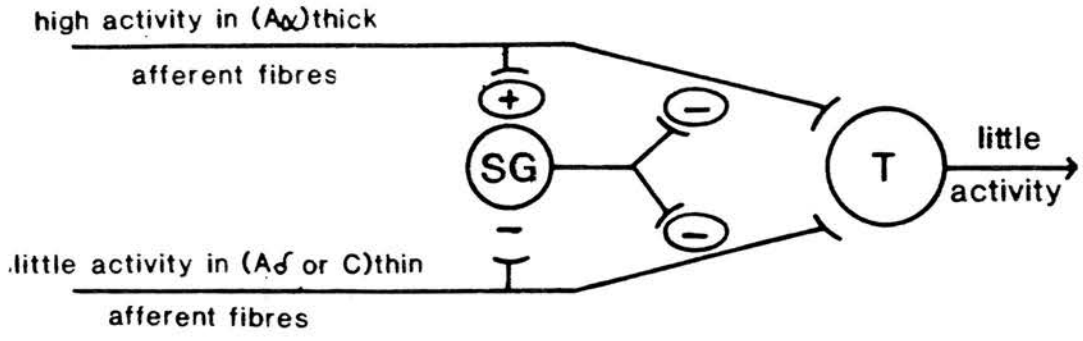
Schematic diagram of the reflex afferent pathway proposed by the 'Gate Control Theory' of Melzack and Wall (1965).

A) Low threshold cutaneous afferent activity was proposed to activate ⊕ SG cells, which in turn inhibited, by presynaptic depolarization ⊖ of the nociceptive afferent fibres, the activity of cells that transmit nociceptive information to higher levels ('T-cells'). Presynaptic depolarisation would result in reduction of neurotransmitter(s) released from the afferents, thereby reducing excitation of dorsal horn neurones (Eccles, 1961).

B) High threshold cutaneous afferent activity was proposed to inhibit ⊖ the activity of SG cells and thus result in reduced presynaptic inhibition+ (hyperpolarization) of afferent fibres. This would result in increased activation of cells that transmit nociceptive information to higher levels ('T-cells').

(Redrawn from Melzack and Wall, 1965)

(A) Low-threshold stimulation



(B) High-threshold stimulation

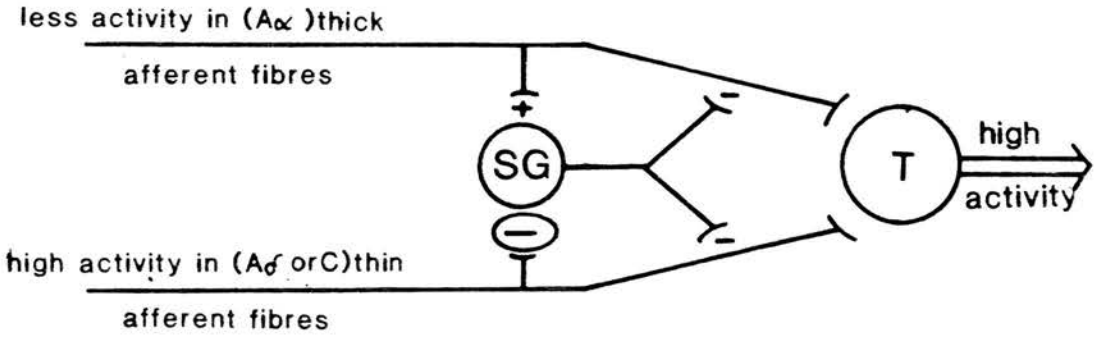
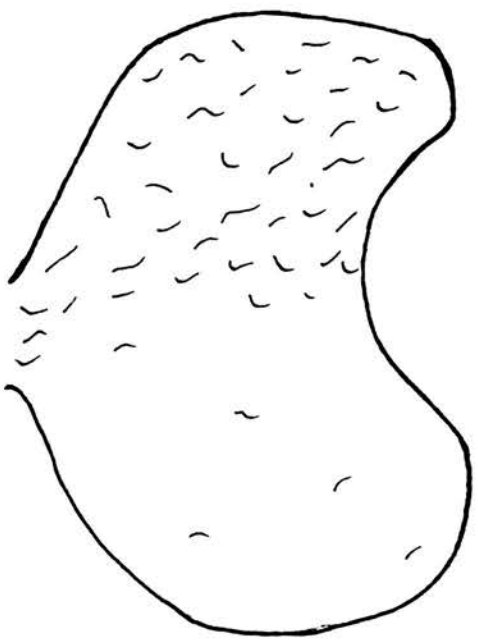


Figure 3

Schematic diagram of the distribution of DA and NA terminals in the lumbar spinal cord of the rat and cat

DA terminals are mainly restricted to the dorsal horn. No distinct laminar distribution has been described for the DA innervation in the cat (Fleetwood-Walker and Coote, 1981) and rat (Skagerberg et al. 1982). NA terminals are distributed throughout the spinal grey matter, concentrated to some extent in the superficial laminae of the dorsal horn in the cat (Fleetwood-Walker and Coote, 1981) and rat (Westlund et al. 1983).

DA



NA

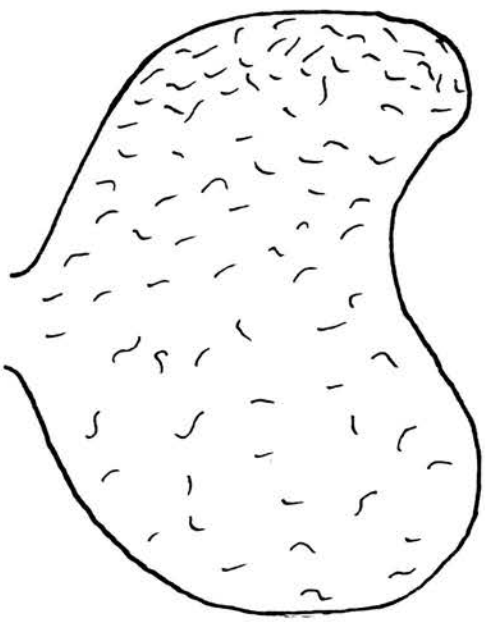
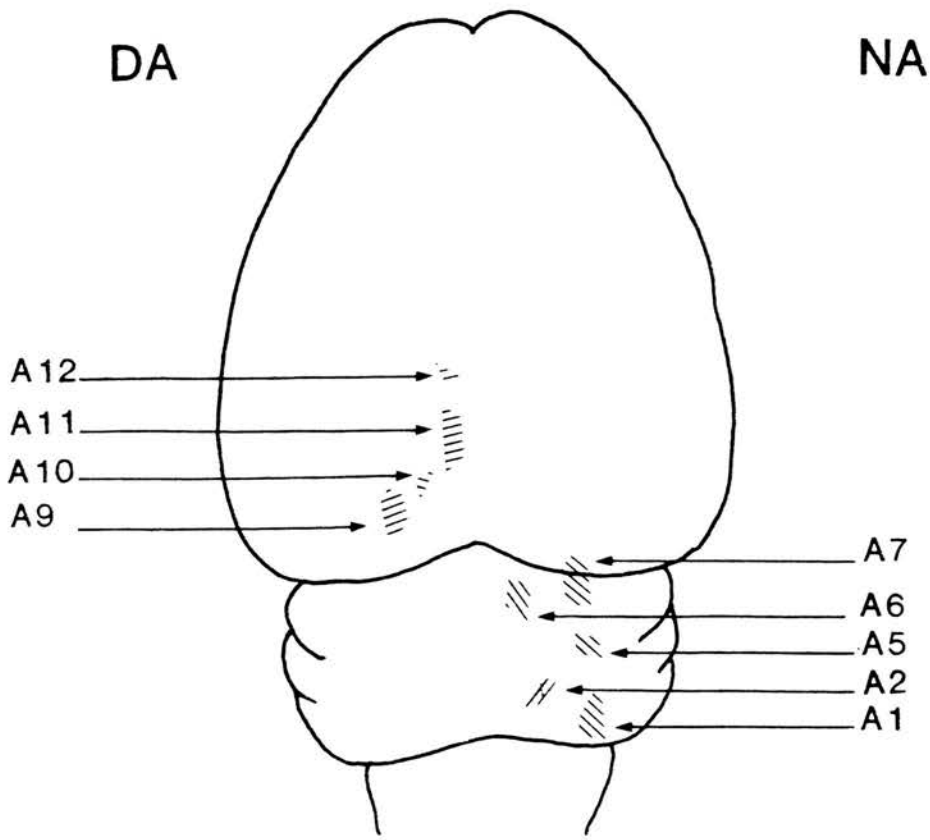


Figure 4

Schematic diagram showing the approximate locations of the major catecholamine cell groups, in the brain of the rat

NA cell groups are shown on the right and DA cell groups on the left.

(Redrawn from Ungerstedt, 1971)


DA
NA
A12
A11
A10
A9
A7
A6
A5
A2
A1

METHODS

A. Surgical procedures.

A(i) The rat preparation:

Adult rats (260-360g) were anaesthetised with α-chloralose (35mg/kg)/ urethane (700mg/kg), administered via a jugular cannula, after induction with halothane. Additional aliquots (0.1ml, 2.5 mg α-chloralose and 24 mg urethane) were given when required. Blood pressure was continuously monitored via a carotid cannula and core temperature maintained at 37 to 38°C. The animal was placed in a stereotaxic frame and held by a tail bar, three pairs of swan-necked clamps, to support the vertebral column and by a mouthpiece and ear bars. A dorsal laminectomy was performed to expose spinal segments, T13 to L3. Skin flaps were tied to the spinal support frame to form a pool around the exposure. To improve the stability of the preparation for extracellular recording from dorsal horn neurones, a 2% agar solution (at 36 to 37°C) was injected under the spinal bone, but above the intact dura, at the rostral end of the laminectomy (Figure 5) and agar was also poured over the exposed spinal cord. Once set, a section of the agar was removed from above the spinal segments L1 and L2, to allow access for the electrophysiological recording of dorsal horn neurones. The dura and pia were retracted and the pool filled with mineral oil to prevent dehydration. Rats were not paralysed. A Y-shaped piece was fitted to the tracheal cannula and humidified oxygen

passed across the open end of the cannula to aid spontaneous respiration (0.1 - 0.2 litres/minute). In experiments in which supraspinal sites were electrically stimulated, a craniotomy was also performed dorsal to the appropriate brain area (approximately 20 mm^2), to allow access.

A (ii) The cat preparation:

Adult cats were anaesthetised with intravenous α-chloralose (60-70 mg/kg) via a jugular cannula, after induction with halothane. Animals were paralysed with gallamine triethiodide ("Flaxedil", 15mg/kg) and artificially respired with room air. The effects of gallamine triethiodide were allowed to wear off periodically to monitor the state of anaesthesia, by looking for the presence of a corneal or nociceptive pinch withdrawal reflex. Additional doses of α-chloralose (20mg/kg) were given when required.

Two dorsal laminectomies were performed: one from C1 to C5, to allow antidromic identification of SCT or PSDC neurones, the other from L3 to S1, for electrophysiological recording of dorsal horn neurones. The animal was transferred to a spinal frame and head holder. The head was fixed using blunt ear bars, bars at the orbits and under the upper jaw. The vertebral column was held rigidly by spinal clamps, at sacral, upper lumbar and thoracic levels. The pelvis was fixed by pins that gripped the iliac crests and then the ipsilateral hindpaw

was attached to a wood block by Plaster of Paris. Skin flaps were tied to the frame to form pools at the cervical and lumbar levels and filled with mineral oil to prevent dehydration. A bilateral pneumothorax was performed to aid stability for recording. Carotid blood pressure was continuously monitored. Rectal and paraffin pool temperatures were maintained at 37 to 38°C and end-tidal CO_2 was kept between 3.5 - 4.0%.

B. Electrophysiological recording and ionophoresis of drugs:

An arc spanning the animal transversely supported the recording microelectrode. The microelectrode was advanced by means of a microdrive in 4 μm steps, at a 1-2° angle from the perpendicular to allow observation of the microelectrode tip as the dorsal surface of the spinal cord was penetrated.

Five and seven-barrel glass microelectrodes were used. The centre barrel (containing 4M NaCl, pH 4.0 to 4.5) was used for extracellular recording and drugs were ionophoresed from the side barrels. The electrodes were constructed from 1.5mm outer diameter, filamented glass tubing. The electrode tip size was 4.0-4.5 μm and the d.c resistance was 5-8 MΩ. The bandwidth of the recording amplifier was 100Hz-1KHz (WPI Instruments,Model M-707). One side barrel contained 1M NaCl (pH 4.0-4.5) for automatic current balancing and current controls using a Neurophore BH2 ionophoresis system (Medical Systems Corporation). To control against the possibility that the excitability of neurones may be altered as a result of potential changes due to effects of unbalanced movement of charged species, such changes were maintained close to zero (0.1 - 0.2 nA) by the technique of current balancing, employed by this system. The algebraic sum of the currents flowing

from the electrode tip was maintained at zero by the neutralization of any extraneous currents via the electrode barrel containing 1M NaCl (pH 4.0-4.5). To check that the effect of an ionophoresed drug on the responses of a neurone was not due to, for example osmotic or pH changes, the ionophoresis of 1M NaCl at similar current levels was used to simulate these conditions, without there being any additional pharmacological effect. The other electrode barrels contained various combinations of the following drugs:

Dopamine, agonists and antagonists; 0.1M Dopamine HCl (Sigma Chemical Co., Dorset), 0.4M RU24213 HCl (Roussel Labs., Swindon); 0.3M SKF38393 HCl (Research Biochemicals Inc., Wayland); 0.01M ($\pm$) sulpiride (Ravizza Labs., Italy), all at pH 4.0-4.5 in aqueous solution.

Noradrenaline, agonists and antagonists; 0.1M or 0.5M (d- or l- or dl-) noradrenaline (bitartrate or HCl salt) (Sigma Chemical Co., Dorset), 0.1M phenylephrine HCl (Sigma Chemical Co., Dorset), 0.1M isoprenaline HCl (Sigma Chemical Co., Dorset), 0.01M (0.1M or 0.5M) clonidine HCl (Sigma Chemical Co., Dorset) 0.1M metaraminol HCl (Sigma Chemical Co., Dorset), 0.1M yohimbine HCl (Sigma Chemical Co., Dorset), 0.05M WB4101 HCl (Ward-Blenkinsop Labs., U.K), 0.0015M prazosin HCl (Pfizer Central Research, Sandwich), 0.1m idazoxan (RX781094) HCl (Reckitt and Coleman, Hull), all at pH 4.0-4.5 in aqueous solution.

Other drugs: 0.1M naloxone HCl (aqueous solution, pH 4.0-4.5, ENDO Research Labs., New York), 0.03M 5-HT creatine sulphate (aqueous solution, pH 4.0-4.5, Sigma Chemical Co., Dorset) and 0.1M D,L-homocysteic acid (DLH, aqueous solution, pH 8.0-8.5, Sigma Chemical Co., Dorset).

One barrel was filled with pontamine sky blue dye (2% in 0.5M NaAc) for marking the positions of recording sites (50 A minutes). All drugs were ejected with cathodal current, except DLH (anodal). Retaining currents

of the opposite polarity (10-15 nA), were applied to minimise drug leakage between tests.

C (i) Identification of supraspinal projecting neurones:

Spinocervical tract neurones were identified and studied in the cat (Figure 6), and spinothalamic tract neurones in the rat (Figure 7).

Projecting dorsal horn neurones were identified by standard antidromic criteria (Lipski, 1981):

1) Collision of antidromic and orthodromic action potentials.
2) High following frequencies (up to 300 Hz).
3) Constant latency ($\pm$ 0.1 msec) and a distinct threshold of activation.

C(ii) Identification of spinocervical tract neurones:

Spinocervical tract (SCT) neurones were identified, in the cat, according to the criteria described by Brown, House, Rose and Snow (1976). Stimuli were applied through arrays of paired silver ball electrodes placed on the ipsilateral, dorsolateral funiculus at spinal segments C1 and C3 (above and below the site of termination of the SCT system) and on the dorsal columns at C4-5 (Figure 6). A restricted lesion of the dorsal columns, at the level of C4, was made to prevent apparent antidromic identification of SCT neurones due to fibres crossing from the dorsal columns.

All SCT cells tested were antidromically activated

from the electrode at the level of C3, but not at the level of C1, or from the dorsal columns. A small sample of neurones, identified as belonging to the PSDC system, were also tested. These neurones were antidromically activated only from the C4/5 dorsal column electrode (Figure 6).

C(iii) Identification of spinothalamic tract neurones:

Spinothalamic tract (STT) neurones were identified, in the rat, using the antidromic criteria described in section C(i). Two arrays of bipolar stimulating (Rhodes Medical Instruments, SNE-100) electrodes were used (Figure 7). Two electrodes, 1mm apart, were placed in the region of the medial lemniscus, where STT are known to pass (Giesler et al. 1976). Stereotaxic coordinates relative to the bregma:(anterior 2.5mm, lateral 2mm, ventral 5mm). These were used to provide a search stimulus of monophasic square wave pulses at 1Hz, 0.4 msec width, 50-150 μA. Once a dorsal horn neurone was found to be activated from the region of the contralateral medial lemniscus, an array of four bipolar stimulating electrodes (within 1 mm^2), placed in the region of the contralateral ventrobasal thalamus, (coordinates relative to Bregma: anterior 3.0mm, lateral 2.5mm and ventral 4.0mm), was used to locate the area of projection within the thalamus more precisely. One electrode of this array, which elicited antidromic activation at the lowest threshold was then identified and this electrode site marked at the end of the

experiment (section C(iv)). In some instances, the correct placement of the bipolar stimulating electrodes within the medial leminiscus, was confirmed at the beginning of the experiment. This was achieved by recording compound action potentials from the area of the medial lemniscus, which could be evoked by electrical stimulation of the contralateral, ventrolateral quadrant of the thoracic spinal cord, using a bipolar stimulating electrode (Figure 8). The bandwidth of the recording amplifier was 8Hz-2.5 KHz (Digitimer Ltd, D3160). The stimulation parameters used were 1Hz, 0.3-0.4 msec width, 50-120 μA. The electrode in the region of the medial lemniscus was moved until the evoked compound action potential was at its largest. This electrode position was then used to provide the search stimulus for STT neurones, as described above.

Neurones, unidentified in terms of their possible supraspinal projections in the rat, were located by generally light tactile stimulation of the hindlimb, whilst searching the ipsilateral dorsal horn with the multibarrelled glass recording electrode.

C(iv) Histological identification of electrode placement:

The position of all stimulating electrodes (including those used to stimulate the regions of dopamine cell groups, A9 and A11, described in methods section E), were marked at the end of the experiment by an iron deposit from the stimulating electrode (30-60

seconds, 30 A D.C.). The animal was killed by an overdose of anaesthetic and the brain was then rapidly removed and fixed in 1% ferrocyanide dissolved in 10% formal saline for 2 to 3 days. After this time, the tissue was sectioned on a cryostat (50 or 100 μm), mounted on gelatinised glass slides, left overnight in saturated formaldehyde vapour and then stained with neutral red. Spinal cord tissue was fixed in 10% formal saline for 12 to 18 hours and then processed as above, to elucidate the recording position of the extracellular electrode, which had been marked with pontamine sky blue, as described earlier.

D. Quantification of cutaneous sensory responses and protocol for testing the effects of ionophoretically-applied drugs

Multireceptive dorsal horn neurones were tested in these studies, in order that the effects on neuronal responses to both noxious and innocuous cutaneous stimuli could be compared on a cell whose inputs were, presumable, capable of being affected by the same general modulatory influences.

Controlled noxious and innocuous stimuli were applied to adjacent sites within the ispilateral, cutaneous receptive field. The receptive fields of the neurones studied were situated mainly on the paw, toes and hindlimbs of both the cat and the rat. The innocuous cutaneous stimulation was provided by a motorized rotating brush (for the duration of 10 to 15 seconds).

The noxious cutaneous stimulation was provided by either a quantifiable pinch (of 10 seconds duration) or a themistor-controlled radiant heat lamp precisely raised from 30°C to 46-48°C within 5 seconds, and maintained at 46-48°C for a chosen duration of between 5 to 10 seconds. The pinch stimulus was quantifiable by means of a scale attached to the pinch mechanism, which was directly related to the pressure exerted (Figure 9). The radiant heat and pinch stimuli were demonstrated to evoke a withdrawal reflex in lightly anaesthetised animals and were used as noxious stimuli. The motorised brush did not evoke a withdrawal response in lightly anaesthetised animals and was used as an innocuous cutaneous stimulus. Activity evoked by ionophoretically-applied DLH was also assessed (5-60 nA for 10 to 15 seconds duration). Control responses were always repeated at least in duplicate and more often in triplicate, and only when they varied by less than 15-20% were the tests carried out. The magnitude of the different responses that were evoked, were matched by altering the positions and the strengths of the stimuli used and were always submaximal. A series of noxious, innocuous and DLH-evoked responses were repeated over 3, 4, or 4.5 minute cycles (Figure 10). Care was taken that the action potentials of the neurone being tested could be clearly discriminated and were continuously monitored throughout the test (Digitimer Spike Processor, D130). Most drugs were applied by ionophoresis for 1 minute before the start of each test cycle (Figure 10B), except

RU24213, which was ionophoresed 30 seconds before each stimulus was applied (Figure 10C). More prolonged ionophoreses of RU24213 caused non-specific spike distortion (results section 10C). After a test had been completed, the neuronal responses were allowed to recover fully (up to 40 minutes). The possiblity that any changes observed during a test may have been due to the order in which the evoked responses were tested, was controlled for by changing the order of the three stimuli during the tests.

Neuronal firing was continuously recorded on FM tape, as well as the firing rate being plotted by computer (400 msec bin width, Cromemco System III) or on a chart recorder (2 second bin width). The analogue signals from the cutaneous stimulators and the Neurophore were recorded simultaneously. Data stored on computer floppy disc was analysed "off-line", by integrating numbers of action potentials in selected epochs for the duration of the stimulus-evoked response as a percentage of control responses. The integrated responses were normalised and expressed graphically, to permit the comparison of the effects of a drug on the different evoked activities of the same neurone and between different neurones. Further summary graphs were calculated by averaging the normalized responses of different neurones which underwent tests using the same time courses and incremented levels of either ionophoretically-applied DA or electrical stimulation in

the region of the A11 DA cell group (Figures 25 and 32).

E. Protocol for the focal electrical stimulation in the regions of the DA cell groups, A9 and A11.

The effects of focal electrical stimulation in the region of either of the DA cell groups, A9 or A11, were tested on the responses of multireceptive dorsal horn neurones in the rat. The electrical stimulation was by either bipolar, or, more usually, monopolar stimulating electrodes, which were stereotaxically placed in the regions required (methods section F). The stereotaxic co-ordinates of the region of the A9 cell group were; anterior 1.5mm, lateral 2.0mm and ventral 7.5mm and for the A11 cell group; anterior 3.0mm, lateral 0.5mm and ventral 4.5mm, with reference to Bregma. The electrical stimulation was begun 15 to 20 seconds before, and left on for the duration of a particular stimulus within the cycle of responses tested (Figure 10A). The stimulation parameters used were: monophasic square wave pulses, 10-100Hz, 0.4msec width, 10-175 μA.

To determine whether an effect, evoked by this electrical stimulation, was mediated by DA at the level of the spinal cord, sulpiride (70-80 nA), a highly selective D_2 DA-receptor antagonist, was ionophoresed in the vicinity of the dorsal horn neurone being tested (Figure 7). The effects of an α_2-receptor antagonist (idazoxan) and naloxone were also tested (results section F(ii)).

To ascertain the optimal stereotaxic locations for electrodes used to stimulate the regions of the A9 or A11 DA cell groups, histofluorescent studies were carried out prior to these electrical stimulation experiments.

F(i) The glyoxylic acid-catecholamine histofluorescent technique.

Rats (260-350g) were deeply anaesthetised with an intraperitoneal injection of sodium pentobarbital (0.1ml/kg). The animal was secured on a grid over a sink, for disposal of the perfused fluid (Figure 12). The thorax was rapidly opened and the ribs retracted to reveal the heart. A small incision was made in the left atrium and a 2mm cannula (connected to the perfusion apparatus) inserted and clamped into place. The perfusion was performed under pressure (0.5-1.5 atomospheres), supplied by a N_2 cylinder, sufficient to allow the rapid circulation of 300mls of fluid within 3 to 4 minutes, according to the glyoxylic acid (GA) technique of Bloom and Battenberg (1976). The perfusion solution used was: 1.5g paraformaldehyde, dissolved in 120mls of phosphate buffer (pH 7.0). 80g $MgSO_4$ was added to the cooled solution and the total volume made up to 150mls with phosphate buffer (pH 7.0). 6g GA and 300mg procaine were dissolved in 120mls phosphate buffer (pH 7.0) and added to the paraformaldehyde solution. The total volume was made up to 300mls with phosphate buffer (pH 7.0) and the solution adjusted to pH 4.8-5.0, if

necessary. The solution was kept on ice until the perfusion was under taken. Procaine, a vasodilator, was added to the solution to aid perfusion.

Effective fixation was assessed by looking for a steady flow of fluid from a cut made in the tail and the animal (including the extremities) being rigid at the end of the perfusion.

Immediately after the perfusion the upper lumbar cord, brain stem and brain were taken as quickly as possible and placed on moistened cork discs. The disc and tissue were then placed in an air-tight metal container, within a vacuum flask, containing dry-ice. The container lid was replaced to prevent dehydration and the flask placed in a freezer overnight.

The tissue was cut, using a cryostat, maintained at -18 to -20^{o}C, in transverse, 20µm thick sections. Care was taken to prevent the tissue from becoming warm, when transferring it from the vacuum flask to the cryostat, or when embedding the cork disc base in mounting medium, as this would have resulted in excessive background fluorescence due to CA leakage in the tissue fluorescence. Serial sections of spinal cord, tissue, or every fourth and fifth section of brain tissue were collected alternately onto one of two sets of glass slides, which had been cooled in the cryostat, for subsequent processing, either for CA-fluorescence, or to be stained for ordinary histological examination. The slides for fluorescence visualization were 0.8-1.0mm

thick (Chance Propper Ltd, BS3836), non-fluorescent type, which had been cleaned of any debris which might fluoresce, by soaking in 2% HCl solution and wiping with absolute alcohol. The slides used for ordinary light microscopy were 1.0-1.2mm thick (Chance Propper Ltd) and were gelatinised before use.

The sections to be processed for CA-fluorescence were rapidly thawed onto the glass slide and immediately placed in the immersion solution kept on ice for 11 minutes, as described in the method of Loren, Bjorkund and Lindvall (1977), with the addition of 350mM sucrose (reported to preserve tissue morphology, Fleetwood-Walker et al. 1983a). 6g GA and 36g sucrose were dissolved in 250mls phosphate buffer (pH 7.0). The total volume was made up to 300mls, with phosphate buffer (pH 7.0) and the pH of the solution adjusted to 7.0.

After immersion, the slides were air dried (37°C) for 5 minutes and then incubated at 100°C, in an oven, for 10 minutes (according to the method of Loren et al. 1977). The sections were then mounted in paraffin, protected with cover-slips, and stored at -20°C.

The sections on 1.0-1.2mm gelatinised glass slides were processed for ordinary light microscopy. They were thawed onto the glass slide and placed in saturated formaldehyde vapour overnight. The following day these sections were stained with neutral red.

F(ii) Examination of tissue sections by ordinary and fluorescent light microscopy.

Sections stained with neutral red were observed using ordinary light microscopy, and camera lucida drawings of brain and brain stem sections were made at approximately 100µm intervals, with the major anatomical landmarks drawn and related to the position of obex or Bregma, on representative figures. These drawings and measurements were used to identify and map the positions of CA-fluorescent cell bodies. CA-histofluorescent sections were visualized under ultraviolet illumination (405/435nm illumination with secondary barrier filter transmission at 475-500nm).

Figure 5

Agar injection around the thoraco-lumbar laminectomy to improve the stability of the rat spinal cord for recording purposes

A 2% agar solution was carefully and slowly injected 0.5-1.0cm beyond the spinal cord and intact dura. Further agar covered the exposed spinal cord and filled the pool surrounding the laminectomy. This procedure improved the stability of the spinal cord, facilitating maintained extracellular recordings from dorsal horn neurones.

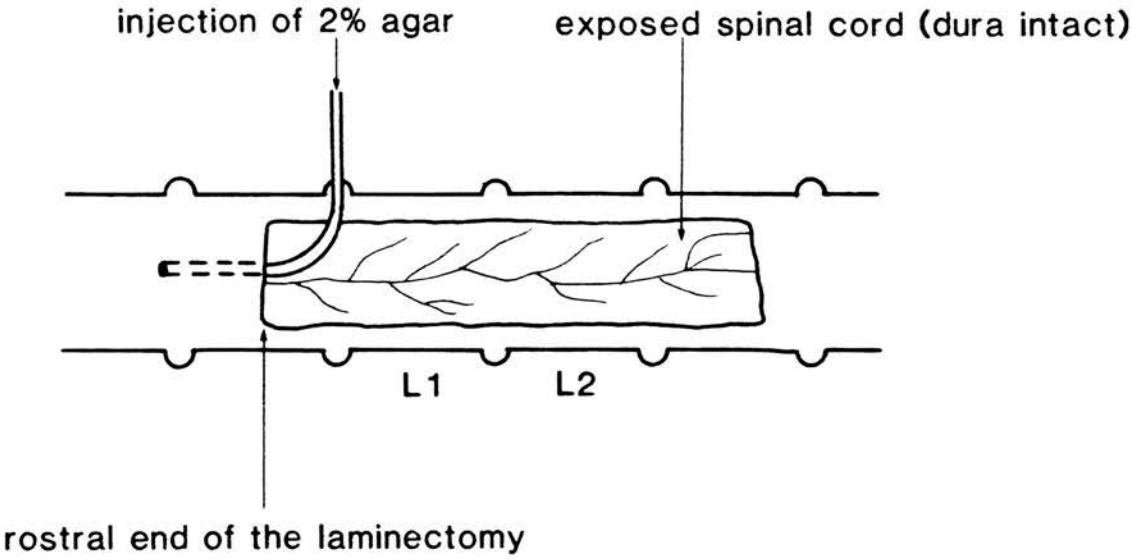

injection of 2% agar
exposed spinal cord (dura intact)
L1
L2
rostral end of the laminectomy

Figure 6

Methods for the identification and testing of SCT neurones, with ionophoretically-applied drugs

Extracellular recordings were made using multibarrelled electrodes in the lumbar dorsal horn of anaesthetized and paralysed cats. Identification of SCT neurones was by standard antidromic criteria (Lipski, 1981), using pairs of silver ball stimulating electrodes which were ipsilaterally placed on: DLF at C1 (S1), DLF at C3 (S2) and dorsal columns at C4 (S3). A discrete surgical lesion (shown by hatching) was made across the dorsal columns at C3/4 to prevent misidentification of neurons from crossing fibres. The effects of various drugs ionophoresed in the vicinity of the SCT neurone tested were assessed on its responses to heat or pinch, brush and DLH-evoked activity (see methods section B). The positions of 15 neurones tested (all identified SCT neurones) are shown on a representative section of the dorsal horn. Approximate locations of the laminae (according to Rexed, 1952; 1954) are shown and all neurones lie within laminae III - V.

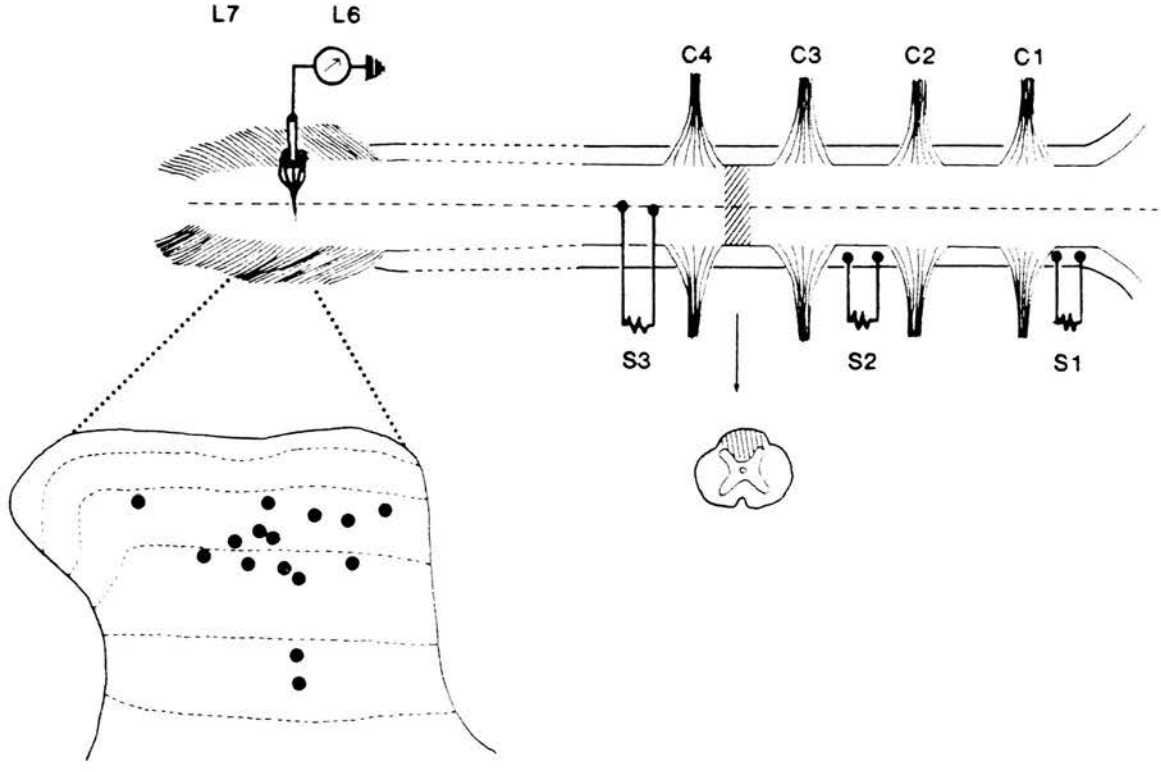

L7
L6
C4
C3
C2
C1
S3
S2
S1

Figure 7

Methods for the identification and testing of STT neurones with ionophoretically-applied drugs

Extracellular recordings were made using multibarrelled glass electrodes in the lumbar dorsal horn of the anaesthetised rat. Identification of STT neurones was by standard antidromic criteria (Lipski, 1981), using two arrays of bipolar electrodes, sterotaxically placed in the region of the contralateral, medial lemniscus (as search stimulus) and the contralateral, ventrolateral posterior thalamus (to locate the site of projection of the neurone). The effects of various drugs ionophoresed in the vicinity of the STT neurone tested were assessed on its responses to brush, pinch and DLH-evoked activity (see methods section B).

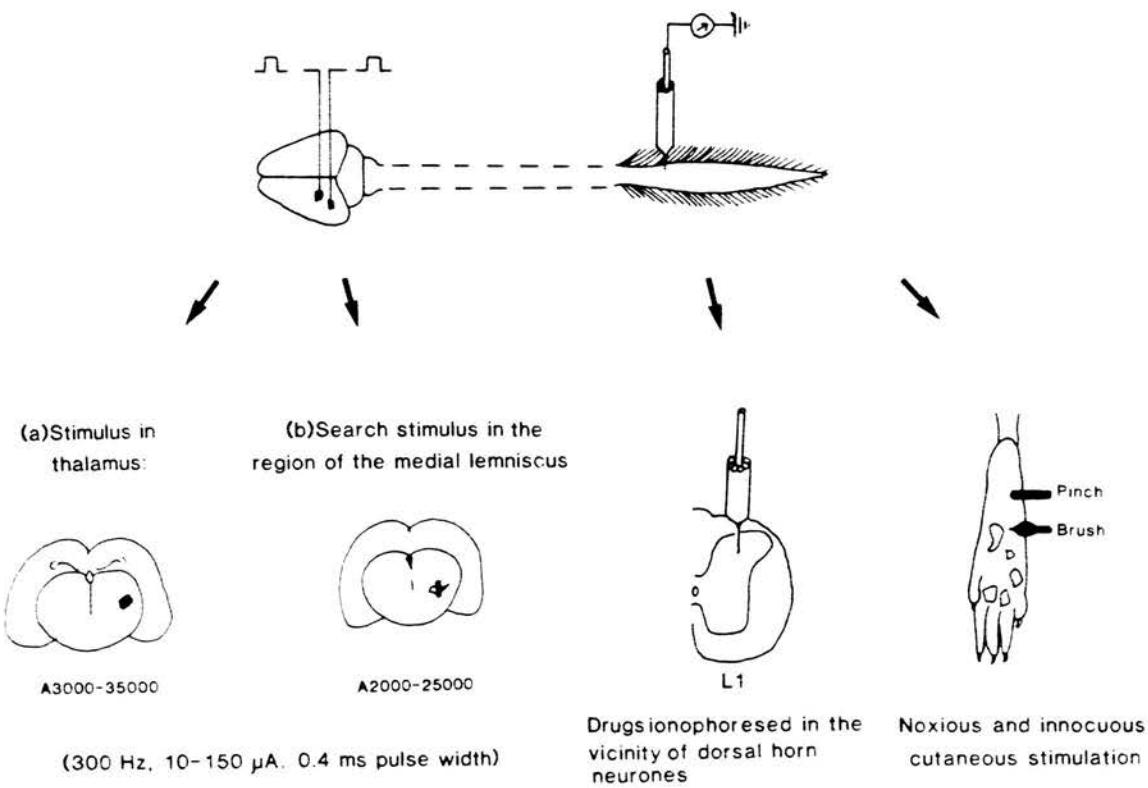

(a)Stimulus in thalamus
(b)Search stimulus in the region of the medial lemniscus
A3000-35000
A2000-25000
L1
Pinch
Brush
(300 Hz, 10-150 μA, 0.4 ms pulse width)
Drugs ionophoresed in the vicinity of dorsal horn neurones
Noxious and innocuous cutaneous stimulation

Figure 8

Compound action potentials recorded by a bipolar (stimulating) electrode stereotaxically placed in the region of the medial lemniscus

The compound action potentials shown (B and C) were evoked by electrical stimulation of the contralateral ventrolateral quadrant of the thoracic spinal cord.

(A) Base line recording, from the region of the medial lemniscus (LM), in the absence of stimulation of the spinal cord.

(B) Compound action potential recorded from the site marked (dark circle), verified histologically as being within the ML, on a transverse section of the brain. The largest compound action potential was recorded from this site and so this placement of the bipolar electrode was used, as a search stimulus, to identify STT neurones.

(C) A much smaller compound action potential was recorded from a site 0.5mm dorsal to the site shown in the diagram (i.e. outside the ML).

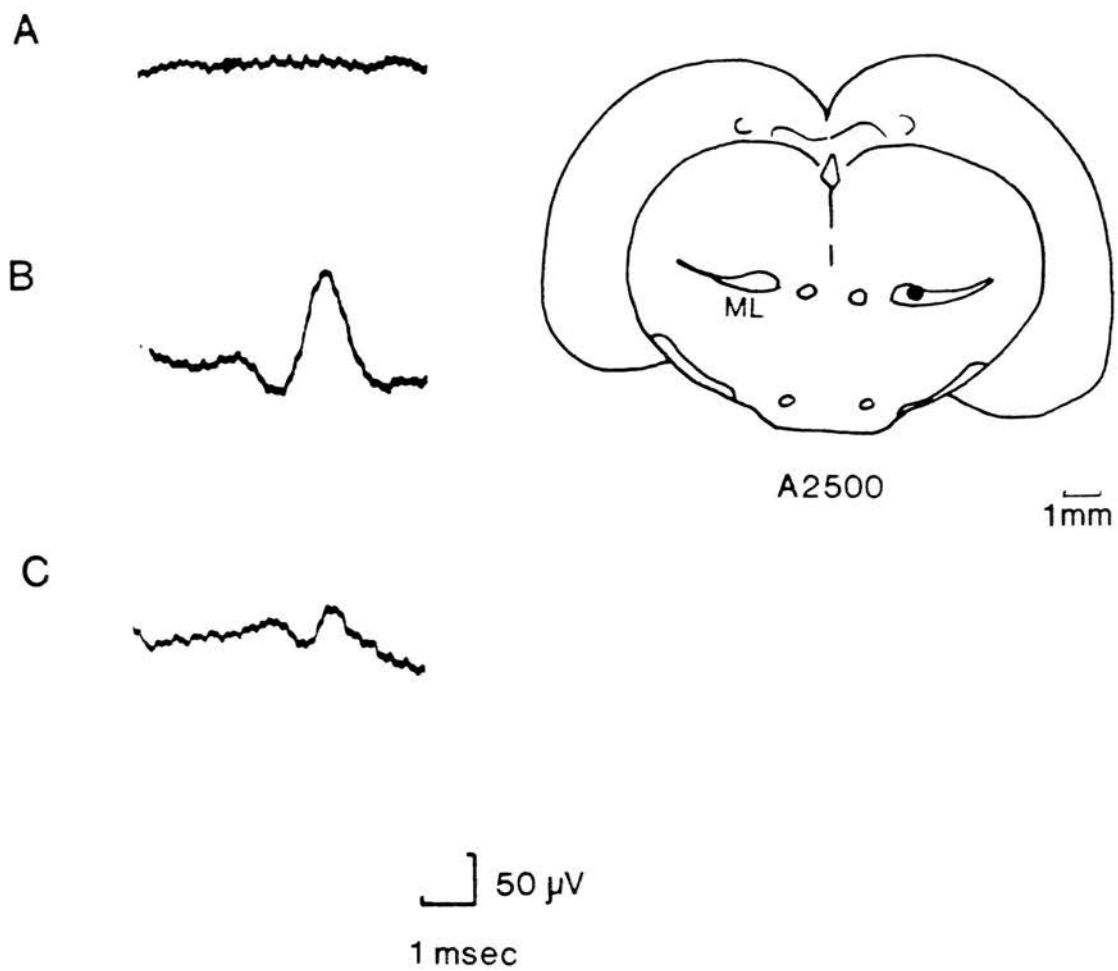

A
B
C
ML
A2500
1mm
50 µV
1 msec

Figure 9

Diagram of cutaneous stimulators used

(A) Noxious pinch: The noxious pinch was quantifiable by means of a graded scale which directly allowed the pincher to be closed to the same level on each test, for a duration of 10 seconds.

(B) Noxious radiant heat: The noxious radiant heat was provided by a thermistor-controlled radiant heat lamp. The surface temperature was measured by a thermocouple, in the center of the heated area, placed within 1-2mm of the cutaneous receptive field. The temperature was raised from 30^{o}C to 46 to 48^{o}C within 5 seconds and held at 46 to 48^{o}C for a duration of 5 to 10 seconds.

(C) Innocuous motorized brush: The noxious motorized brush rotated consistantly, gently stimulating the receptive field for a duration of 10 seconds.

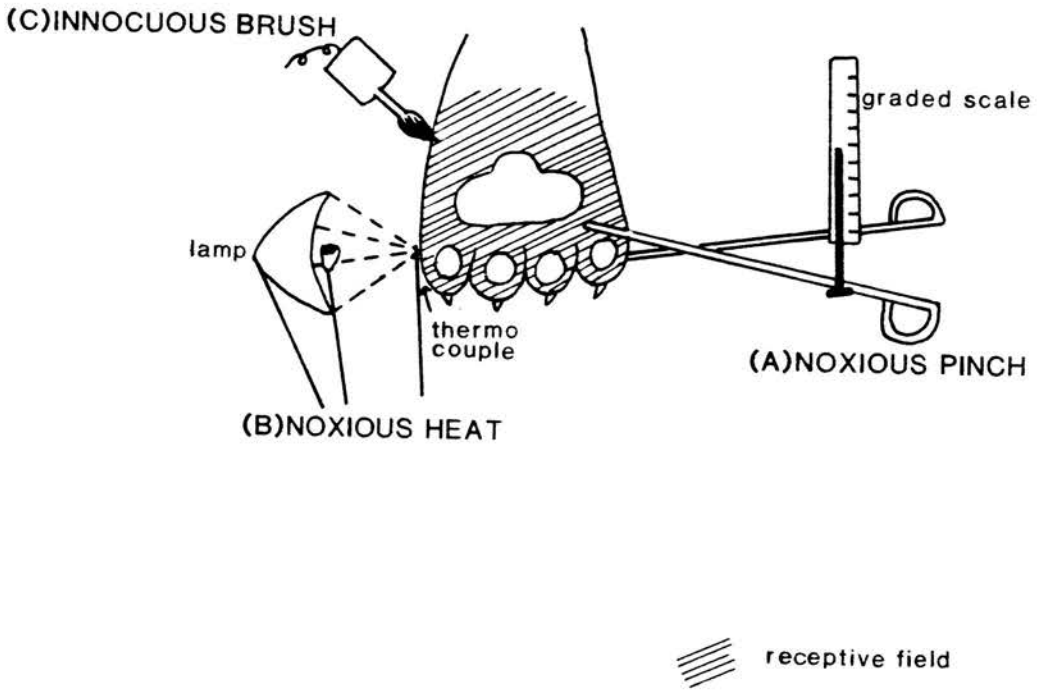

(C)INNOCUOUS BRUSH
graded scale
lamp
thermo couple
(A)NOXIOUS PINCH
(B)NOXIOUS HEAT
receptive field

Figure 10

Protocol used for testing multireceptive dorsal horn neurones, in the rat and cat

The upper trace in each section represents the duration of the stimulus: innocuous brush (B), ionophoretically -applied DLH (D) and noxious pinch or radiant heat (P), which evoked activity in the multireceptive dorsal horn neurone.

(A) Electrical stimulation in the region of the A9 or A11 groups (see methods section E), was started 20 seconds before and left on for the duration of each of the above stimuli (10 seconds). In these tests the stimuli, B, D and P, were applied at 1 minute intervals over a 3 minute cycle.

(B) Continuous ionophoresis of drugs (NA or DA) was started 1 minute before B, D and P were tested (at 1 minute intervals) therefore, in these tests, the overall cycle lasted 4 minutes.

(C) Intermittent ionophoresis of the D_2 DA-agonist, RU24213, was started 30 seconds before and continued for the duration of each stimulus. Each stimulus was repeated at 90 second intervals, therefore the overall cycle lasted 4.5 minutes.

Protocols used for testing multireceptive dorsal horn neurones

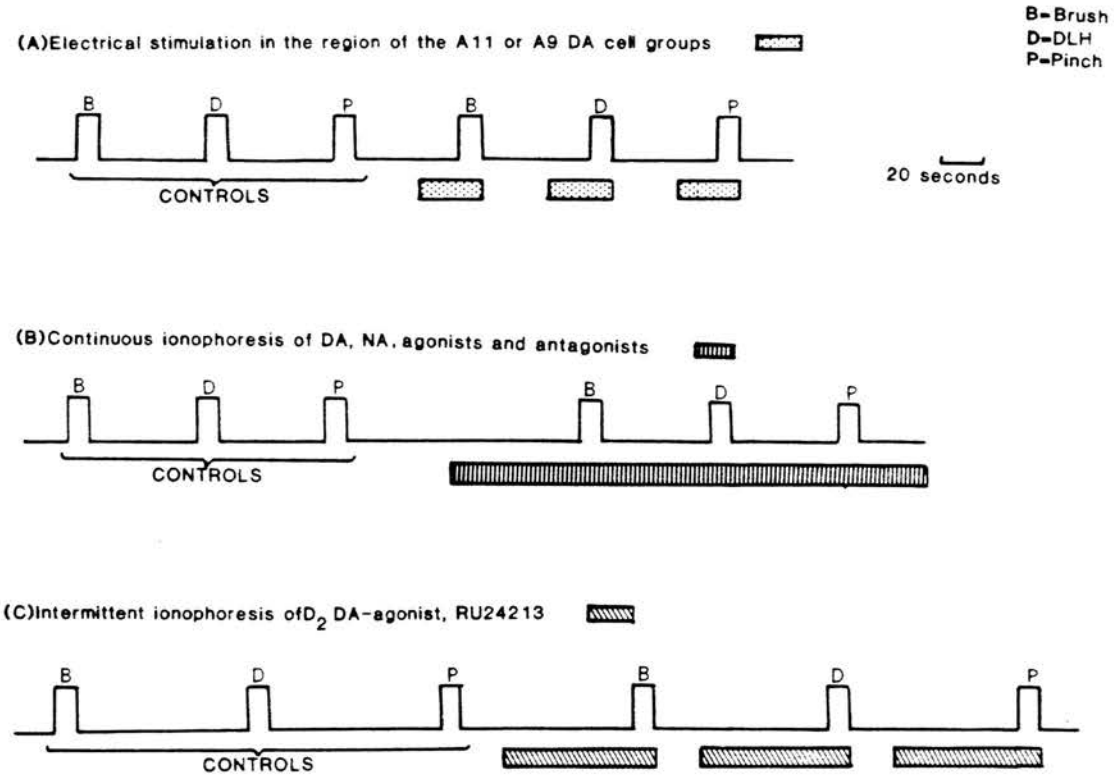
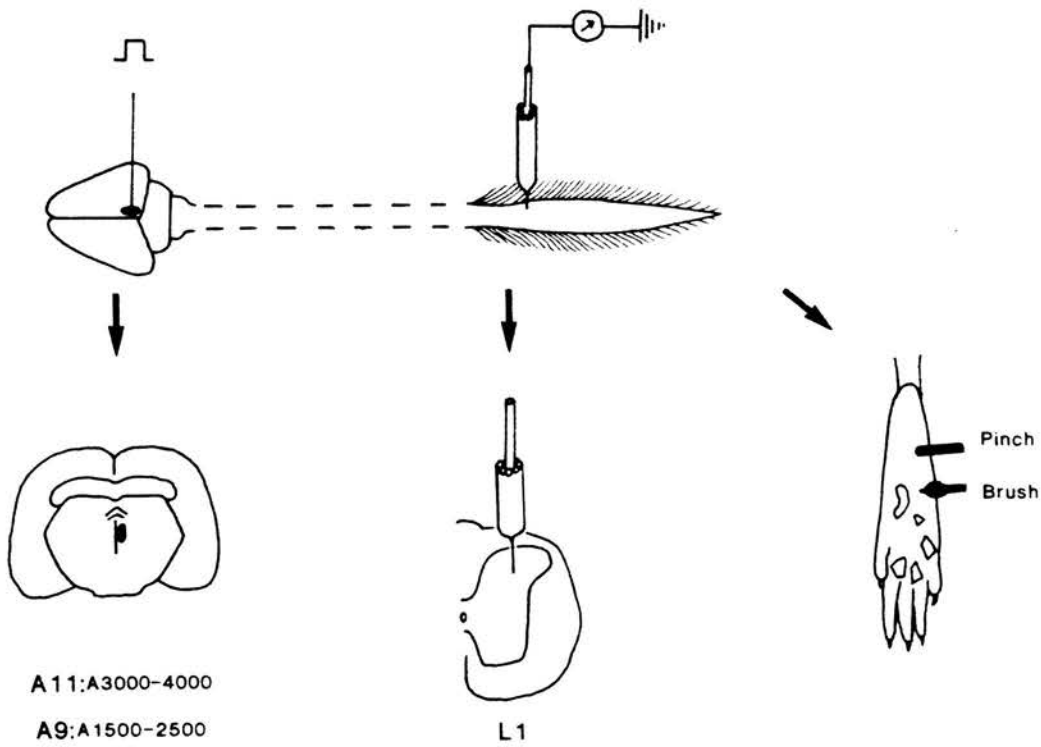


Figure 11

Methods for the assessment of stimulus-evoked effects, from the regions of the A11 or A9 DA cell groups, on multireceptive dorsal horn neurones in the rat

The effect of electrical stimulation in the region of either the A11 or A9 DA cell groups was assessed on the neuronal responses of dorsal horn neurones, to brush, pinch and DLH-evoked activity. The action of sulpiride, ionophoretically-applied in the vicinity of the dorsal horn neurone, on any stimulus-evoked effect was then assessed.



A11 or A9 area stimulation
(10-175 μA)
(10-100 Hz)

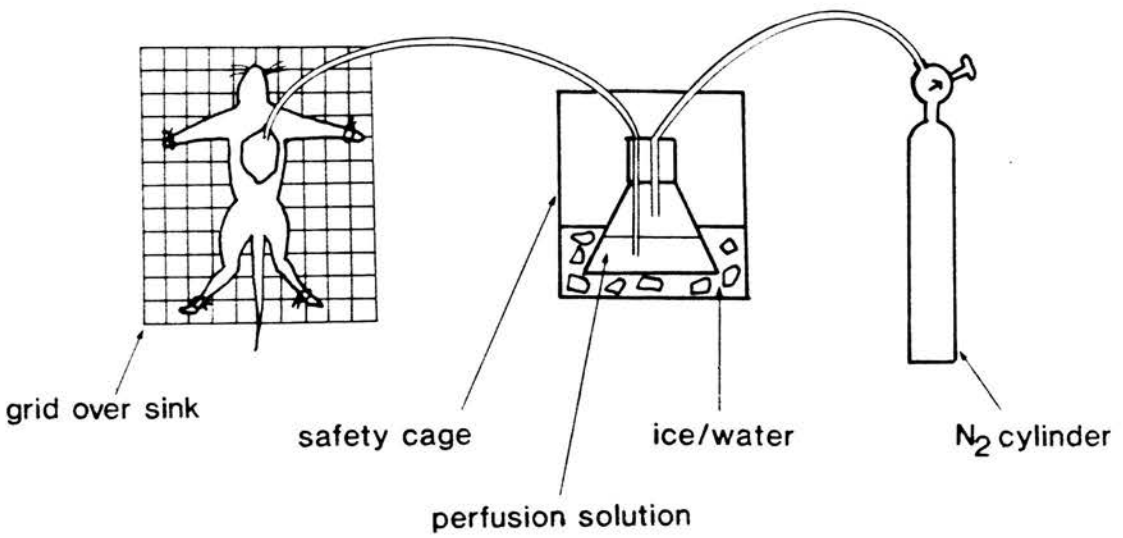
Sulpiride/Dopamine/DLH
ionophoresed in the vicinity
of dorsal horn neurones

Noxious and innocuous
cutaneous stimulation

Figure 12

Method of high pressure perfusion of the rat, for the glyoxylic acid histofluorescence techniques

The nitrogen (N_2) cylinder provided pressure (0.5 - 1.5 atomospheres) to force 300 mls of ice cold perfusion solution (methods section F) through the deeply anaesthetised rat, within 3-4 minutes. The animal was secured to a grid over a sink for the disposal of perfused fluid. The perfusion fluid was administered, via a left atrial cannula, connected to a high pressure container flask (within the safety cage).


grid over sink
safety cage
perfusion solution
ice/water
N_2 cylinder

RESULTS

A. Characteristics and receptive field properties of neurones tested.

A(i) In the cat:

The properties and locations of SCT neurones were very similar to those reported previously (Cervero et al. 1977 ; Brown, 1981). Neurones were antidromically activated from stimulating electrodes on the ipsilateral DLF, at the level of C3 (Figure 6), following frequencies up to 300Hz, with latencies constant to within 0.1 msec. The mean latency was 5.4 msec and the mean conduction velocity 54 m/sec, whilst the mean threshold for activation was approximately 270 mV (Table 1). Antidromic identification was confirmed by collision testing (example shown in Figure 13).

The cutaneous receptive field location and properties of SCT neurones were very similar to those reported previously (Brown, 1981). The receptive fields of the neurones studied were all on the paws and toes of the ipsilateral hindlimb and remained topographically constant throughout the recording period (over 2 hours). The multireceptive neurones tested, had prominent excitatory responses to both noxious and innocuous cutaneous stimuli and corresponded to class II and III subtypes (Brown, 1981), having both low and higher threshold responses to innocuous stimuli. The region of

the receptive field most intensely activated by noxious cutaneous stimulation, was often found to be located at the centre of the area most responsive to innocuous cutaneous stimulation. Inhibitory receptive fields were rarely observed and were not studied here. The nociceptive responses of these neurones were tested by the use of a thermistor-controlled radiant heat lamp which raised the skin temperature from 30°C to 46 to 48°C within 5 seconds and maintained this for 5 to 10 seconds duration.

Cyclical repetition of this noxious heat stimulus, or a quantified noxious pinch (for a duration of 10 seconds), the motorised brush (as an innocuous stimulus) and DLH-evoked activity were set up to give consistent response (within 10-15%) which could be reproduced even over prolonged testing periods (over 2 hours), in both the cat and rat.

Positions of pontamine sky blue marks corresponding to the recording sites of 15 SCT neurones are shown in Figure 6. All these neurones lay within laminae III-V of the dorsal horn, in agreement with the report of Brown (1981) as to the location of the majority of SCT neurones.

A(ii) In the rat:

The properties and location of STT neurones tested in the rat were very similar to those previously described in the rat (Giesler et al. 1976) and monkey

(Price et al. 1978). Neurones were antidromically activated from 2 arrays of bipolar electrodes, placed in the region of the medial lemniscus (used as a search stimulus) and in the region of the ventrobasal thalamus (Figure 7). The majority of dorsal horn neurones tested were antidromically activated from the region of the ventrolateral posterior nucleus (VLP, Figure 14). These neurones followed stimulation frequencies of up to 300 Hz. For a sample of 10 STT neurones the mean latency was 4.8 msec and the mean conduction velocity was 16 m/sec (calculated assuming that their axons projected directly to the thalamus, from the spinal cord). The mean threshold for antidromic activation of STT neurones from the thalamus was approximately 100μA (Table 2). Collision testing of an STT neurone is shown in Figure 15.

The cutaneous receptive fields of both the STT neurones, and neurones unidentified in terms of their possible ascending projection, were very similar and in agreement with those described in previous studies in the rat (Geisler et al. 1976; Menetrey et al. 1977). All receptive fields were located on the paws or toes of the ipsilateral hindlimb and remained topographically constant throughout the recording period (often over 2 hours). Examples of the receptive field areas (for both the rat and cat) are shown in various figures. The multireceptive dorsal horn neurones had prominent excitatory responses to both noxious and innocuous

cutaneous stimuli. Reproducible neuronal responses could be evoked on repetition (once, every 3 minutes) of the noxious pinch stimulus in both the rat and the cat. However the noxious radiant heat stimulus resulted in a marked reduction in the nociceptive response in the rat preparation. No tissue damage was observed to the skin area and indeed this effect has been noted elsewhere (Headley et al., personal communication). The effect may have been due to an activation of descending inhibitory influences. Therefore the quantifiable noxious pinch stimulator was routinely used in the rat (Figure 9).

Positions of pontamine sky blue marks, corresponding to the recording sites of 19 dorsal horn neurones (both STT and unidentified neurones) investigated in the rat, are shown in Figure 16 to be located mainly within the deeper dorsal horn laminae. It appears that the population of STT neurones may be located slightly more ventrally than the population of unidentified dorsal horn neurones, although, admittedly, it was a small population that was studied.

B. Effects of ionophoretically-applied NA.

B(i) Multireceptive neurones:

The effects of ionophoretically-applied NA were tested mainly on SCT neurones, although the findings were confirmed in a small sample of PSDC neurones in the cat

and also in a sample of dorsal horn neurones in the rat. Generally 45 to 60 seconds of continuous ionophoresis of NA revealed the modulatory effects described below (using the protocol shown in Figure 10B). In agreement with other studies using ionophoretically-applied NA (Headley et al. 1978; Belcher et al. 1978), NA did not cause any change of rapid onset (<20 seconds, although clearly this may depend greatly on the precise locus of action).

The predominant effect of ionophoretically-applied NA was a selective inhibition of the response of the neurone to noxious cutaneous stimulation, whilst the responses to brush, DLH-evoked and spontaneous activity were unaffected, in 40/41 SCT and 3/5 PSDC neurones tested. A typical example of the effect of ionophoretically-applied NA, on the continuous firing record of a multireceptive SCT neurone is shown in Figure 17. Recovery of the nociceptive responses, to within 10-20% of control values took up to 25 minutes after the cessation of ionophoresis of NA. Records, such as those shown in Figure 17, were analysed by integrating numbers of action potentials in selected epochs for the duration of the stimulus-evoked response. The integrated responses were normalised as a percentage of control responses and expressed graphically in current-response curves (Figure 18). The degree of selectivity of the effect of ionophoretically-applied NA is shown, numerically, in Table 3. The responses to brush, DLH-evoked and spontaneous activity were not

significantly different from control values (P>0.05, Students' t-test on raw data, or Sign test R statistic on normalized data), at currents of NA (mean=14.6nA, n=16) which had been calculated to reduce the responses to noxious cutaneous stimuli to 50% of control values. Ionophoretically-applied 1M NaCl, at similar currents, had no effect on the responses of these neurones, in which NA had been shown to be effective. At higher ejection currents of NA (50-100nA), a gradual and general inhibition of all types of evoked and spontanteous activity became apparent.

These selective antinociceptive effects were obtained using the l-NA bitartrate salt as either 0.1M or 0.5M solutions. The effects using l-NA HCl (0.1M or 0.5M) were indistinguishable from those obtained with the bitartrate salt. Whilst the receptor-active stereoisomer, l-NA, also reproduced the selective effect, but the d-NA isomer did not have any effect (Figure 19), even with up to 5 fold greater ejection currents, on 5 neurones tested.

B(ii) Neurones responding only to innocuous cutaneous stimulation:

Ionophoretically-applied NA (40-50nA) had no apparent effect on either the brush, DLH-evoked or spontaneous activity, on 4/6 SCT and 4/4 PSDC neurones which responded only to innocuous cutaneous stimuli (Figure 20). On 2/6 SCT non-nociceptive neurones, NA had

a weak depressant effect on all types of activity tested, but this was with ejection currents greater than 40nA.

C. Effects of ionophoretically-applied NA receptor agonists and anatagonists:

C(i) α_2-selective agonists:

Two α_2-agonists were tested in the same manner as NA, clonidine and metaraminol. It has been hypothesised that in peripheral tissue two subtypes of α_2-receptor may exist, although the evidence is somewhat equivocal (Mottram, 1982). To test whether there is a distinct involvement of one particular α_2-receptor subtype in the selective antinociceptive action of NA, two α_2-agonists, namely, clonidine (an imidazoline derivative) and metaraminol (a phenylethylamine derivative) were used. Both these agonists are selective for α_2-receptors (Tanaka and Starke, 1980; U'Prichard, Bechtel, Rouot and Snyder, 1979; Wikberg, 1978) but supposedly discriminate the subtypes according to Mottram (1982).

Ionophoretically-applied clonidine (10-60 nA) mimicked the selective antinociceptive action demonstrated by NA. Nociceptive responses (to radiant heat tested in the cat and pinch tested in the rat) were inhibited, whilst responses to brush, DLH or spontaneous activity, were unaffected. This effect was demonstrated on 12/15 SCT neurones in the cat and 5/5 unidentified dorsal horn neurones in the rat (Figures 18 and 21). In the 3

remaining SCT neurones, clonidine had little or no effect on the responses tested (even with up to 60-80 nA of ejection currents).

In the cat, concentrations of 0.1M and 0.5M clonidine were used and caused selective antinociceptive effects with similar ejection currents. In the rat, however, these concentrations of clonidine (and also noradrenaline) tended to cause distortion and reduction of action potential height to an extent which prevented controlled testing of the responses, even with low ejection currents (10-25nA). A lower concentration of clonidine (0.01M), however, caused a selective antinociceptive inhibition of unidentified multireceptive dorsal horn neurones (Figure 21), without affecting the action potentials.

In 2/3 SCT neurones, the antinociceptive effect of NA was also mimicked by ionophoretically-applied metaraminol (a partially α_2-selective NA analogue; Wikberg, 1978), with a clear margin of selectivity. No evidence was apparent to support the hypothesis of α_2-receptor subtypes.

The selective action of clonidine was not attenuated by the presence of ionophoretically-applied naloxone (an opiate antagonist) or sulpiride (70-80 nA), a highly selective D_2 DA-receptor antagonist (Seeman, 1981), in 4/4 neurones tested in the rat. This obviates the possibility that the actions of the $\alpha_2$agonist clonidine were mediated via either an opiate or a dopaminergic receptor.

C(ii) α_1-selective agonist:

Phenylephrine has a pronounced affinity for α_1-, rather than α_2- adrenoreceptors (Wikberg, 1978; U'Prichard et al. 1979; Tanaka and Starke, 1980) and caused a non-selective inhibition of all types of responses tested in 6/11 multireceptive SCT neurones in the cat and 6/6 unidentified multireceptive neurones in the rat (Figure 18). In a further 3 SCT neurones, up to 50 nA of phenylephrine was ineffective. The remaining 2 SCT neurones showed a partial selectivity, where the response to noxious heat appeared to be inhibited to a greater degree than the responses to brush or DLH-evoked activity. In contrast to the marked actions of NA or clonidine, a clear-cut selectivity was not observed with phenylephrine. To further test the possiblity that phenylephrine was causing a direct postsynaptic inhibition, neuronal activity was evoked to a steady rate by continual ionophoresis of the excitant amino-acid, DLH, and the effect of ionophoretically-applied phenylephrine tested. Phenylephrine (up to 80 nA) failed to caused any inhibition of the DLH-evoked activity of 3/5 SCT neurones and in 2/5 SCT neurones, only modest inhibition was observed. What effect there was could not be reversed by ionophoretically-applied antagonists (prazosin or WB4101) and thus phenylephrine was not thought to exert direct receptor specific effects on the SCT neurones tested (results section C(iv)).

C(iii) β-selective agonist:

Isoprenaline, a potent and selective β-agonist (U'Prichard et al. 1979) was tested on 6 multireceptive SCT neurones. Upto 60-80 nA of isoprenaline failed to significantly effect any type of activity tested, on 4 of these neurones (Figure 18). On 2 neurones, a very minor generalized inhibition was observed at higher currents (80nA).

C(iv) Effects of ionophoretically-applied NA antagonists:

The selective antinociceptive effect of NA was attenuated by the presence of ionophoretically-applied α_2-antagonists, yohimbine (Langer, Massingham and Shepperson, 1981) and idazoxan (Chapelo, Doxey, Myers and Roach, 1981). From current-response curves, the percentage antagonism was calculated as; the percentage recovery of nociceptive response in the presence of both agonist and antagonist, divided by the total inhibition of nociceptive response in the presence of the agonist alone x100; thus the degree of reversal of the agonist effect by the antagonist was assessed. Acute reversal (mean=49% antagonism, n=5) of the effect of NA was seen with idazoxan in 5/5 SCT neurones (Figure 22), but not with yohimbine (2/2 SCT neurones), although the time-course of recovery of the nociceptive response was markedly shortened by a factor of 2 to 3. Ionophoresis of either α_2-antagonist prior to the NA test (for 12-20 minutes at currents of 20-50nA) always reduced the

inhibition of the nociceptive response, in comparison with the control test. Under these conditions, idazoxan and yohimbine produced a mean reversal of the NA effect of 82% (n=5) and 75% (n=2) respectively, with no apparent change in control responses. In contrast, the selective α_1-antagonists, prazosin (Cambridge, Davey and Massingham, 1977; Tanaka and Starke, 1980) and WB4101 (Tanaka and Starke, 1980; Massingham, Dubocovich, Shepperson and Langer, 1981) did not produce any reversal of the effect of NA when tested on 5 SCT neurones, using either acute or chronic application (upto 80nA for 20 minutes). However, at higher currents some non-specific spike distortion and excitation may have masked an effect. As described in results section C(ii) prazosin or WB4101 did not reverse a modest inhibition of DLH-evoked activity caused by ionophoretically-applied phenylephrine, in tests in which non-specific spike distortion did not occur.

D. Effects of ionophoretically-applied DA.

D(i) Multireceptive neurones:

A selective antinociceptive action of DA was observed in 12/18 SCT neurones in the cat, 8/8 STT neurones and 19/21 unidentified dorsal horn neurones in the rat. Generally 45 to 60 seconds continuous ionophoresis of DA revealed these effects (using the protocol shown in Figure 10B). In agreement with other

studies using ionophoretically-applied CAs (Belcher et al. 1978; Willcockson et al. 1984), DA (and NA) did not cause any change of rapid onset (<20seconds). NA and DA were shown to have pharmacologically distinct receptor-mediated actions (results section C(i)).

An example of the effect of ionophoretically-applied DA on a multireceptive SCT neurone is shown on a continuous firing record, in Figure 23. The nociceptive response recovered to within 10-20% of control values 12 minutes after cessation of ionophoresis of DA. Both multireceptive STT and SCT neurones have been classified into subclasses having either low or higher thresholds to innocuous cutaneous stimuli (Menetrey et al. 1977; Brown, 1981). However, in the total population of neurones studied (including neurones with low and higher thresholds to innocuous cutaneous stimuli), the response to innocuous brush stimulation was consistently unaffected by DA (Figure 24A-D and Table 4). Similar observations were made for the action of ionophoretically-applied NA (Table 3). The degree of selectivity appeared similar for all multireceptive SCT, STT and unidentified neurones tested, as shown, numerically, in Table 4. The responses to brush, DLH-evoked and spontaneous activity were not significantly different from control values ($P>0.05$, Students' t-test on raw data, or Sign test R statistic on normalized data), at currents of NA which had been calculated to reduce the nociceptive responses of SCT

(mean=22.8nA, n=15), STT (mean=24.4nA, n=8) and unidentified (mean=21.2nA, n=19) neurones to 50% of control values. The degree of selectivity of the effect of DA is shown on a summary graph (Figure 25), calculated from twelve dorsal horn neurones in the rat, tested using the same currents of ionophoretically-applied DA (i.e. 5, 10, 15, 20nA, etc., over 4 minute cycles, Figure 10B).

The selective inhibition of the nociceptive response by either NA or DA was observed irrespective of the order of application of the stimuli or the magnitude of the responses tested. Duplicate or triplicate observations of control responses and the effects of ionophoretically-applied DA or NA were similar, if the test was repeated after recovery.

In only 3/18 SCT neurones in the cat and 2/21 neurones in the rat did ionophoretically-applied DA have no effect (at currents upto 60nA). Using currents of 60-100nA some non-selective depression of all types of activity was observed in these cases.

D(ii) Nocispecific neurones:

DA, ionophoresed in the vicinity of 3/3 nocispecific neurones inhibited the responses to noxious pinch in the rat, whilst the response to DLH-evoked and spontaneous activity were unaffected (Figure 26A).

D(iii) Neurones responding only to innocuous cutaneous stimulation:

In 2 neurones in the rat and 1 SCT neurone in the cat, there was little or no effect on either brush, DLH-evoked or spontaneous activity with up to 100nA of DA (Figure 26B).

E. Effects of ionophoretically-applied DA agonists and antagonists.

E(i) D_2 DA-receptor agonist:

Ionophoretic application of the selective D_2 DA-receptor agonist, RU24213 (Euvrard, Ferland, DiPaolo, Beaulieu, Labrie, Oberlande, Raynaud and Boissie, 1980) potently mimicked the selective antiociceptive action of DA (Figure 27A). RU24213 had a more rapid onset of action than that observed for either DA or NA. Only 30 seconds duration of ionophoresis of RU24213, at 5-20nA ejection currents, caused potent selective inhibition of the nociceptive response. More prolonged ionophoresis (>60 seconds) resulted in spike distortion, preventing further testing. Using shorter time courses of application (30 seconds), RU24213 consistently, selectively and potently inhibited the nociceptive responses of all 6 unidentified neurones tested in the rat. Recovery, after cessation of ionophoresis of RU24213, of the nocicepetive response to within 10-15% of control levels was rapid, within 5 minutes.

E(ii) D_1 DA-receptor agonist:

The selective D_1 DA-receptor agonist, SKF38393 (Setler, Sarau, Zirkle and Saunders, 1978) did not affect any of the responses of all 6 multireceptive neurones tested in the rat, when ionophoresed at currents ranging from 50 to 100nA for upto 10 minute (Figure 27B).

E(iii) D_2 DA-antagonist, sulpiride:

The highly selective D_2 DA-receptor antagonist, sulpiride (Seeman, 1981) consistently reversed the selective inhibition of the nociceptive response caused by DA, on 2/2 multireceptive SCT neurones and 17/17 neurones in the rat, including 7/7 STT neurones (Figure 28 and 29).

Sulpiride was effective when ionophoresed either acutely during an on-going DA test or chronically before a DA test; causing in both cases a mean of 80% antagonism in these experiments (Figure 25).

The inhibition of the nociceptive responses of 2 nocispecific neurones caused by DA was also reversed by sulpiride (Figure 26).

The level of ionophoretic current (nA) required to eject sufficient sulpiride to demonstrate an acute reversal of the selective antinociceptive action of ionophoretically-applied DA, was found to be finely balanced, between 70-85 nA. 40-60nA of ejection current of sulpiride usually prevented further inhibition of the nociceptive response by DA, but did not reverse an

already established inhibition of DA. Ejection currents greater than 90nA, passed through the electrode barrel containing sulpiride, often resulted either in blockade of the electrode or non-specfic spike distortion. Therefore, the optimal levels of sulpiride ejection current for reversal of the action of DA were found to be 70-85nA (for 3 to 6 minutes) and were consistently effective. The same levels were used when investigating the effects of focal electrical stimulation of the regions of the A9 and A11 DA cell groups, described in the following results section F.

Sulpiride, ionophoresed alone in the absence of DA, at 70-80nA for 5 to 10 minutes did not alter the magnitude of the responses to brush, DLH or noxious pinch, or the level of spontaneous activity (within 10-15% of control levels) when tested on 4/4 multireceptive dorsal horn neurones in the rat.

1M NaCl, ionophoresed using currents up to 100nA for 5 to 10 minutes, had no effect on any of the responses of 5/5 multireceptive dorsal horn tested in the rat, nor did it modify the selective action of DA.

F (i) Focal electrical stimulation experiments:

Optimal stereotaxic placements of stimulating electrodes, positioned in the regions of the A9 and A11 DA cell group, were ascertained in a glyoxylic acid-histofluorescence study described in methods section F(i) and results section H.

A11 DA cell group: The predominant effect of focal electrical stimulation in the region of the A11 DA cell group in the rat, was a selective inhibition of the nociceptive response of multireceptive dorsal horn neurones. The response to brush, DLH-evoked and spontaneous activity were unaffected. Maximal effects were elicited within a limited time course of 15 to 20 seconds and the nociceptive response recovered to within 10-20% of control values, 3 to 6 minutes after ceasing electrical stimulation. This selective antinociceptive effect was observed in all 17 multireceptive neurones tested (Figure 30 and 31).

A range of electrical stimulation currents (10-175μA) and frequencies (10-100Hz) were tested. In general, lower frequencies (10-33Hz) required higher current levels to inhibit the nociceptive responses, although responses to brush, DLH-evoked and spontaneous activity were unaffected with up to 175μA (10-33Hz; Figure 31). The selective effect was demonstrated using either monopolar or bipolar stimulating electrodes. A summary graph, calculated from 16 neurones tested using the same increments of stimulation currents (10, 20, 40μA, etc., at 100Hz over 3 minute cycles, as shown in figure 10A), shows the highly selective action of stimulation in the region of the A11 DA cell group (Figure 32).

It was found that the selective inhibitory effects described above were only evoked from the region

immediately encompassing the cells of the A11 cell group, as described by Bjorklund and Nobin (1973) and Hokfelt and Kuhar (1985) and confirmed in the present study (results section H). This restricted region is shown in Figure 33. Movement of the stimulating electrodes by 0.5-1.0mm, out of the region of the A11 DA cell group, abolished the selective stimulus-evoked effect, using stimulation parametres previously shown to be effective within that region.

Electrical stimulation in the region of the A11 DA cell group did not affect the evoked responses or spontaneous activity of 2 dorsal horn neurones which responded only to innocuous cutaneous stimuli.

F(ii) Effect of sulpiride:

Sulpiride, ionophoresed in the vicinity of the multireceptive dorsal horn neurones, reversed the selective inhibition of nociceptive responses evoked by electrical stimulation in the region of the A11 DA cell group (70-85nA for 3 to 6 minutes). The mean percentage antagonism in these experiments was 81% (Figure 32) calculated from all 17 neurones tested. 1M NaCl, 0.1M naloxone and 0.1M idazoxan were also tested using the protocol found to be effective for sulpiride in reversing the effects of stimulation in the region of the A11 DA cell group (results section E(iii)). None of these, applied individually, showed any tendency to mimic the blockade of sulpiride on 7 neurones tested.

In 15 neurones, both ionophoretically-applied DA and electrical stimulation in the region of the A11 DA cell group were demonstrated to have a similar selective antinociceptive effect.

F(iii) A9 DA cell group:

Electrical stimulation (10-175μA, 10-100Hz) in the region of the A9 DA cell group did not affect the evoked responses or spontaneous activity of any of the 5 multireceptive dorsal horn neurones tested (Figure 34).

G. Effect of ionophoretically-applied 5-HT:

Using the same protocol described for NA and DA (Figure 10B), ionophoretically-applied 5-HT failed to reproduce the clear-cut selective antinociceptive action of either of these two substances. At higher currents (>30nA), the responses of all 3 multireceptive neurones tested in the rat were non-selectively depressed (Figure 35). Selectivity was only seen to a marginal degree in 1 neurone out of 3.

H. Glyoxylic-acid histofluorescence study:

The histofluorescence technique employed, is reported to result in fluorescence of all monoamines (Loren et al. 1976) and was used here to stereotaxically map CA cell groups in the rat (with particular interest in the DA containing groups).

A9: The A9 cell group was visualised in a diagonal band

corresponding to the region of the substantia nigra pars compacta (Figure 34); stereotaxic co-ordinates (with reference to bregma), anterior 1.5-2.0mm, lateral 2.0-3.0mm and ventral 7.5-8.0mm (Figure 36).

A10: Located alongside the rostral end of the A9 cell group, fluorescent cell bodies were occasionally observed, scattered medially to the pars compacta and presumed to belong to the A10 cell group.

A11: Rostral to the A9 cell group, a third group of fluorescent cell bodies was observed, located within 0.5-1.00mm from the midline (Figure 39), medial to the fasiculus retroflexus. A distinct delineation between the A9 and A11 cell groups and the medial location of A11 cells was established (Figure 33). Stereotaxic co-ordinates of the A11 group (with reference to the bregma) were anterior 3.0-4.mm, lateral 0.5-1.0mm and ventral 4.5-5.5mm.

CA groups, previously defined by others as noradrenergic in immunocytochemical studies (Westlund et al. 1981; 1983) were also observed in this study.

A1 and A2: The cells of the A1 group formed a crescent-shape extending from the lateral pole of the lateral reticular nucleus (Figure 36). The A2 cell group was located dorsal and lateral to the central canal and the nucleus of the hypoglossal (XII) nerve (Figure 36).

A5: Rostral to the A1 and A2 cell groups were cells of the A5 cell group situated lateral and dorsal to the superior olivary nucleus and medial to the facial nerve VII (Figure 37).

A6 and A7: A dense cluster of histofluorescent cells, closely corresponding to the area of the locus coeruleus, formed the A6 cell group (Figure 38). The cells of the sub-coeruleus A7 cell group were more scattered, generally in the region of the parabrachial nucleus (Figure 38). In contrast to most of the other cell groups described, whose axes extended longitudinally, the A7 sub-coeruleus cell group was observed to extend laterally, in more rostral sections.

Table 1

Latencies and thresholds for antidromic activation of a sample of 10 of the SCT neurones recorded in the cat

Neurone Number	Latency (msec)	Threshold (mv)
22/4	4.4	370
24/5	6.0	330
36/1	6.8	200
36/2	5.8	240
36/4	4.5	220
37/2	4.4	310
37/3	3.8	250
37/4	4.0	200
38/1	8.0	240
40/2	5.8	300
Mean $\pm$ SD	5.4 $\pm$ 1.3	263 $\pm$ 53

Figure 13

Antidromic identification of an SCT neurone by collision testing

Spontaneous action potentials were used to trigger the antidromic stimulus, after a delay here of 3.2 ms, shown by the dotted line and large arrow.

(a) Shows 6 superimposed sweeps of an antidromic action potential, recorded at the cell body.

(b) The delay until triggering the action potential (shown by the small arrow) is reduced below the critical period for this cell and collision has occurred.

(c) Shows the reappearance of the antidromic action potential when the original delay is restored.

(d) Shows the cancellation when a second spontaneous potential has occurred within the critical period.

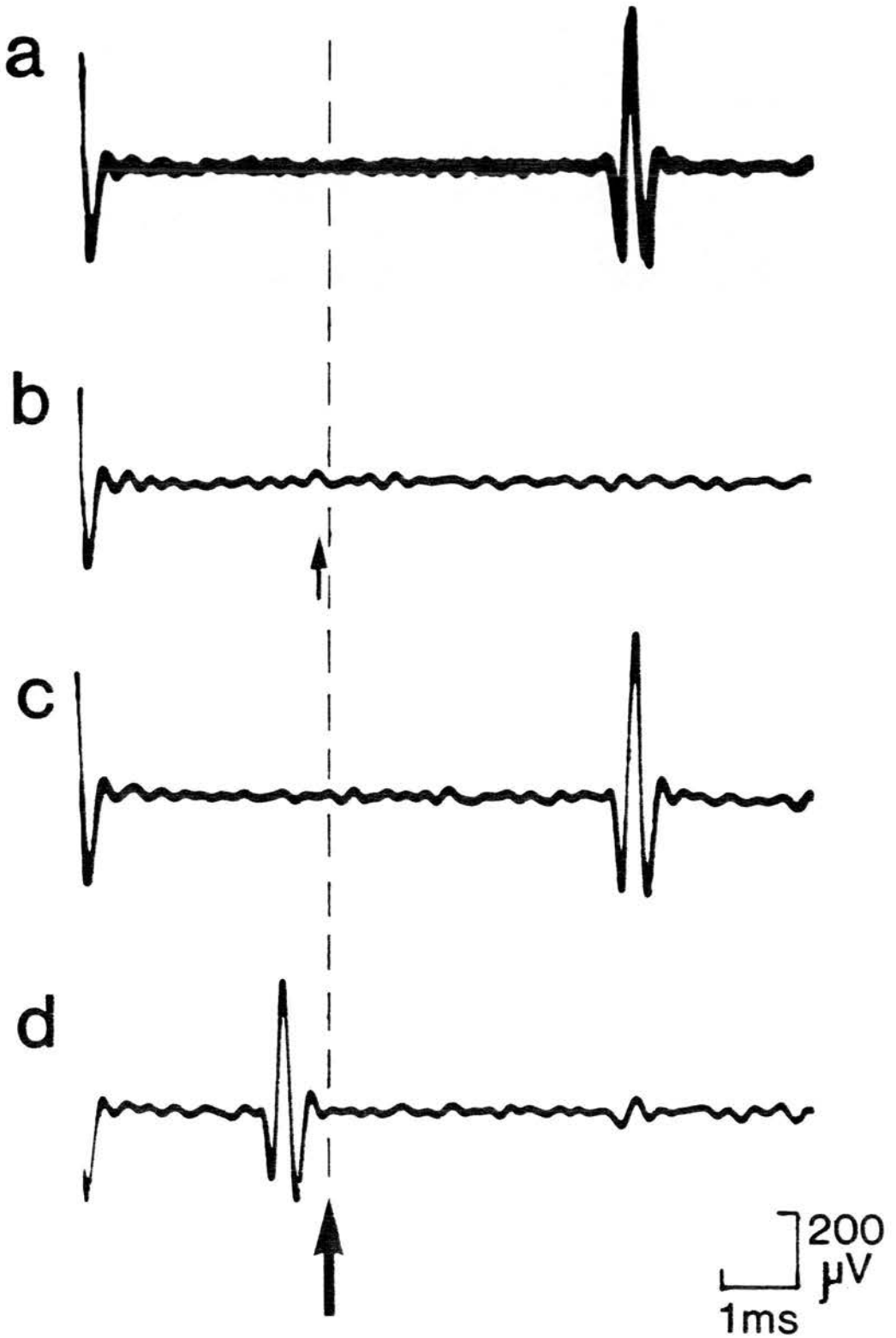

a
b
c
d
200
μV
1ms

Figure 14

The positions of the bipolar electrodes used to identify STT neurones in the rat

Shown on a representative transverse section. The majority of neurones were antidromically activated from the region of the contralateral, ventrolateral posterior nucleus (VLP) of the thalamus. The positions of the bipolar electodes used were marked by iron deposit.

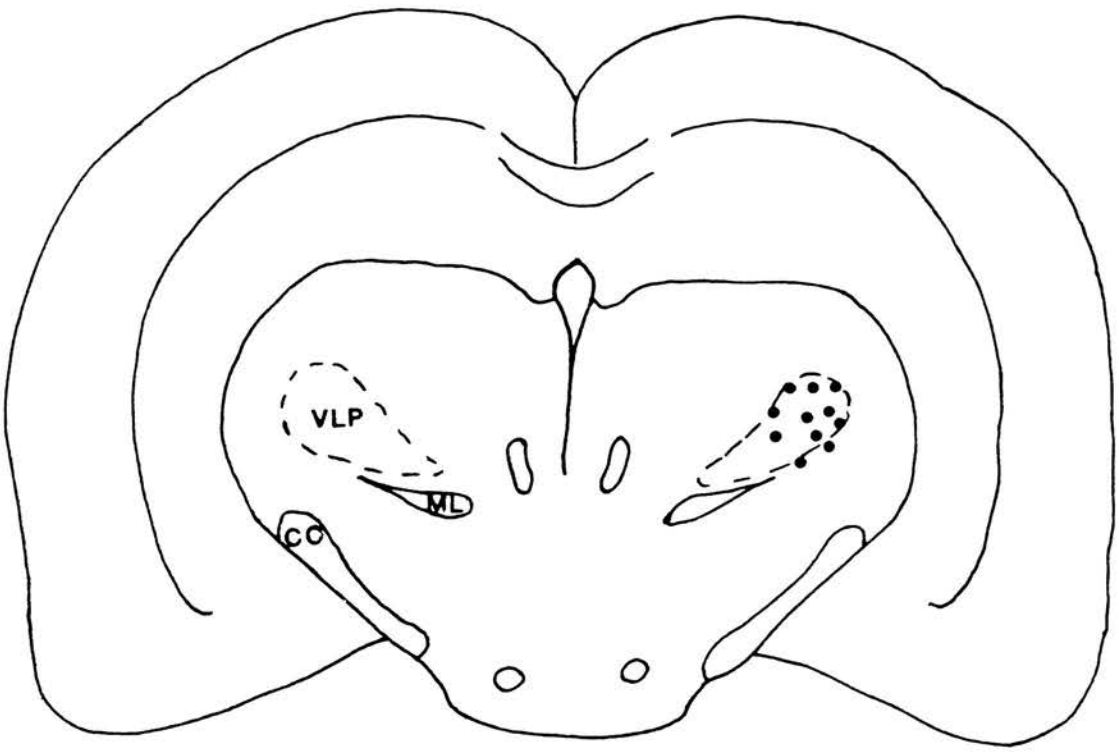

VLP
ML
CC
A3000–3500
1mm

Table 2

Latencies and thresholds for activation of a sample of 10 STT neurones

Neurone Number	Latency (msec)	Threshold (μA)
29	5.6	100
30	6.4	120
31	4.9	80
33	5.1	160
34	5.9	100
35	5.2	80
36	4.0	120
38	5.9	70
41	5.3	120
42	5.8	90
Mean $\pm$ SD	4.8 $\pm$ 1.7	102 $\pm$ 26

Figure 15

Antidromic identification of an STT neurone by collision testing

Spontaneous action potentials were used to trigger the antidromic stimulus, after a delay here of 3.5 ms, shown by the dotted line and the large arrow:

(A) Shows 5 superimposed sweeps of an antidromic action potential, recorded at the cell body (expanded time scale).

(B) The antidromic potential was triggered 3.5 ms after the spontaneous action potential.

(C) The delay until triggering the action potential (shown by the small arrow) is reduced below the critical period for this cell (3.4 ms) and collision occurred.

(D) Shows cancellation when a second spontaneous potential occurred within the critical period.

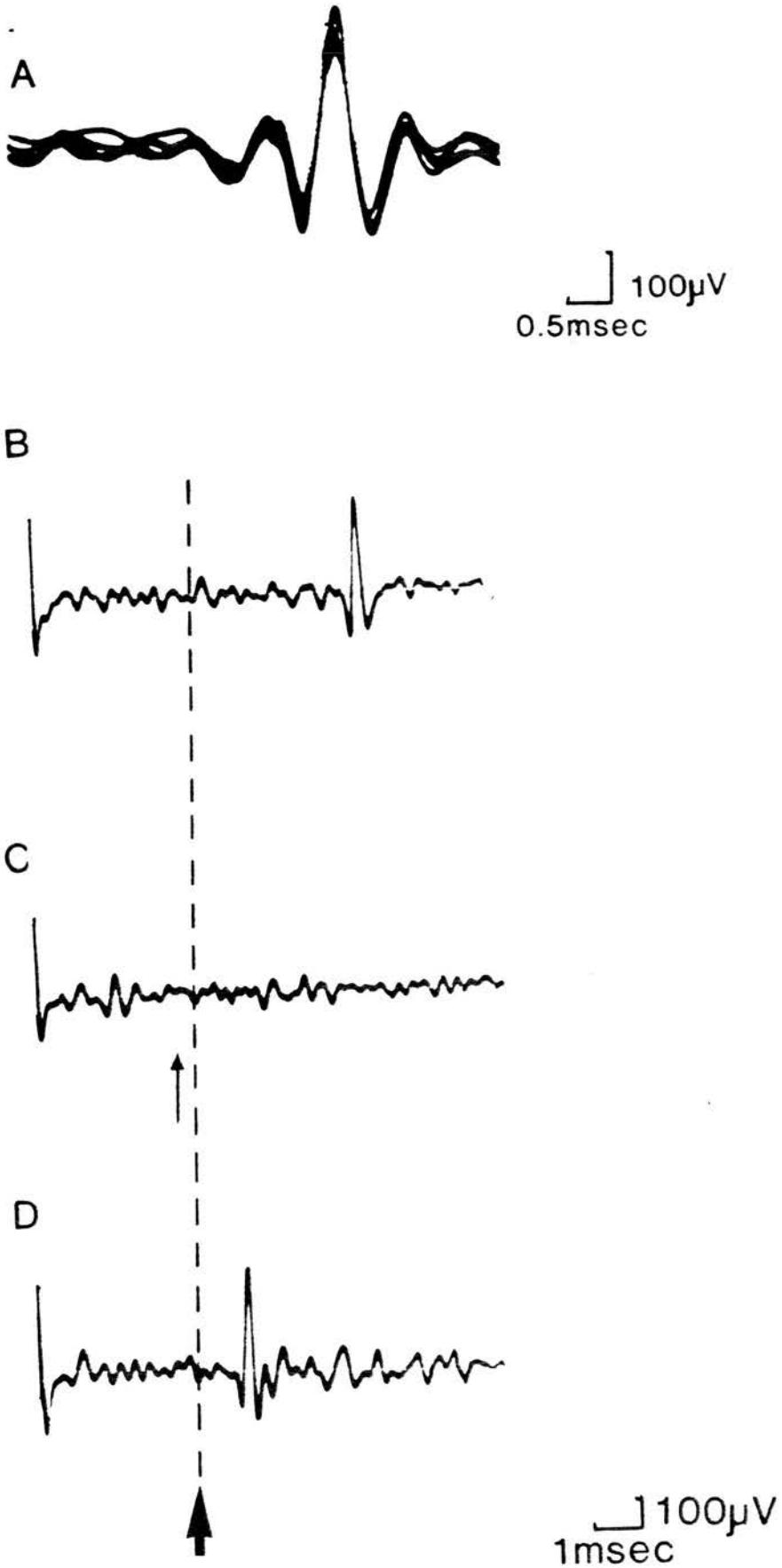

A
100μV
0.5msec
B
C
D
100μV
1msec

Figure 16

The recording sites of 19 dorsal horn neurones in the rat

Extracellular recording sites were marked with pontamine sky blue and are shown on a representative transeverse section. Filled circles represent the positions of STT neurones and open circles represent the positions of neurones unidentified in terms of possible ascending projections. From the small sample of neurones tested, STT neurones are apparently located generally ventral to unidentified dorsal horn neurones, in the deeper dorsal horn.

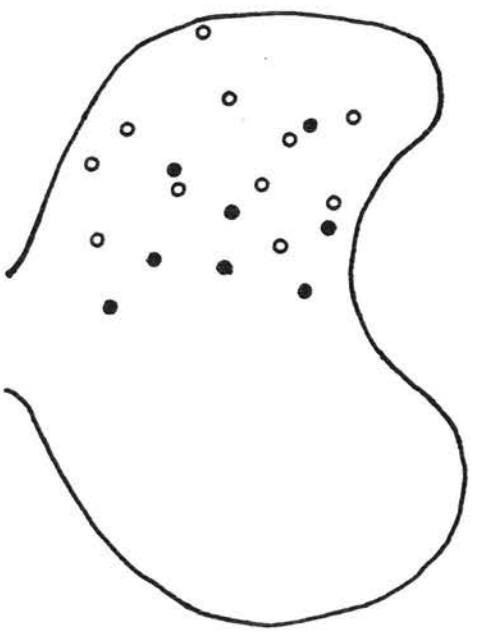


Figure 17

Selective antinociceptive effect of ionophoretically-applied NA on a multireceptive SCT neurone

Continuous records of firing rates of a neurone plotted as the number of action potentials per 2 seconds bins against time. Prominent excitatory responses to brush, DLH and noxious heat are shown on the top row. Typical effects of ionophoretically-applied NA are shown in the middle row, where NA selectively inhibited the response to noxious heat with no prominent change in the other responses. Effects were maximal after about 1 minute of inophoresis. The nociceptive response recovered to control levels 30 minutes after cessation of NA ionophoresis.

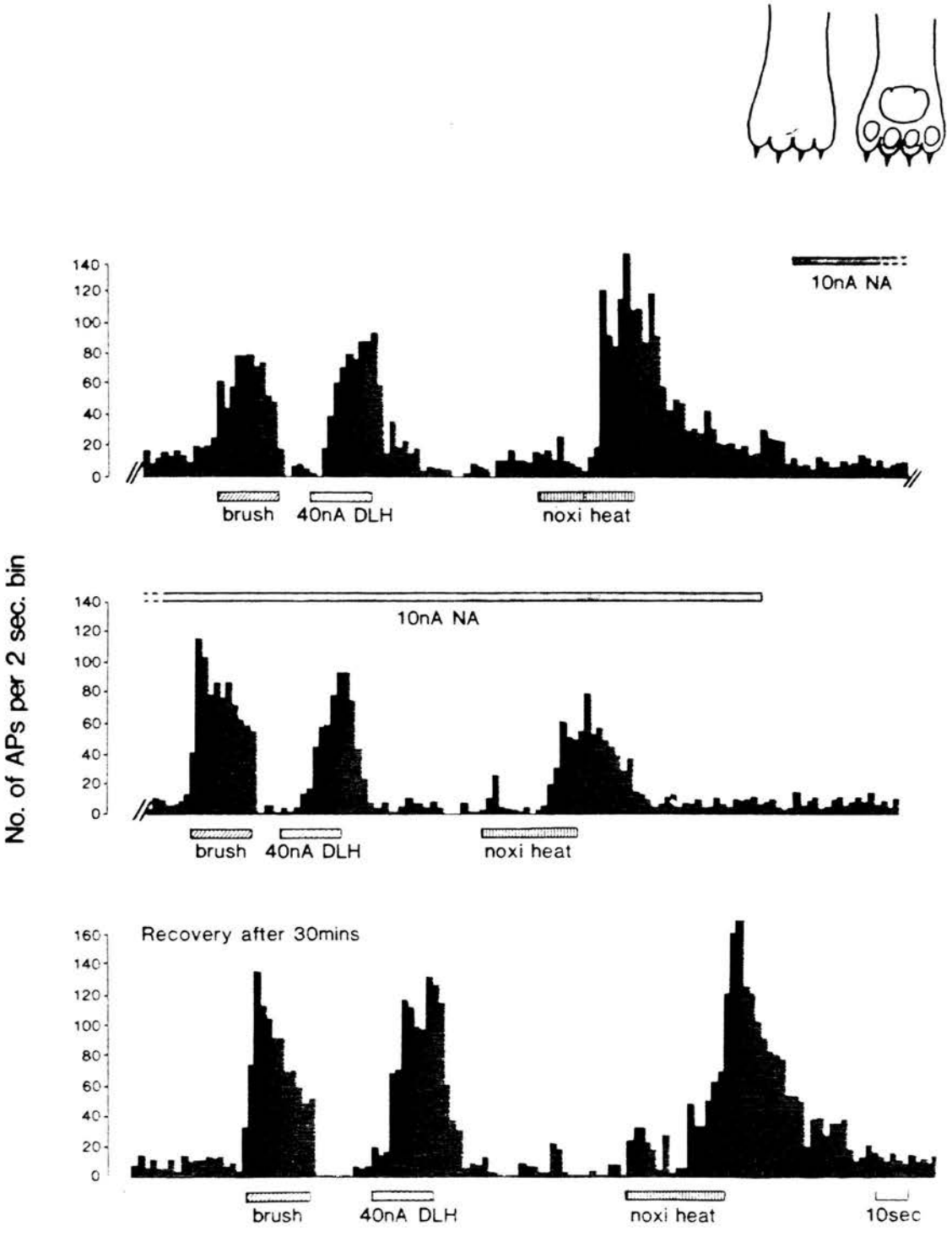

No. of APs per 2 sec. bin
140
120
100
80
60
40
20
0
10nA NA
brush
40nA DLH
noxi heat
10nA NA
brush
40nA DLH
noxi heat
160
140
120
100
80
60
40
20
0
Recovery after 30mins
brush
40nA DLH
noxi heat
10sec

Figure 18

Effects of ionophoretically-applied NA and α_2-, α_1- and β- agonists, on a multireceptive SCT neurone

(A) NA selectively inhibits the response to noxious radiant heat, whilst the responses to brush and DLH are unaffected.

(B) The α_2-agonist, clonidine, mimiced the selective antinociceptive action of NA.

(C) The α_1-agonist, phenylephrine, caused a non-selective depression of all responses.

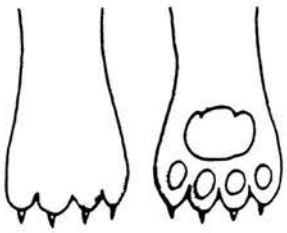
(D) The β-agonist, isoprenaline, had little effect on any of the responses tested.

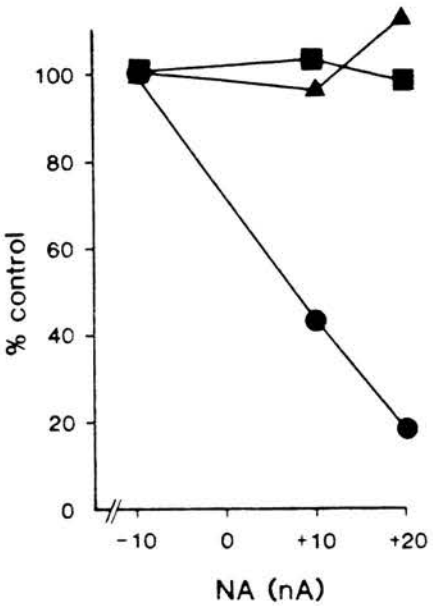
Key:

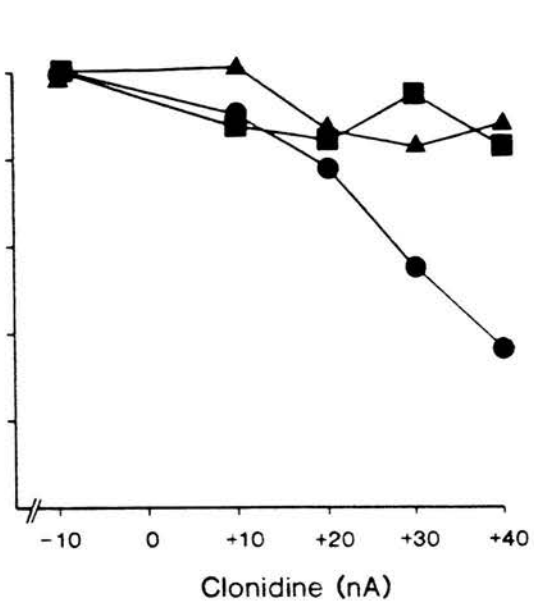
● Noxious radiant heat

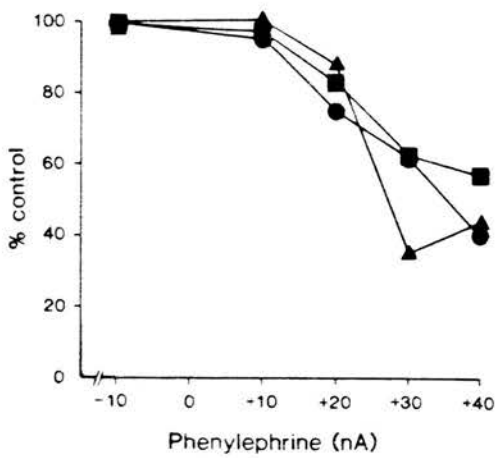
■ Ionophoretically-applied DLH

▲ Innocuous brush




100
80
60
40
20
0
% control
-10
0
+10
+20
NA (nA)


-10
0
+10
+20
+30
+40
Clonidine (nA)


100
80
60
40
20
0
% control
-10
0
+10
+20
+30
+40
Phenylephrine (nA)

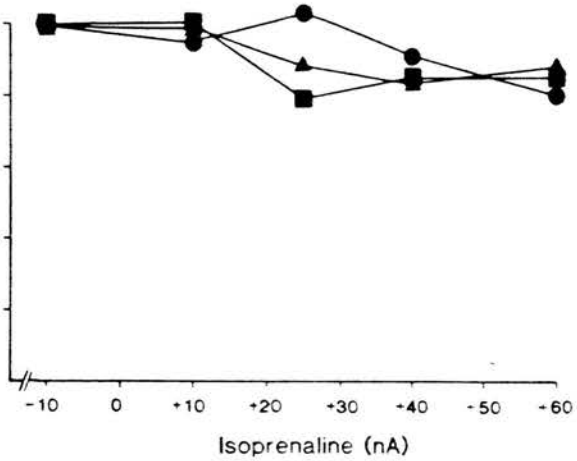

-10
0
+10
+20
+30
+40
-50
-60
Isoprenaline (nA)

Table 3

Gives an indication of the degree of selectivity of ionophoretically-applied NA on the responses of a sample of 16 multireceptive SCT cells tested in the cat

Ionophoretic current of NA for 50% inhibition of the response	Percentage of controls at current giving 50% inhibition of heat response		
Heat	Brush	DLH	Spontaneous activity
$14.6^{+}3.5$nA (0-39)	$101.5^{+}6.1$	$93.3^{+}5.6$	$89.3^{+}8.6$

At the currents (nA) of NA, estimated to give 50% reduction in the noxious heat response, there was no significant change from control levels in any other type of activity (students matched paired t-test on raw data, or Sign test R normalized data). The mean and standard error values are shown.

Figure 19

Effects of ionophoretically-applied l-NA and d-NA, on a multireceptive SCT neurone

(A) The receptor active stereoisomer l-NA caused a selective antinociceptive effect.

(B) d-NA did not effect the evoked responses tested, with five-fold higher ejection currents than those used for l-NA.

Key:

● Noxious radiant heat

■ Ionophoretically-applied DLH

▲ Innocuous brush

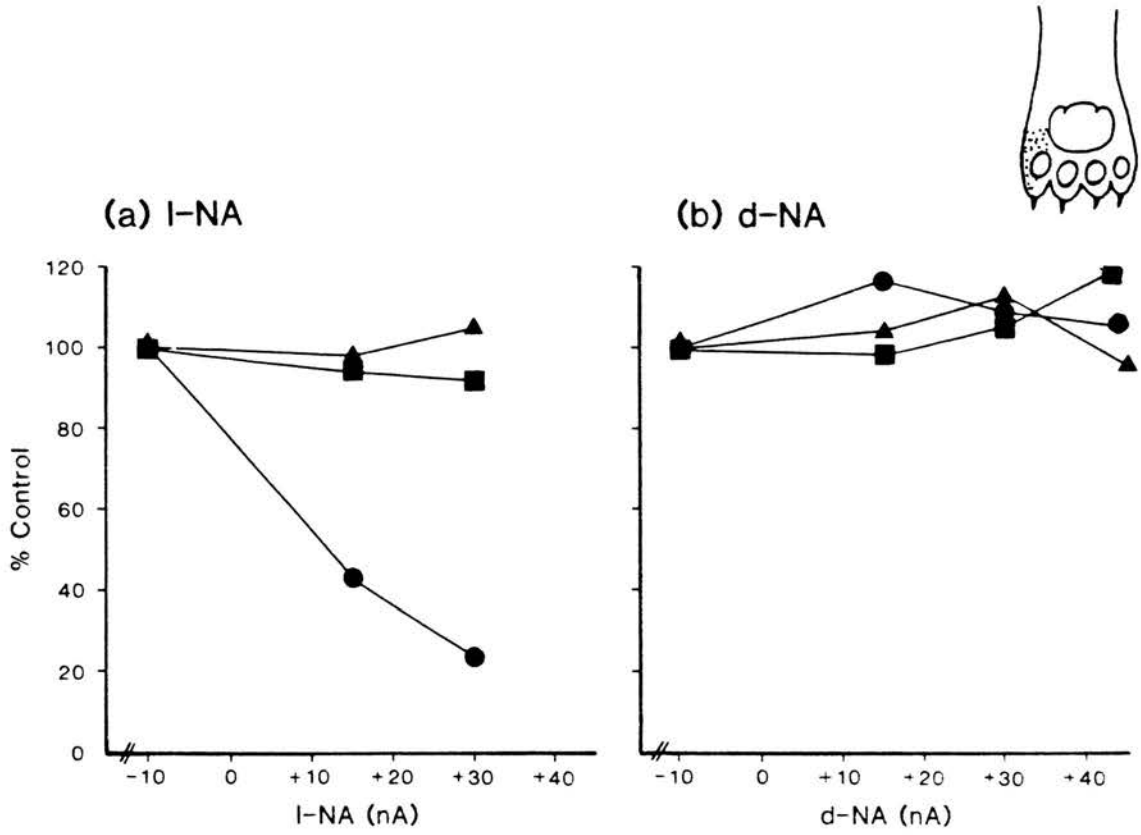

(a) l-NA
(b) d-NA
% Control
120
100
80
60
40
20
0
-10
0
+10
+20
+30
+40
l-NA (nA)
d-NA (nA)

Figure 20

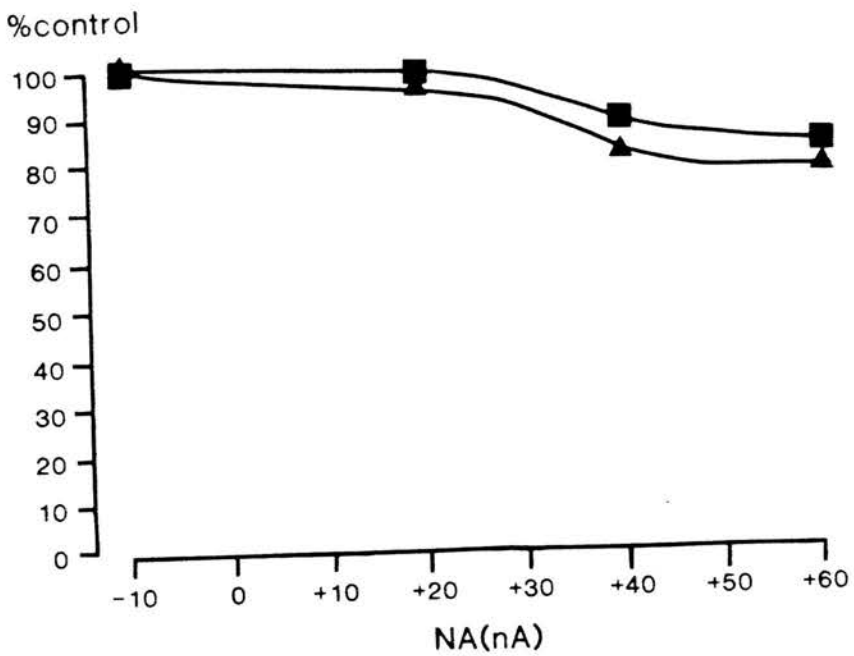
Effect of ionophoretically-applied NA on an SCT neurone which responded only to innocuous cutaneous stimulation

NA, at currents up to 40-50nA, typically had no marked effect (i.e. 20% change) on the evoked response of these cells.

Key:

● Innocuous brush

■ Ionophoretically-applied DLH


%control
100
90
80
70
60
50
40
30
20
10
0
-10
0
+10
+20
+30
+40
+50
+60
NA(nA)

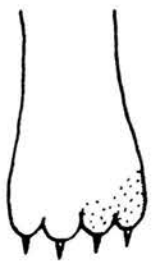


Figure 21

The selective antinociceptive effect of ionophoretically-applied clonidine on two multireceptive dorsal horn neurones in the cat (A) and rat (B)

(A) Ionophoretically-applied clonidine (0.1M) selectively inhibited the response of a SCT neurone to noxious radiant heat to less than 50% of control values with 30 nA of ejection current. The responses to brush and DLH were unaffected by continuous ionophoresis of clonidine.

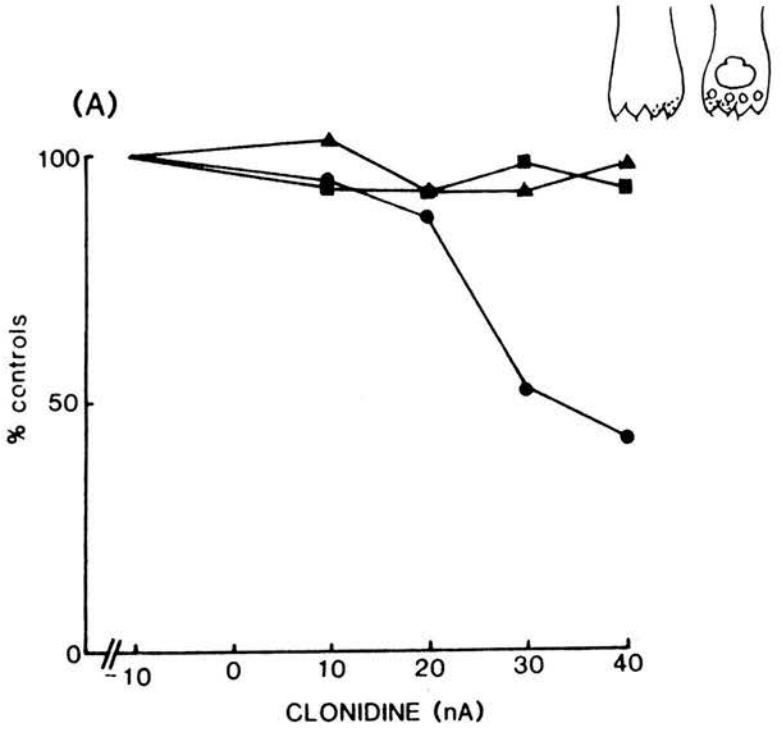
(B) Ionophoretically-applied clonidine (0.01M) selectively inhibited the response of a multireceptive dorsal horn neurone in the rat to noxious pinch, to less than 20% of control values with I5 nA of ejection current, whilst the responses to brush and DLH were unaffected. After the third test (at 12 minutes), ionophoresis of clonidine was ceased and in the following test (at 15 minutes), it was seen that the response to noxious pinch had recovered to 80% of control values, whilst the responses to brush and DLH were still not significantly different from control values.

A comparison of these diagrams with Figure 17 shows that the α_2-agonist clonidine mimicked the action of ionophoretically-applied NA.

Key: ● (A) Noxious radiant heat (B) Noxious pinch

■ Ionophoretically-applied DLH

▲ Innocuous brush


(A)
100
50
0
% controls
−10
0
10
20
30
40
CLONIDINE (nA)

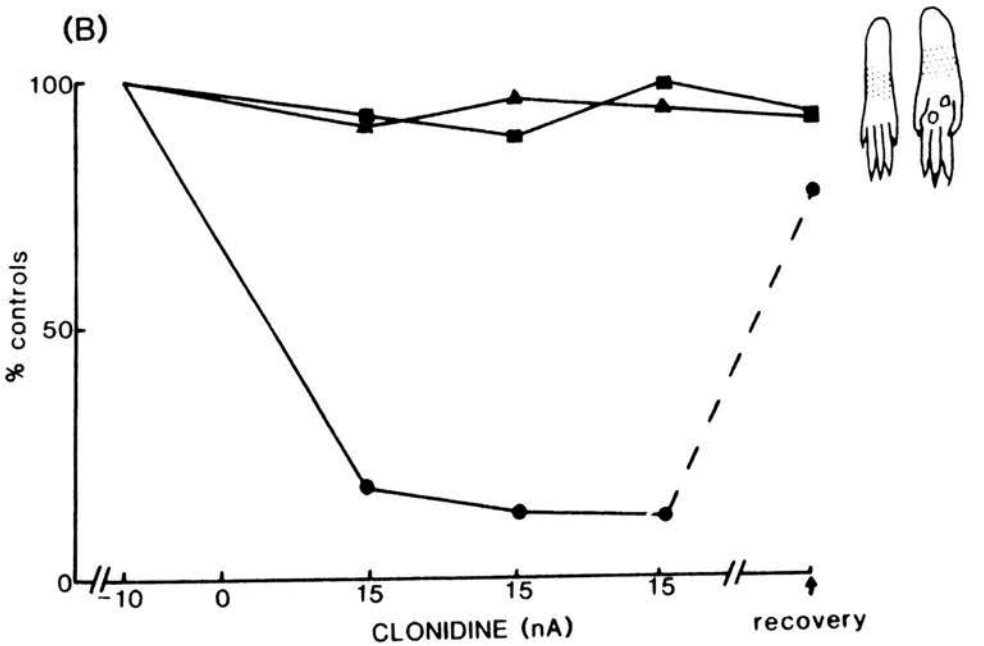

(B)
100
50
0
% controls
−10
0
15
15
15
recovery
CLONIDINE (nA)

Figure 22

Antagonism of the selective NA effect by an α_2-specific antagonist idazoxan, on multireceptive SCT neurones

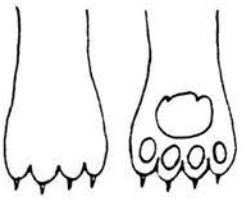
(a) The marked inhibition of the response to noxious heat of a multireceptive SCT neurone by NA (20 nA) was reversed by the acute ionophoretic-application of the α_2-antagonist idazoxan (RX).

(b) In four tests NA (20 nA) was found to selectively inhibit the nociceptive response to 10% of control values. However, in the presence of idazoxan, chronically ionophoresed 5-10 minutes before the subsequent NA test, the potency of NA was greatly reduced (40 nA required to inhibit the nociceptive response to 25% of control levels).

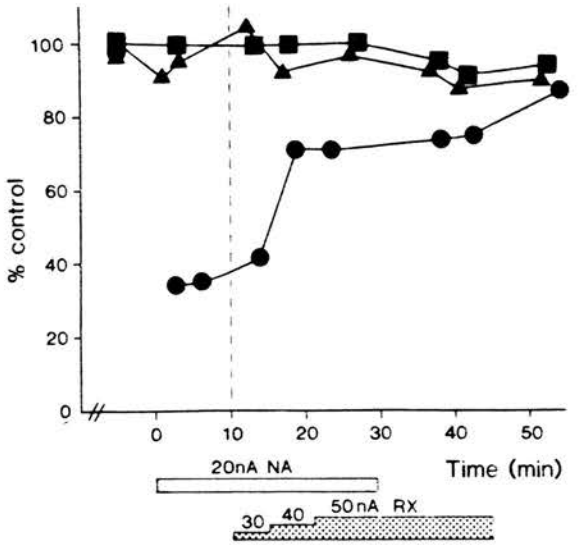
Key: ● Noxious radiant heat

■ Ionophoretically-applied DLH

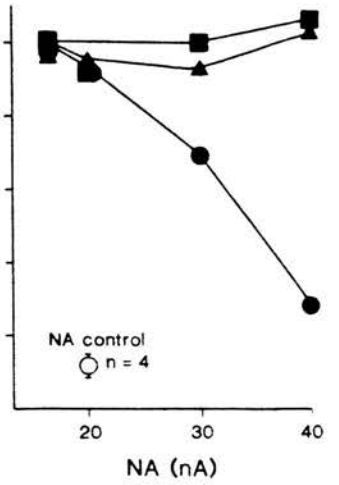
▲ Innocuous brush



(a) Acute RX



(b) Chronic RX (15 mins application prior to NA)



C 29/83 n47

Figure 23

Selective antinociceptive effect of ionophoretically-applied DA, on a multireceptive SCT neurone

Continuous records of firing rate of a neurone plotted as the number of action potentials per 400 msec bins against time. Prominent excitatory responses to brush, DLH and noxious heat can be seen on the top row. Typical effects of ionophoretically applied DA can be seen on the lower row, where DA selectively inhibited the response to noxious heat, to less than 40% of controls values. The response to brush and DLH were still at least 85% of control values during the DA test, after 15 minutes of continual ionophoresis of 40 nA DA. 12 minutes after the DA ionophoresis had ceased, the response to noxious heat had recovered to control levels (brush and DLH responses were unchanged, not shown).

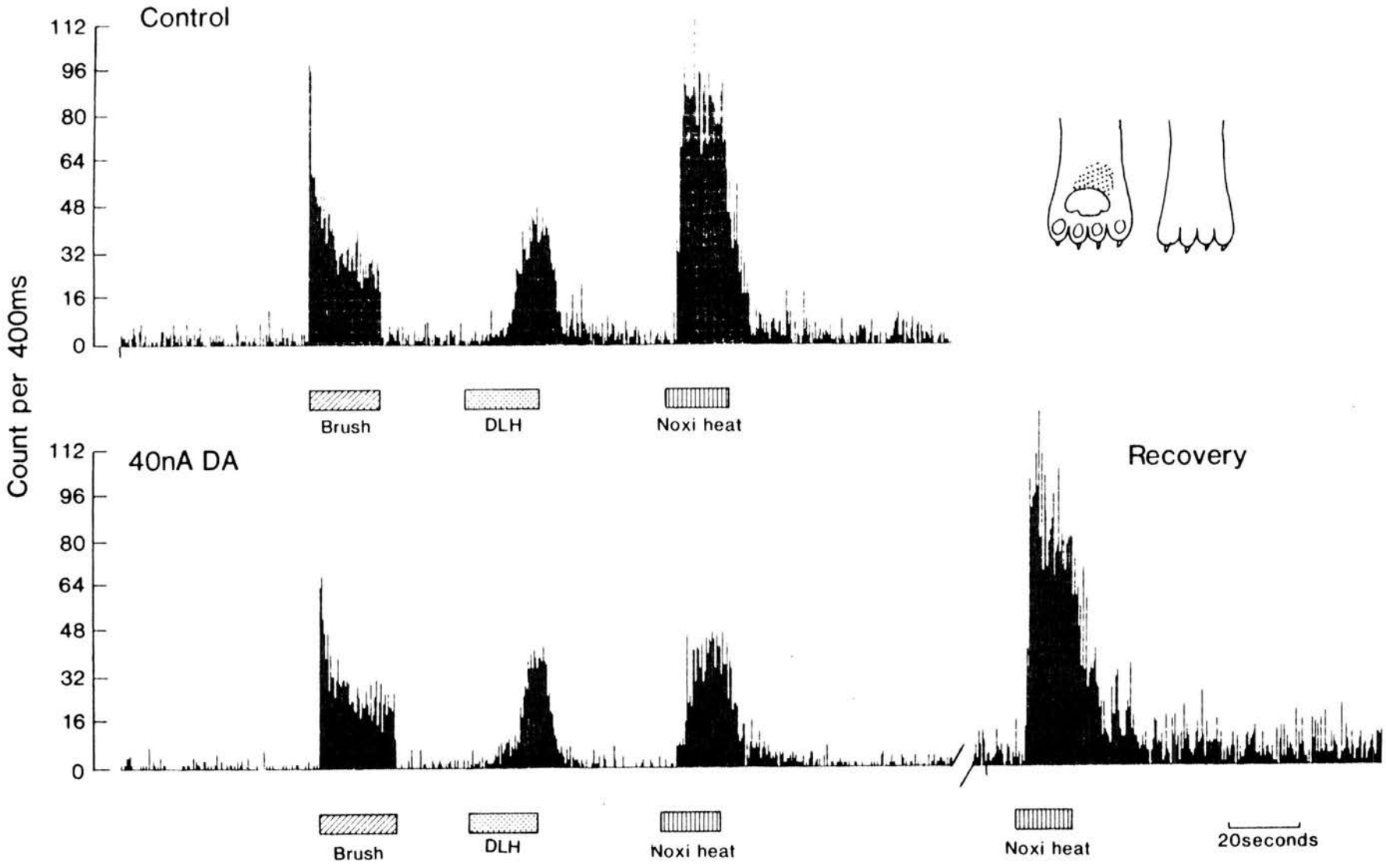

Control
112
96
80
64
48
32
16
0
Count per 400ms
Brush
DLH
Noxi heat
40nA DA
Recovery
Noxi heat
20seconds

Figure 24

Selective antinociceptive effects of ionophoretically-applied DA, on multireceptive dorsal horn neurones

The multireceptive neurones tested were unidentified (A and B) and STT neurones (C) in the rat and a SCT neurone (D) in the cat. In each case, DA selectively and potently inhibited the nociceptive responses, with low ejection currents (10-20 nA), whilst the responses to brush and DLH were unaffected.

Key: ● Noxious pinch (A, B and C) and noxious heat (D)

■ Ionophoretically - applied DLH

▲ Innocuous brush

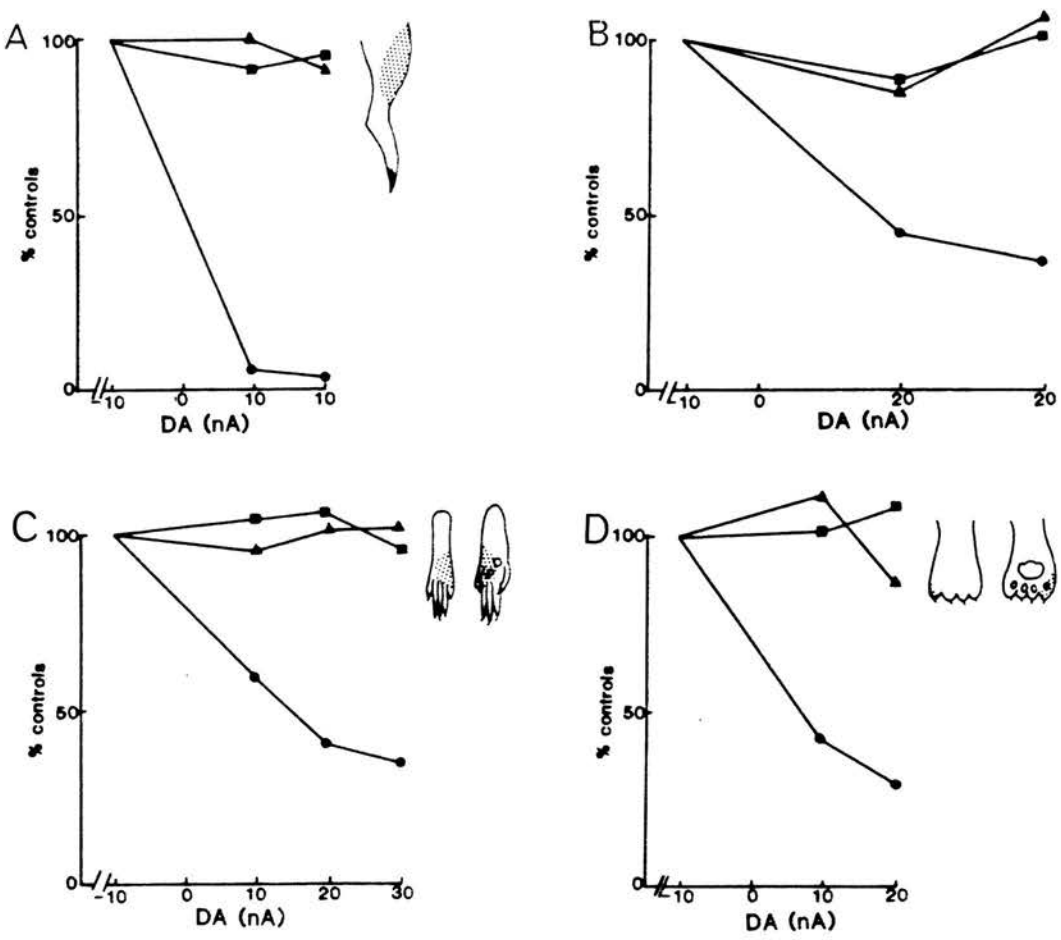

A
100
50
0
% controls
-10
0
10
10
DA (nA)
B
100
50
0
% controls
-10
0
20
20
DA (nA)
C
100
50
0
% controls
-10
0
10
20
30
DA (nA)
D
100
50
0
% controls
-10
0
10
20
DA (nA)

Table 4

Gives an indication of the degree of selectivity of the action of ionophoretically-applied DA on multireceptive dorsal horn neurones in the cat and rat

Types of dorsal horn neurones tested	Ionophoretic current of DA for 50% inhibition of the nociceptive response	Percentage of controls at current giving 50% inhibition of nociceptive response		
		Brush	DLH	Spontaneous activity
SCT (Cat) n=15	22.8 ± 3.5 (0-49)	88.8 ± 2.3	101.0 ± 2.3	96.4 ± 4.5
STT (Rat) n=8	24.4 ± 4.1 (0-49)	93.0 ± 1.6	98.1 ± 3.4	104.6 ± 5.2
Unidentified in terms of possible ascending projection (Rat) n=19	21.2 ± 4.9 (0-49)	92.7 ± 2.7	95.8 ± 3.0	98.2 ± 2.2

At currents (nA)of DA estimated to give 50% reduction of the nociceptive responses, there was no siginificant change from control levels in any other type of activity (Students matched paired t-test on raw data, or Sign test R on normalized data). The mean and standard error values are shown.

Figure 25

Summary graph showing the effect of ionophoretically-applied DA

The graph shows the mean values ($\pm$ SEM) of the responses to brush, DLH and pinch, of 12 multireceptive dorsal horn neurones in the rat. Each neurone was tested using the same currents of ionophoretically-applied DA (i.e. 5, 10, 15, 20 nA) over 4 minute cycles (Figure 10B). On the far right the consistent reversal of the action of DA, by the D_2 DA-antagonist, sulpiride (70-85 nA) is shown.

Key:

- ● Noxious pinch
- ■ Ionophoretically-applied DLH
- ▲ Innocuous brush

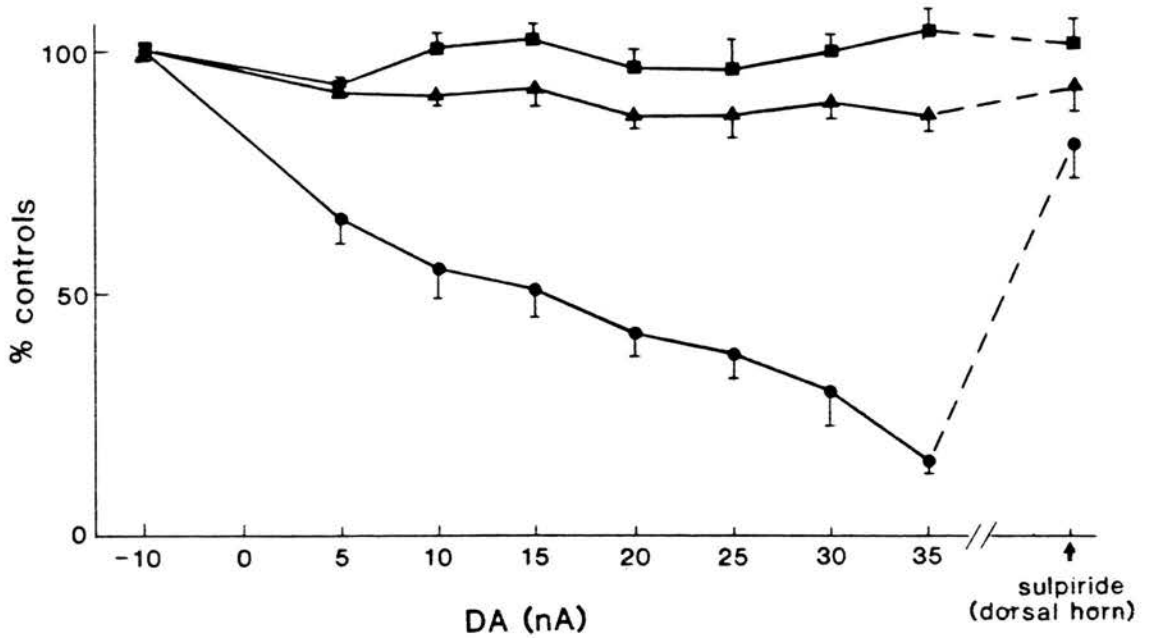

100
50
0
% controls
-10
0
5
10
15
20
25
30
35
DA (nA)
sulpiride
(dorsal horn)

Figure 26

Effects of ionophoretically-applied DA on a non-nociceptive and a nociceptive specific dorsal horn neurone in the rat

(A) DA (30 nA) inhibited the nociceptive response of a nociceptive specific STT neurone, whilst DLH-evoked and spontaneous activity (not shown) were unaffected. The inhibition of the nociceptive response was reversed in the presence of ionophoretically-applied sulpiride, a D_2 DA-antagonist.

(B) DA (up to 80 nA) did not alter the brush, DLH-evoked (or spontaneous) activity of a non-nociceptive neurone.

Key:

- ● Noxious pinch
- ■ Ionophoretically-applied DLH
- ▲ Innocuous brush

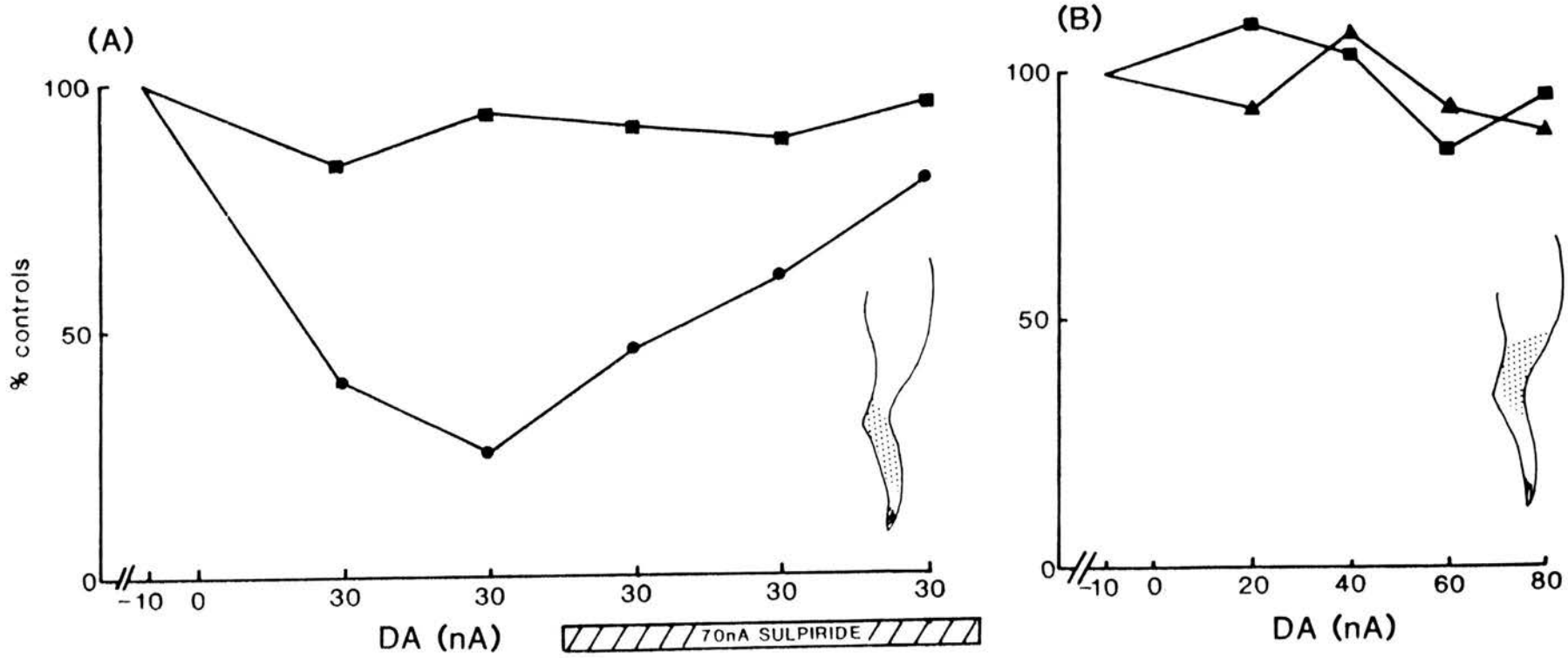

(A)
100
50
0
% controls
−10
0
30
30
30
30
30
DA (nA)
70nA SULPIRIDE
(B)
100
50
0
−10
0
20
40
60
80
DA (nA)

Figure 27

Effects of ionophoretically-applied D_1 and D_2DA-receptor agonists, on a multireceptive dorsal horn neurone in the rat

(A) The selective D_2 DA-agonist, RU24213, potently mimicked the selective antinociceptive action of DA (using the protocol shown in Figure 10C).

(B) The selective DA D_1-agonist, SKF 38393 (up to 90nA, using the protocol shown in Figure 10B) did not affect the evoked responses tested.

Key:

● Noxious pinch

■ Ionophoretically-applied DLH

▲ Innocuous brush

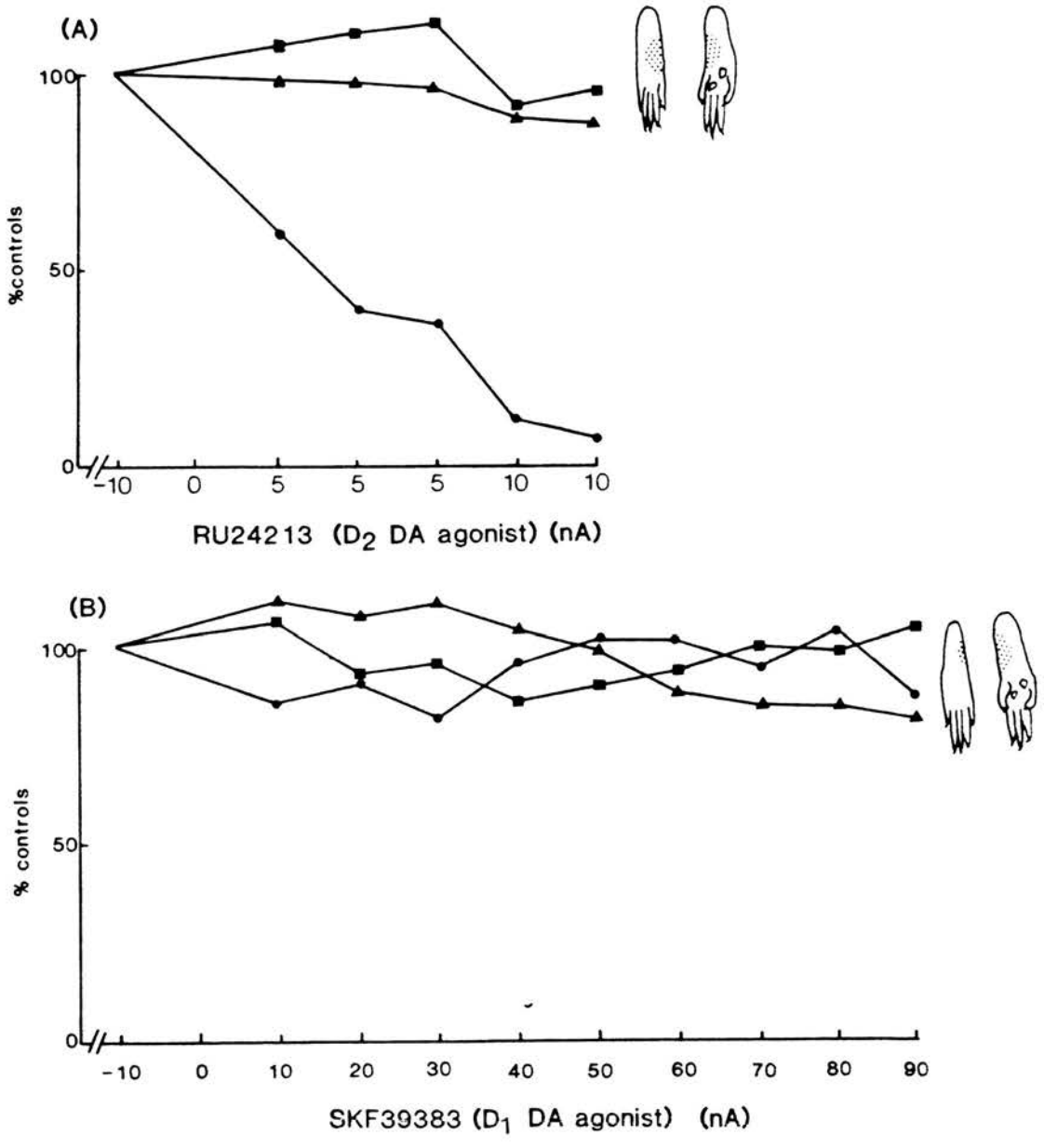

(A)
100
50
0
%controls
-10
0
5
5
5
10
10
RU24213 (D2 DA agonist) (nA)
(B)
100
50
0
% controls
-10
0
10
20
30
40
50
60
70
80
90
SKF39383 (D1 DA agonist) (nA)

Figure 28

Selective antinociceptive effect of ionophoretically-applied DA and antagonism with D_2 DA-receptor antagonist sulpiride

Continuous records of firing rates of a neurone plotted as the number of action potentials per 400 msec bins against time. Prominent excitatory responses to brush, DLH and noxious pinch are shown on the top row. Typical selective antinociceptive effects of ionophoretically-applied DA (40nA) are shown in the middle row. The marked inhibition of the nociceptive response was reversed by the acute ionophoretic-application of the D_2 DA-antogonist, sulpiride, is shown on the bottom row.

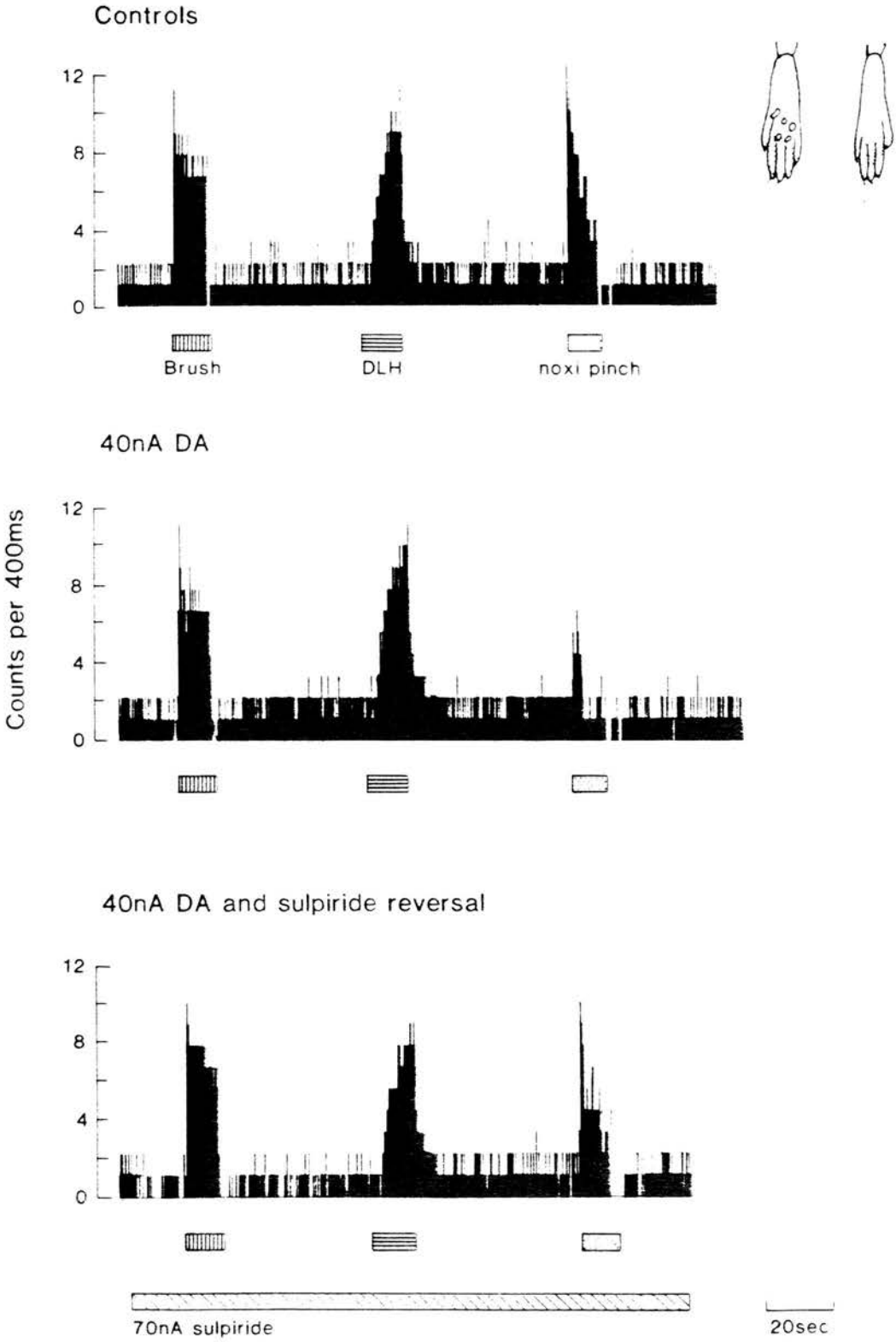

Controls
12
8
4
0
Brush
DLH
noxi pinch
40nA DA
Counts per 400ms
12
8
4
0
40nA DA and sulpiride reversal
12
8
4
0
70nA sulpiride
20sec

Figure 29

Antagonism of the selective effect of DA by D_2 DA-receptor antagonist sulpiride

(A) On the first test (a) 20 nA DA inhibited the nociceptive response to less than 50% of the control values. Complete recovery occurred 20 minutes after the cessation of the test.

The presence of sulpiride, however, ionophoresed at 70 nA for 20 minutes before and during the second DA test, completely antagonised the action of DA (b).

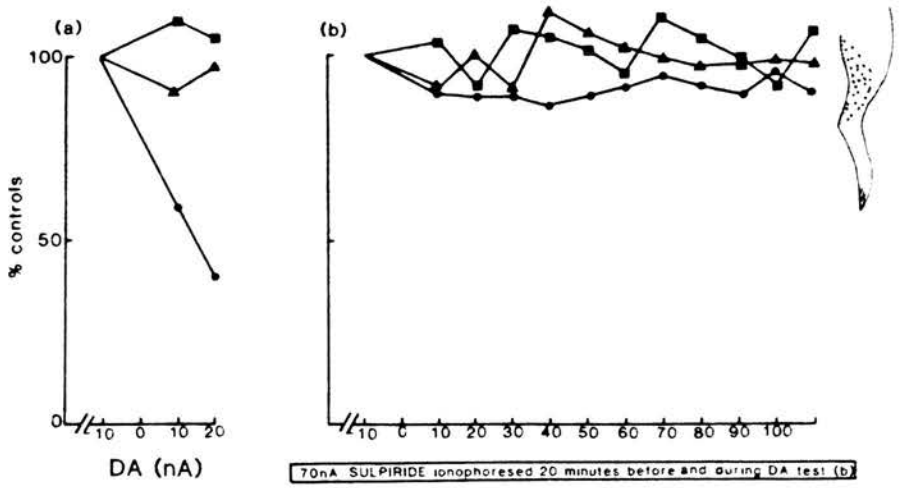
(B) and (C) Sulpiride, ionophoresed during a DA test, reversed the selective antinociceptive effect of DA and the nociceptive response recovered to within 20% of control value.

In all 3 examples shown, the multireceptive neurones tested, in the rat, was identified as the STT neurone.

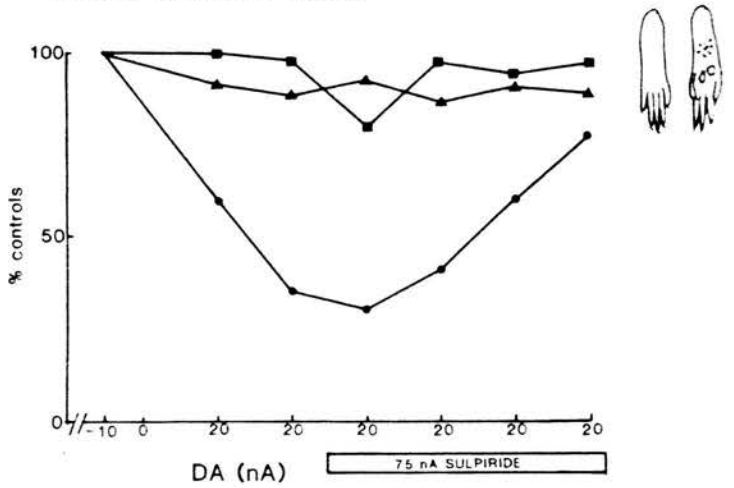
Key:

- ● Noxious pinch
- ■ Ionophoretically-applied DLH
- ▲ Innocuous brush

(A)Chronic application of sulpiride



(B)Acute application of sulpiride



(C)Acute application of sulpiride

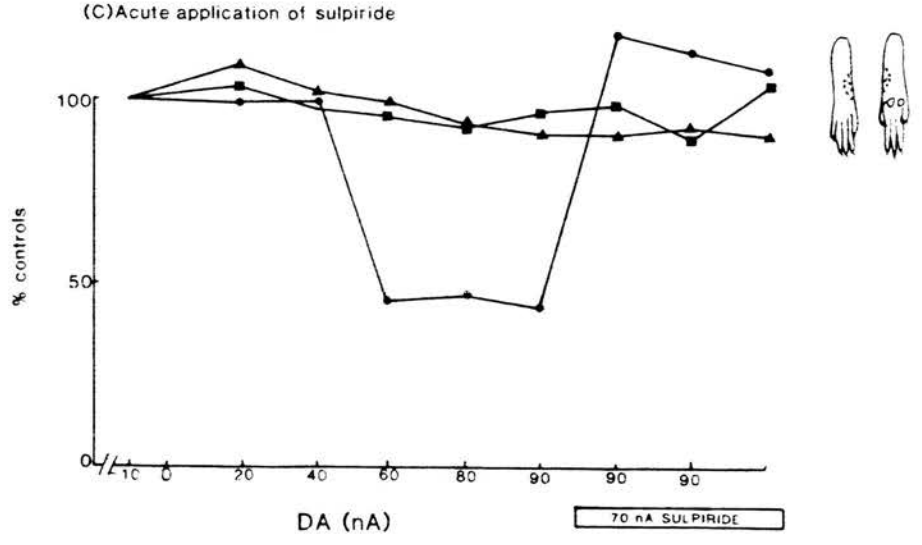


Figure 30

Selective antinociceptive effect of focal electrical stimulation in the region of the A11 DA cell group

Continuous records of firing rates of a neurone plotted as the number of action potentials per 400ms bins against time. On the top row, prominent excitatory responses to brush, DLH and pinch stimulation, of a multireceptive dorsal horn neurone are shown. Focal electrical stimulation in the region of the A11 DA cell group markedly and selectively inhibited the response to pinch, whilst the responses to brush, DLH-evoked and spontaneous activity were unaffected, as shown in the centre row. This effect was reversed when the DA antagonist sulpiride was ionophoresed in the vicinity of the dorsal horn neurone tested (bottom row). This effect was typical of 17 separate neurones, tested in the rat.

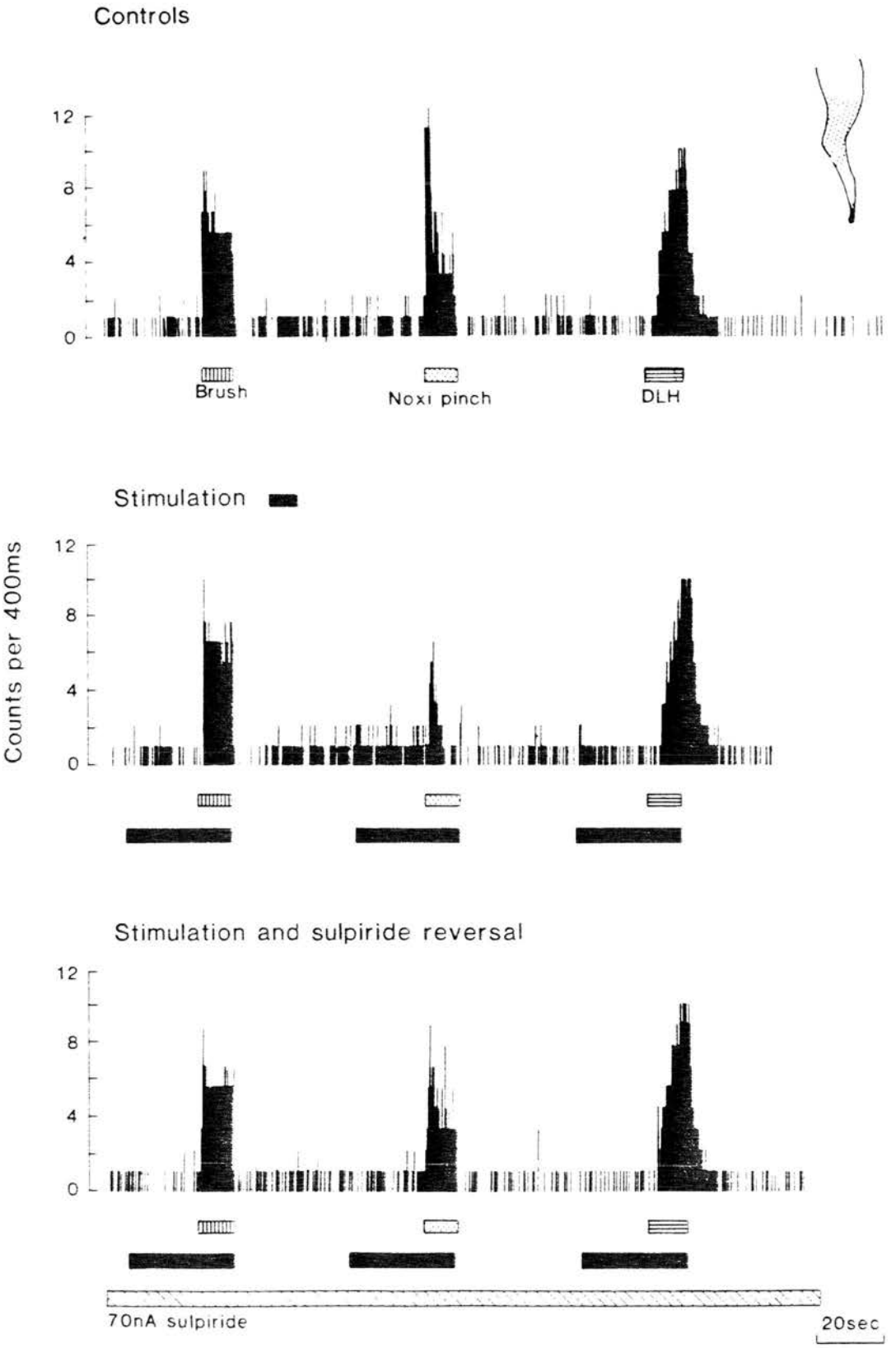

Controls
12
8
4
0
Brush
Noxi pinch
DLH
Stimulation
Counts per 400ms
12
8
4
0
Stimulation and sulpiride reversal
12
8
4
0
70nA sulpiride
20sec

Figure 31

Focal electrical stimulation in the region of the A11 DA cell group, using different stimulation parameters

(A) and (B) show the effect of electrical stimulation on the same neurone at different frequencies of stimulation, (A) at 100Hz and (B) at 33Hz. Using the same levels of stimulation current (10-50 μA) it may be seen that the response to noxious pinch is slightly less potently inhibited at low frequencies of stimulation (B). (C) and (D) show the effect of electrical stimulation, on different dorsal horn neurones, at 100Hz and 10Hz respectively.

Although higher stimulation currents were required when lower stimulation frequencies were used, in all cases a selective antinociceptive effect was evoked from the region of the A11 DA cell group. The inhibitions of the response to noxious pinch were consistently reversed by ionophoretically-applied sulpiride, as shown in (A), (C) and (D).

Key:

- ● Noxious pinch
- ■ Ionophoretically-applied DLH
- ▲ Innocuous brush

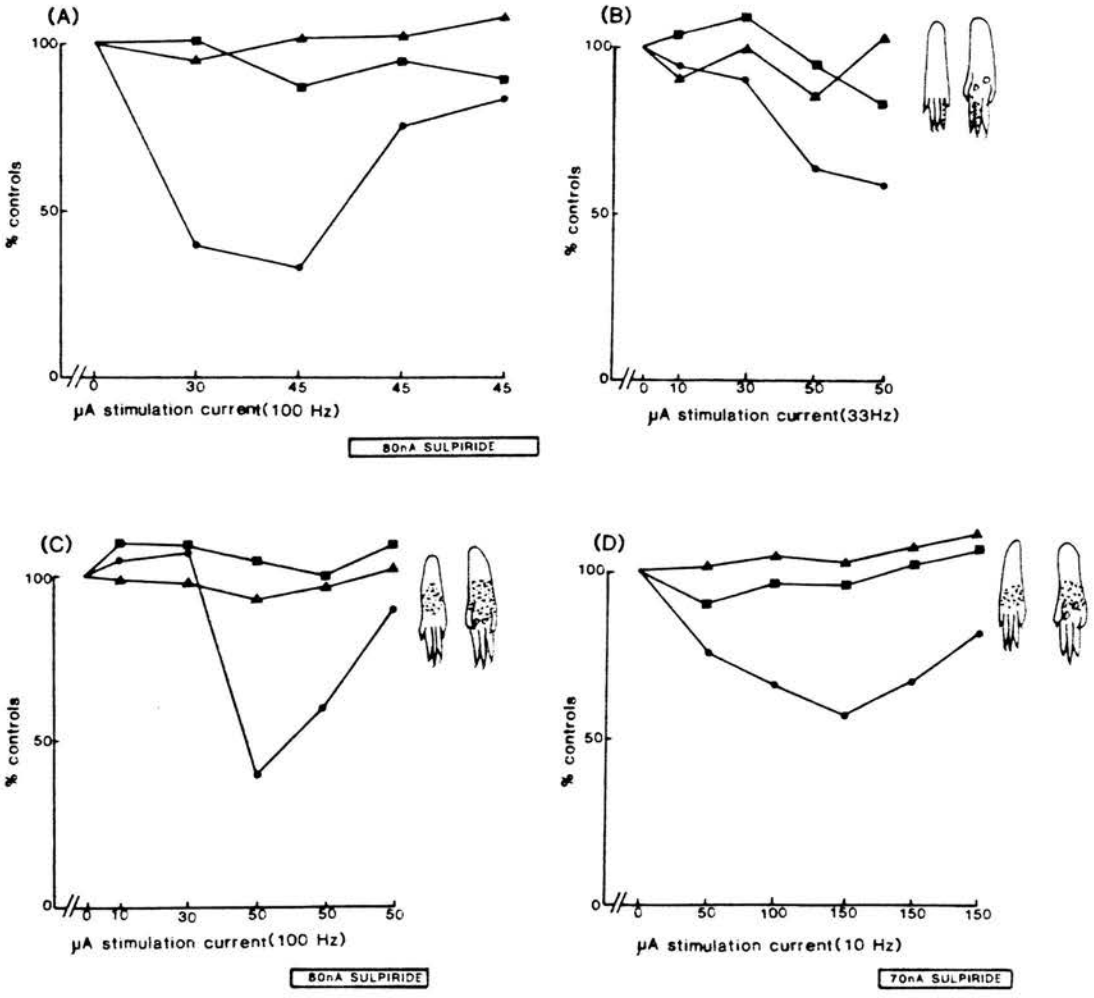

(A)
100
50
0
% controls
0
30
45
45
45
μA stimulation current(100 Hz)
80nA SULPIRIDE
(B)
100
50
0
% controls
0
10
30
50
50
μA stimulation current(33Hz)
(C)
100
50
0
% controls
0
10
30
50
50
50
μA stimulation current(100 Hz)
80nA SULPIRIDE
(D)
100
50
0
% controls
0
50
100
150
150
150
μA stimulation current(10 Hz)
70nA SULPIRIDE

Figure 32

Summary graph showing the effect of focal electrical stimulation, in the region of the A11 DA cell group

The graph shows the mean values (± SEM) of the responses to brush, DLH and pinch, of 17 multireceptive dorsal horn neurones, with 10, 20, 40, etc μA of stimulation current (100Hz). On the far right, the consistent reversal of the selective antinocicepetive action of electrical stimulation of the region of the A11 DA cell group, by ionophoretically-applied sulpiride (70-85 nA) is shown.

Key:

- ● Noxious pinch
- ■ Ionophoretically-applied DLH
- ▲ Innocuous brush

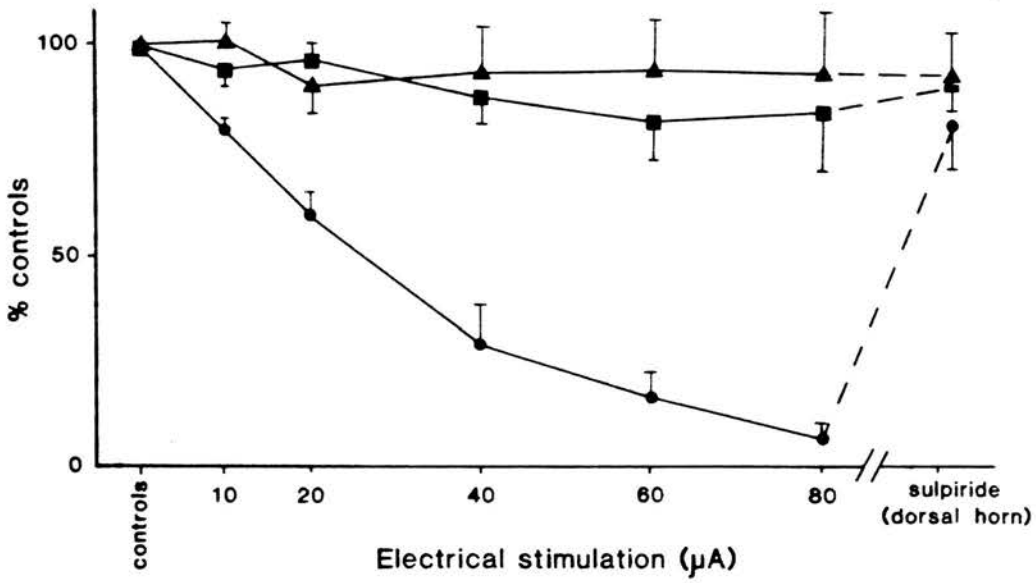

100
50
0
% controls
controls
10
20
40
60
80
sulpiride
(dorsal horn)
Electrical stimulation (μA)

Figure 33

Stimulation sites, in the region of the A11 DA cell group

Sites, marked by iron deposit, showing the stereotaxic placement of stimulating electrodes are drawn on representative transverse sections of the rat brain. Filled circles (●) represent sites from which a selective antinociceptive effect, on multireceptive dorsal horn neurones, was evoked by electrical stimulation. Open circles (○) represent sites from which no apparent effect was evoked by electrical stimulation. The sites from which a selective antinociceptive effect was evoked by electrical stimulation were found to be restricted to the region encompassing the cells of the A11 DA cell group (as previously described by Bjorklund and Nobin, 1973; Jacobowitz and Palkowitz, 1974 and in the present study), shown by hatching.

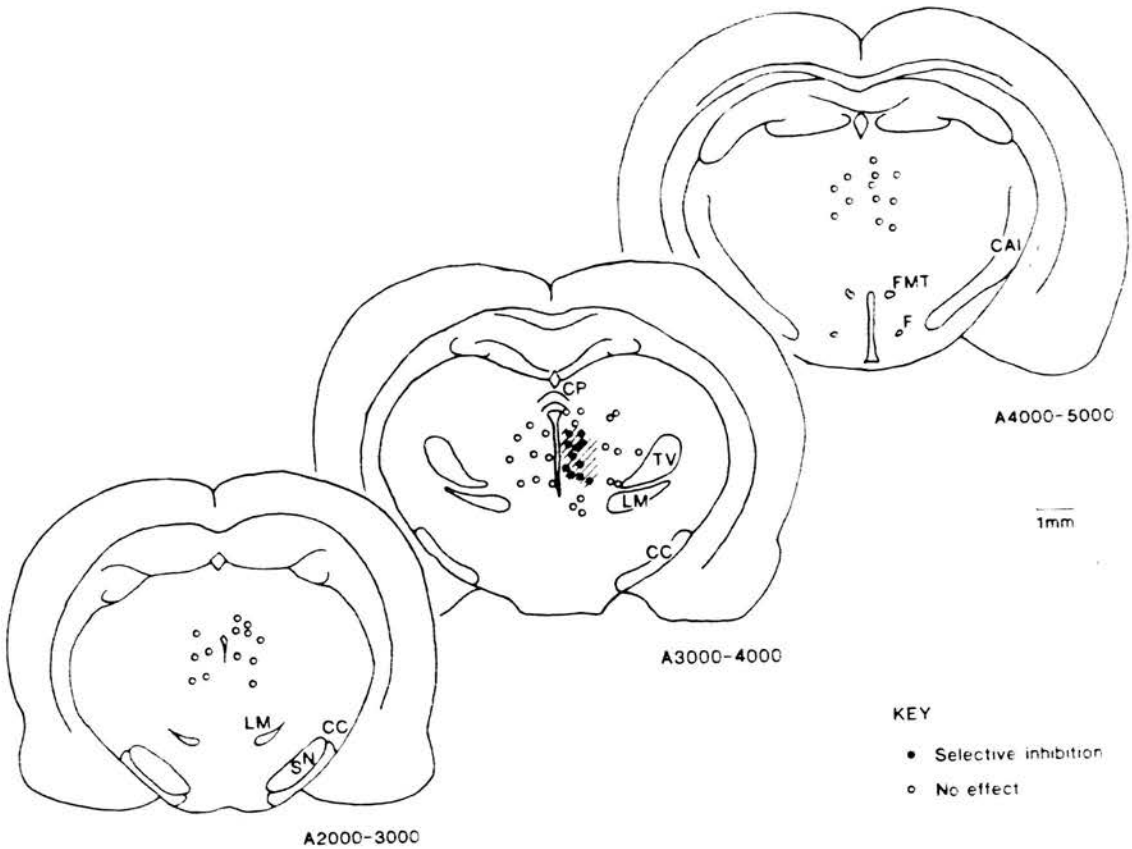

CAI
FMT
F
A4000-5000
CP
TV
LM
CC
1mm
A3000-4000
LM
CC
SN
A2000-3000
KEY
• Selective inhibition
○ No effect

Figure 34

Stimulation sites, in the region of the A9 DA cell group

Electrical stimulation in the region of the A9 DA cell group did not effect the evoked responses of 5 multireceptive dorsal horn neurones. Stimulation sites, marked by iron deposits, show the stereotaxic placement of the stimulating electrodes are drawn on representative transverse sections of the rat brain. The hatching shows the region of the A9 DA cell group (as previously described by Ungerstedt, 1971; Jacobwitz and Palkowitz, 1974 and in the present study).

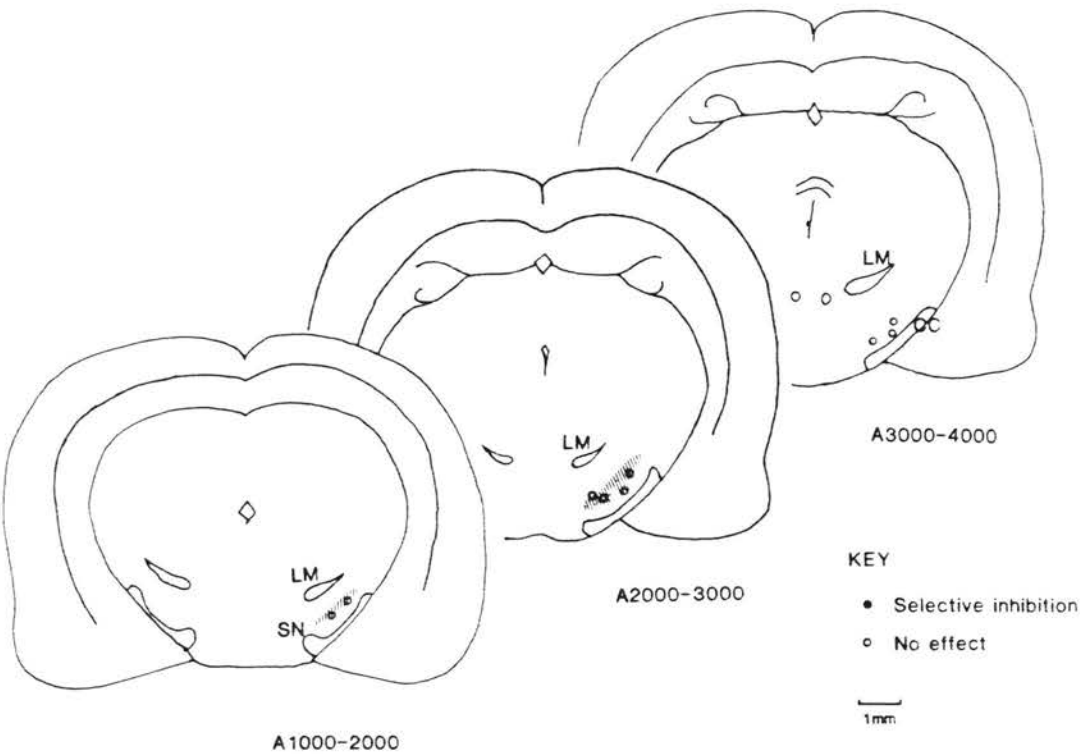

LM
OC
A3000-4000
LM
A2000-3000
LM
SN
A1000-2000
KEY
Selective inhibition
No effect
1mm

Figure 35

Effect of ionophoretically-applied 5-HT, on a multireceptive dorsal horn neurone in the rat

5-HT failed to reproduce the clear-cut selective antinociceptive action of either NA or DA. At higher currents ($\geqslant$ 30nA) the responses to brush, DLH- and pinch were non-selectively depressed.

Key:

- ● Noxious pinch
- ■ Ionophoretically-applied DLH
- ▲ Innocuous brush

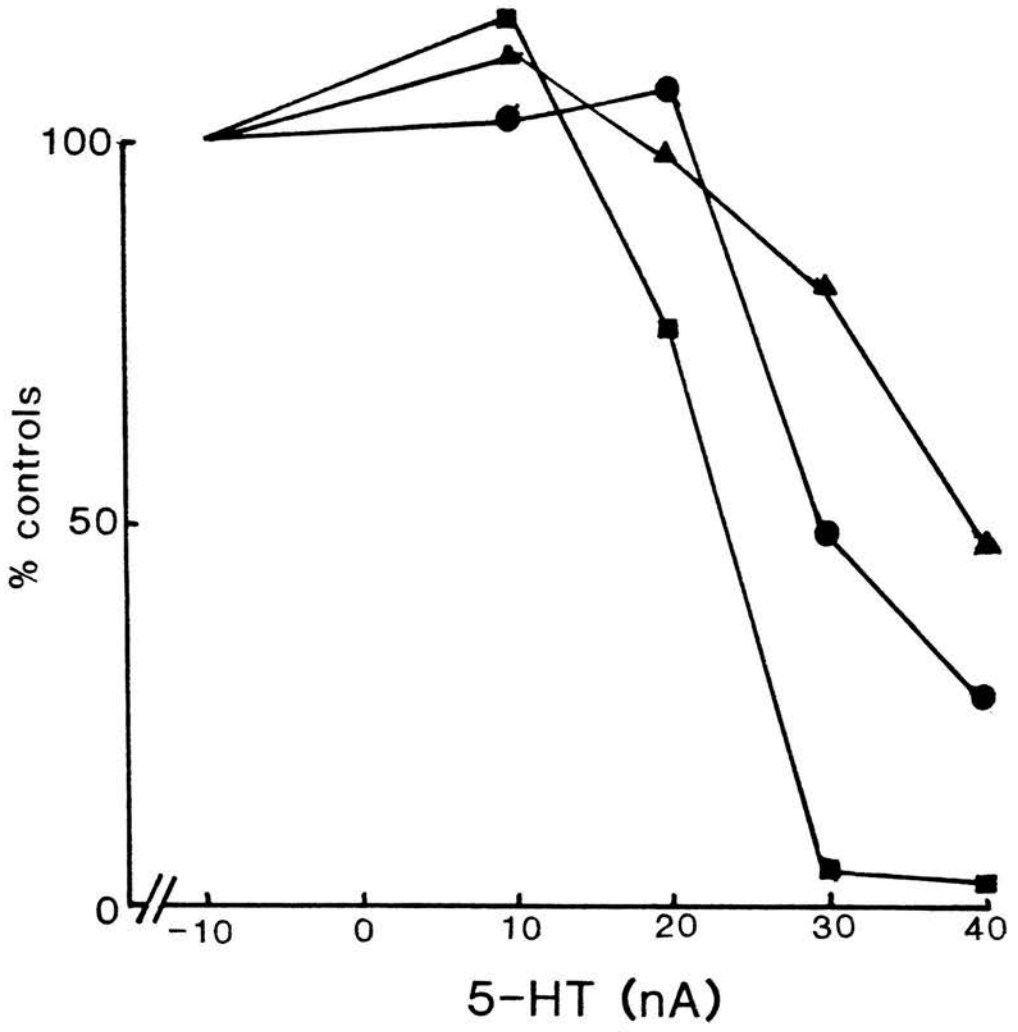

100
50
0
% controls
-10
0
10
20
30
40
5-HT (nA)

Figure 36

A1 and A2 cell groups

The positions of A1 and A2 cell groups are shown on a representative transverse section of the brain of the rat. CA-histofluorescent cells mapped in these areas are regarded primarily as noradrenergic (e.g. Westlund, et al. 1983).

Abbreviations:

FC - Fasciculus cuneatus

LRN - Lateral reticular nucleus

NTST - Nucleus tractus solitarii

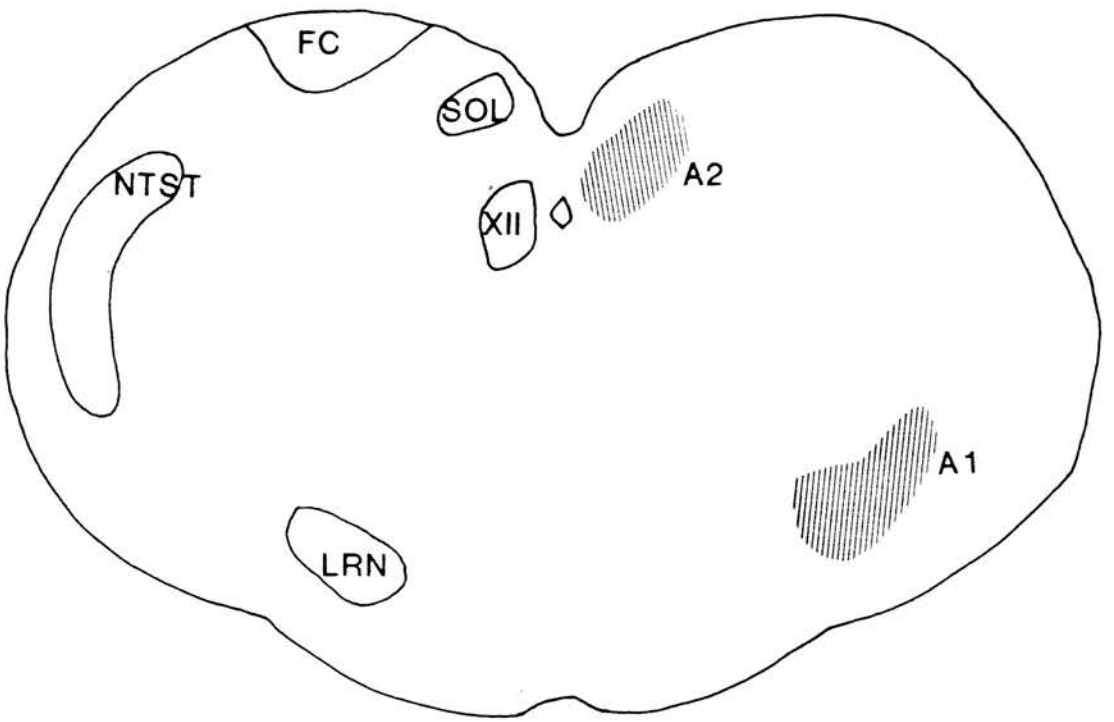

FC
SOL
NTST
XII
A2
A1
LRN
P7000-8000
1mm

Figure 37

A5 cell group

The position of the A5 cell group is shown on a representative transverse section of the brain of the rat. CA-histofluorescent cells mapped in this area are regarded primarily as noradrenergic (e.g. Westlund et al. 1983).

Abbreviations:

FLM - Fasciculus longitudinalis medialis

ML - Medial lemniscus

NRF - Nucleus retrofacilias

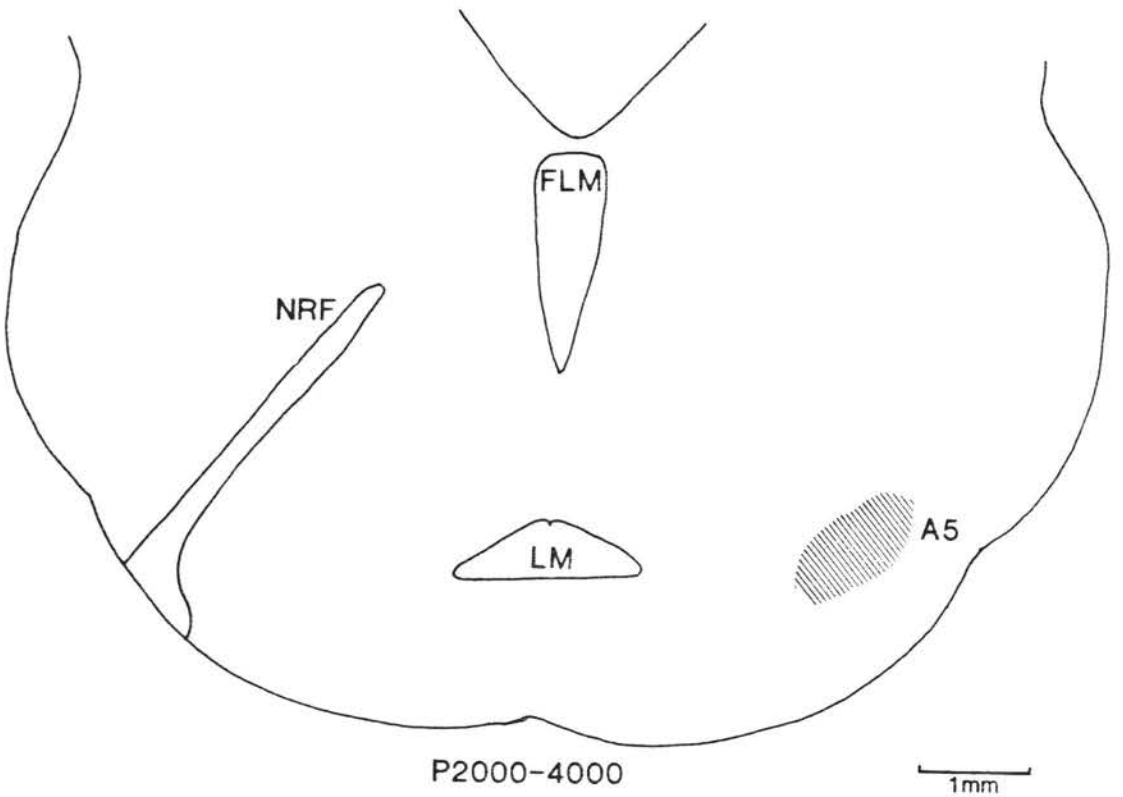

FLM
NRF
LM
A5
P2000-4000
1mm

Figure 38

A6 and A7 cell groups

The position of the A6 cell group is shown, in close proximity to the locus coeruleus. The A7 (sub-coeruleus) cell group is also shown, on a representative transverse section of the brain of the rat. The CA-histofluorescent cells mapped in the area are regarded primarily as noradrenergic (e.g., Westlund et al., 1983).

The CA-histofluorescent cells of the A7 cell group were scattered in and around the area shown by the hatching and the A7 cell group was observed to be extremely diffuse.

Abbreviations:
FLM - Fasciculus longitudinalis
ML - Medial lemniscus
LC - Locus coeruleus
NTST - Nucleus tractus solitarii

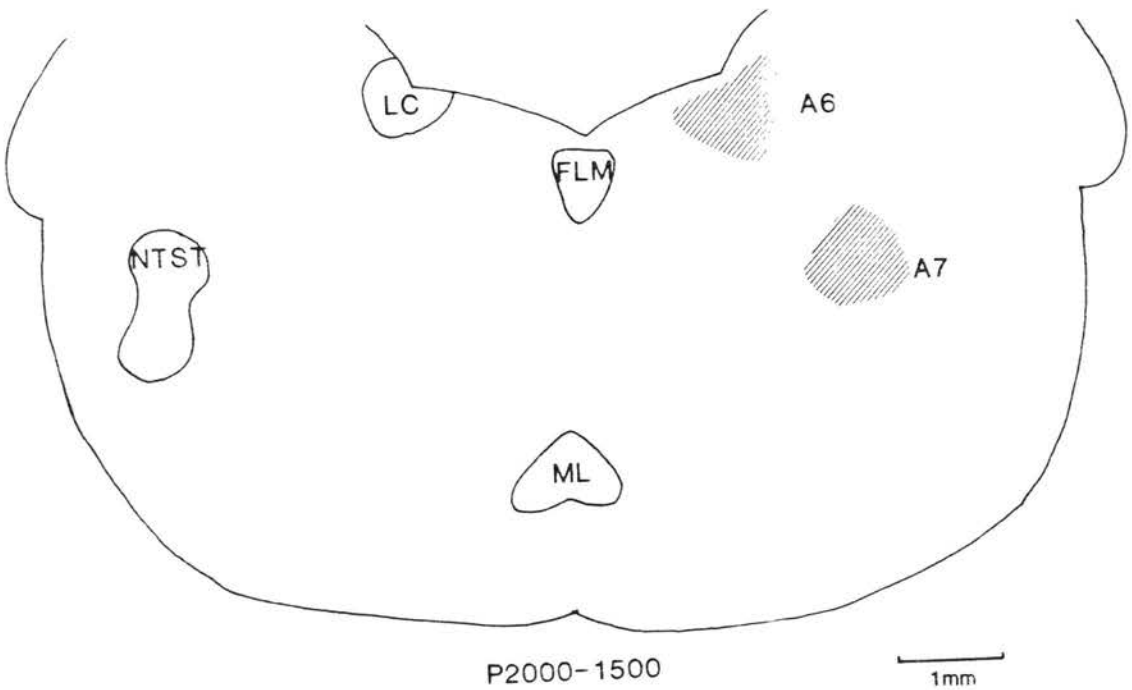
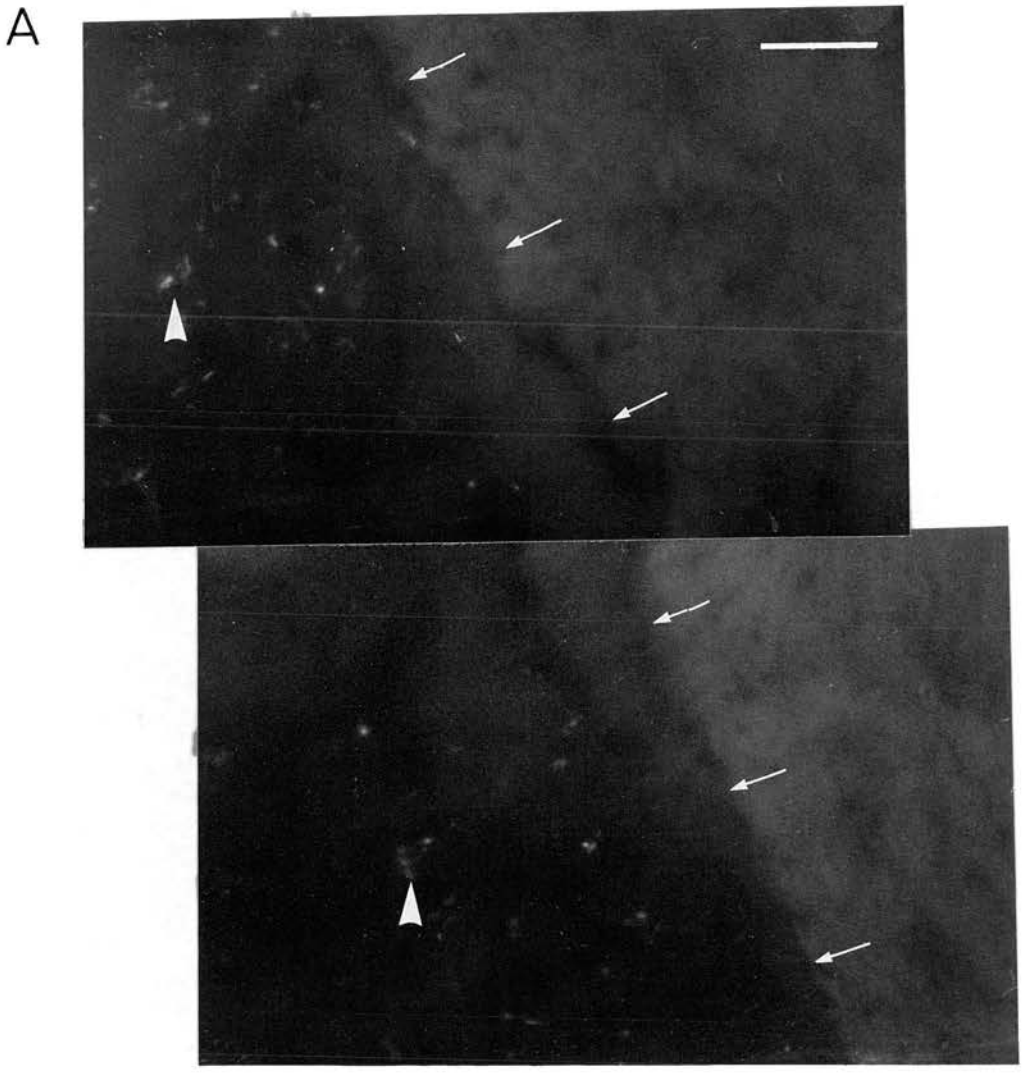

LC
A6
FLM
NTST
A7
ML
P2000-1500
1mm

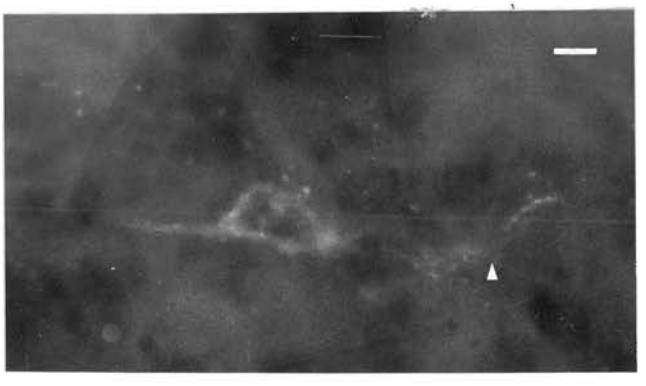
Figure 39

Photomicrographs of CA-histofluorescent cells in the A11 cell group of the rat

A. Shows two histofluorescent cell bodies in the A11 cell group (marked by arrow-heads) in close proximity to the midline (marked by arrows as described by Bjorklund and Nobin (1973) and Hokfelt et al. (1979). Magnification x60, scale bar represents 100 μm.

B. Shows the ventral histofluorescent cell body pictured in A at a higher magnification (x250, scale bar represents 10 μm). The axon was observed to bifurcate in a T-shaped manner, described as characteristic of cells in the A11 DA group (Bjorklund and Nobin, 1973).


A


B

DISCUSSION

The results presented in this study provide strong support for selective antinociceptive actions mediated by DA and NA at the level of the dorsal horn. Further experiments provided the first demonstration of a selective antinociceptive effect, elicited from the region of the A11 DA cell group and mediated by DA at the spinal level. These findings are discussed in the light of previous studies investigating the catecholaminergic control of somatosensory transmission and the implications of such actions on transmission of nociceptive information to supraspinal levels.

A. Antinociceptive action of NA and DA at the level of the dorsal horn.

The predominant effect of ionophoretically-applied NA and DA on the multireceptive somatosensory neurones tested in this study, was a selective inhibition of the response to noxious cutaneous stimuli, whilst the responses to brush, DLH-evoked and spontaneous activity were unaffected. These findings of a functionally selective antinociceptive action of the CAs at the level of the spinal cord are supported by observations in recent behavioural studies (Reddy and Yaksh, 1980; Jensen and Smith, 1983a; Jensen and Yaksh, 1984a).

Previous investigations of the effects of ionophoretically-applied NA, gave contrasting views as to

the locus of action of NA in the dorsal horn (Belcher et al. 1978; Headley et al. 1978). Belcher et al. (1978) observed a depression of all types of evoked activity of multireceptive neurones in response to ionophoretically-applied NA and therefore proposed that NA had a postsynaptic (direct) action on the neurones tested. The results presented here for both NA and DA (results section B(i) and D(i)), however, do not support a simple postsynaptic action on multireceptive dorsal horn neurones. The selective inhibition of one type of modality input implies a more indirect, effectively presynaptic action, on the pathways relaying nociceptive input to the neurone being tested. It is not possible to elucidate whether substantially different populations were studied, by Belcher et al. (1978) and another group, Satoh, Kawajiri, Ukai and Yamamoto (1979) (who also reported a non-selective action by ionophoretically-applied NA on dorsal horn neurones in the rabbit), compared to the results of the present study. However, Belcher et al. (1978) and Satoh et al. (1979) used higher ionophoretic currents (up to 100 nA) levels at which, a generalized depression of all activity was also observed to be caused by NA in the present study. Furthermore, it is unclear whether the action of NA reported by Belcher et al. (1978) was mediated by a specific adreno-receptor, since the α-antagonist, phentolamine, failed to reverse the non-selective effects.

The selective antinociceptive action of

ionophoretically-applied NA, (since confirmed by Davies, Johnston, Miller, Quinlan and Sheardown, 1984), implies a presynaptic, rather than a postsynaptic locus of action. This observation was also described by Headley et al. (1978), although the margin of selectivity was less distinct than that observed in the present study. The different margins of selectivity attributed to NA, may be due to the different populations of neurones sampled, or may simply have been revealed by more rigorously selective use of comparisons through a range of ionophoretic currents undertaken in the present study.

The action of ionophoretically-applied DA, although similar to that of NA, was shown to be pharmacologically distinct. Sulpiride, a highly selective DA antagonist (Seeman, 1981), failed to attenuate the selective inhibition of nociceptive responses of multireceptive dorsal horn neurones caused by clonidine, but it caused a rapid and consistent reversal of the selective antinociceptive action of DA (results section C(ii) and E(iii)).

Willcockson et al. (1984) and Cahusac and Hill (1982) have previously investigated the action of ionophoretically-applied DA, on STT neurones in the monkey and on trigeminal neurones in the rat respectively. In the former study, the action of DA was reported to be non-selective, inhibiting both non-nociceptive and nociceptive neuronal responses. However, the ejection currents used were at levels also

found to cause general depression of all neuronal activity in the present study (50-100nA). From the abstract of Cahusac and Hill (1982) it cannot be discerned whether the action of DA was selective or not. Barasi and Duggal (1985b) recently reported that intravenously-administered apomorphine selectively reduced the responses of dorsal horn neurones to noxious but not innocuous stimulation. The results of Barasi and Duggal (1985b), Headley et al (1978) and those of the present study suggest that both NA and DA have an indirect, presynaptic action on multireceptive dorsal horn neurones in the rat and cat. It seems likely that selective antinociceptive actions of NA and DA do occur widely, but may be difficult to detect without the use of strictly controlled protocols.

The possibility of an inhibitory action by either NA or DA generated at a remote dendritic site, receiving a specific nociceptive input remains. If noradrenergic receptors were restricted to individual dendrites receiving a nociceptive input, separate from non-nociceptive inputs, the nociceptive response might be selectively reduced. Although, in such a case one might also predict a concomitant reduction of spontaneous activity, even at lower ejection currents, which was not seen (Table 3 and 4). It would be expected that a direct antinociceptive action of NA (or DA) would be inhibitory (e.g. Marshall and Engberg, 1979) and result in inhibitory postsynaptic potentials which may be recorded

intracellularly. However, it is most likely that selective antinociceptive actions of both NA and DA are mediated at a presynaptic locus of action, because neither spontaneous nor DLH-evoked activity were reduced by ionophoretically-applied NA or DA (10-40 nA). Furthermore, this view is supported by electron microscopic investigation of the dendritic trees of SCT neurones, which revealed a marked lack of synapses with terminations containing dense core vesicles, characteristic of monoamines (Maxwell, 1985 personal communication). SCT neurones formed a major proportion of neurones tested in this study.

The innocuous cutaneous brush stimulus used, most probably activated both polysynaptic and monosynaptic inputs to the neurones tested (at least with respect to SCT neurones; Hongo and Koike, 1975; Brown, 1981) and as the response to this submaximal stimulus was unaltered, the action of NA and DA is most likely to have been exerted on a specific nociceptive pathway, rather than non-specifically depressing all polysynaptic inputs. The precise receptor site(s) mediating the selective antinociceptive effect caused by ionophoretic-application of NA or DA could be at any point from primary afferent terminals, through a number of antecedent neurones in the polysynaptic nociceptive input to the neurones tested.

Many fine, unmyelinated Aδ - and C-fibres appear to terminate in the superficial dorsal horn (Ralston and Ralston, 1979; Rethelyi, 1977; Light and Perl, 1979b),

whilst the dendrites of one population of neurones investigated in the present study (SCT cells) are reported to extend dorsally only into lamina III (Brown, 1981). Jeftinija, Semba and Randic (1981) reported that ionophoretically-applied NA increased the threshold for antidromic activation of C-fibre afferents from dorsal rootlets, consistent with a reduction in the excitability of C-fibre afferents, presumably including both nociceptive and non-nociceptive C-fibre afferents. It was not, however, possible to reverse this effect with either ionophoretically-applied phentolamine or intravenously-administered yohimbine. NA has also been reported to decrease the inward calcium current of the soma membrane of cultured chick sensory neurones (Dunlap and Fishbach, 1979). A similar action *in vivo* may provide NA with a mechanism for presynaptic modulation of transmitter release. Jeftinija *et al*. (1981), however, did not determine whether the action of NA was direct, on the C-fibre afferents tested, or indirect via dorsal horn neurones possibly, in turn modulating presynaptic excitability of C-fibre afferents. Ryan, Hackman, Wohlberg and Davidoff (1985), on finding that dorsal root hyperpolarization caused by DA, in an isolated frog spinal cord prepartion, was abolished in the presence of ouabain, suggested that the dopaminergic action on primary afferents was an indirect synaptic action mediated by dorsal horn interneurones. An indirect action on primary afferents is also supported by

behavioural studies, in which it was reported that the tail-flick response was unaffected by intrathecally-administered DA in rats with intact neuraxes, whilst a potent analgesic action was exerted on the hot-plate or acetic acid-writhing responses (Jensen and Smith, 1983a ; Jensen and Yaksh, 1984a). If DA had a direct inhibitory action on primary afferent terminals, both spinally and supraspinally-mediated nociceptive responses would be expected to be reduced in behavioural studies. An indirect action by NA on primary afferents, is also suggested by the finding that degenerating afferent fibres do not appear in close proximity to noradrenergic terminal (Satoh, Kashiba, Kimura and Maede, 1982).

A wealth of evidence indicates that the SG is involved in the modulation of sensory transmission through the spinal cord (Melzack and Wall, 1965; Cervero and Iggo, 1978). Synaptic contacts between SG neurones and descending catecholaminergic tracts therefore may be envisaged. A potent selective antinociceptive action of NA, ionophoresed in the SG, on deeper dorsal horn neurones has been demonstrated (results not shown) in unpublished observations by Fleetwood-Walker et al. (1985). Autoradiographic studies have visualized receptor sites for DA and NA, especially in the superficial dorsal horn laminae, at the lumbar level of the spinal cord (Scatton et al. 1984; Dashwood, Fleetwood-Walker, Gilbey, Mitchell and Spyer, 1985). Gobel (1980) suggested that some axonal endings which had

similar ultrastructural characteristics to NA- (or 5HT-) containing nerve terminals were observed on the dendrites of stalked or islet cells in the SG. There are reports that unidentified neurones in the superficial dorsal horn may either be excited (Todd, 1982) or hyperpolarized (North and Yoshimura, 1984) by NA. In the latter, *in vitro* study, the majority of SG neurones were hyperpolarized by NA, an effect presumably mediated via an 2-adrenoreceptor (as it was antagonised by either phentolamine or yohimbine, but not prazosin or propranol). North and Yoshimura (1984) also reported a small population of SG neurones on which NA evoked excitatory postynaptic potentials mediated via an α_1-adrenoreceptor. These studies did not identify the different classes of neurones present in the SG.

The possible functions of SG neurones have been the subject of speculation and two views, by no means exclusive, have arisen. The SG has been proposed to be a relay nucleus for information from primary afferent fibres. This view was supported by Kumazawa and Perl (1978) and Light *et al*. (1979a; 1979b) who emphasised a close correlation between the anatomical distribution of afferent terminals in the superficial dorsal horn and the functional properties of SG neurones. This view assumes that some SG neurones are excitatory in nature. However, the demonstration of a population of SG neurones which were inihibited by cutaneous stimuli (Hentall, 1977; Cervero *et al*. 1978) prompted Cervero and Iggo (1978) to

propose the SG neurones act as inhibitory interneurones on larger dorsal horn neurones. Thus the SG may act as both a relay nucleus and a nucleus of interaction and modulation of cutaneous sensory information. Interactions between descending NA or DA tracts and the SG may well be involved in the modulation of spinal processing. However, their relation in terms of mechanism to the selective antinociceptive effects, described in the present study, are difficult to establish.

The site of the selective action of NA (or DA) might be closer to the deeper dorsal horn neurones tested in this study than laminae I and II. This is suggested by the observations of Headley et al. (1978), that a prominent selective effect of NA occurred in the region of lamina IV as well as when ejected more dorsally. This is supported by the present study in which low ejection currents of NA and DA (10-40nA) ionophoresed in the vicinity caused a potent selective antinociceptive action on multireceptive neurones, located in the deeper dorsal horn laminae, in both the rat and cat.

It is of interest that ionophoretically-applied 5-HT was found not to cause selective antinociceptive effects, but caused a general depression of all types of evoked and spontaneous neuronal activity with 30-50nA of ejection current (results section G). This is particularly intriguing, considering previous reports that intrathecally-administered serotonin has a

behavioural antinociceptive effect (Yaksh and Wilson, 1979). The results presented do, however, agree with previous reports as to the relatively non-selective action of ionophoretically-applied 5-HT (30-100nA) on multireceptive dorsal horn neurones (Headley et al, 1978; Belcher et al. 1978). This non-selective action reported in electrophysiological studies, however, may be due to a compound action of 5-HT on several specific subtypes of serotonergic receptors, reported to exist in the CNS, and may be clarified by the use of more receptor-specific agonists (see Bradley and Costa, 1984).

Putative intraspinal antinociceptive neurotransmitters, such as GABA, glycine or the opioids, may be involved in the catecholaminergic antinociceptive actions at the spinal level. The marked selective inhibition of nociceptive responses by NA was found to be unaffected in the presence of ionophoretically-applied bicuculline and strychnine, suggesting that the noradrenergic action was independent of local GABA- and glycine-mediated inhibitory effects (Fleetwood-Walker, Hope, Mitchell and Molony, 1983b). Morphine and related opioid compounds have analgesic and antinociceptive actions, which appear to be exerted at both spinal and supraspinal sites (Barton, Basbaum and Fields, 1980). At least 3 opioid peptide receptor subtypes have been distinguished and proposed to be located in the dorsal horn (Kosterlitz, Paterson and Robson, 1982). Each receptor subtype, μ, κ and δ, has been suggested to have

independent analgesic actions (Schmauss and Yaksh, 1984; Schmauss, Shimohigashi, Jensen, Rodbard and Yaksh, 1985). The failure of naloxone to attentuate the action of clonidine (results section C(ii)), implies that the antinociceptive adrenergic actions are not mediated via a local opioid system. However, other studies (Fleetwood-Walker, Mitchell, Hope and Molony, 1985) have reported that the selective antinociceptive effect evoked by ionophoretically-applied dynorphin$_{(1-13)}$ (probably a K receptor-mediated effect) was reversed by an α_2-antagonist. Therefore it may be speculated that segmental antinociceptive (opioid) effects may be mediated at least in part through the activation of descending (noradrenergic) tracts. Further evidence (data not shown) indicates that the antinociceptive effects of μ and δ-preferring agonists show no apparent interaction with catecholaminergic neurotransmission at the spinal level. The finding that pretreatment by continuous intrathecal-administration of morphine can attentuate the analgesia induced by intrathecally-administered NA (Milne, Cervenko, Jhamandas, Loomis and Sutak, 1985) suggests that opioid-noradrenergic cross-tolerance had developed in the rat and supported the above proposal (Fleetwood-Walker et al. 1985). Morphine is likely to act predominantly through u type receptors, though there will be some effects on K sites. The results of Milne et al. (1985) suggest a functional synergy between the spinal opioid receptor and α_2-

receptor actions. This may be at least in part, explained by our postulated K modulation of NA input. Although a functional intraction of α_2/μ- mediated action may also occur, its location is as yet undefined. Opioid mechanisms are not thought to be involved in dopamine-mediated antinociception, at the spinal level (Jensen and Yaksh, 1983a). Any possible secondary mediator(s) of the selective antinociceptive actions of ionophoretically-applied DA and NA remain unknown, although the various neuropeptides present in the dorsal horn provide a number of candidates.

B. Receptors mediating the action of NA and DA.

Previous ionophoretic studies did not investigate the possible different dopaminergic or adrenergic receptor subtypes, which may mediate the effects described.

Behavioural studies implied the involvement of an α_2- adrenoreceptor in the mediation of the analgesic effects attributed to intrathecally-administered NA. The selective antinociceptive action of NA was reported to be mimicked, for example, by the α_2-agonist clonidine (Yaksh and Reddy, 1981; Howe et al. 1983) although an α_1-agonist, phenylephrine, was also reported to have effects similar, but much less potent effect than NA (Reddy and Yaksh, 1980). Ionophoretically-applied clonidine and metaraminol, both selective α_2-agonists (U´Prichard et al. 1979; Wikberg, 1978; Tanaka and

Starke, 1980) were found, in the present study, to mimic the action of NA, whilst the selective α_1-agonist, phenylephrine (Tanaka and Starke, 1980) and B-agonist, isoprenaline (U'Prichard et al. 1979), did not. Autoradiographic studies have demonstrated α_2- and α_1-receptor sites throughout the lumbar dorsal horn, with a higher concentration of α_2- sites in the superficial dorsal horn (Dashwood, et al. 1985), but do not give any indication as to the possible site of action of ionophoretically-applied NA, in the present study. A distribution similar to that described for α_2- receptor sites is reported for DA receptor sites (Scatton et al. 1985).

The receptor subtype possibly mediating the selective antinociceptive action of intrathecally-administered DA was not investigated in behavioural studies, in which the $D_{1/2}$ DA-agonist, apomorphine, was used (Jensen and Smith, 1983a). In the present study, the highly selective D_2 DA-receptor antagonist, sulpiride, (Seeman, 1981) was shown to consistently reverse the action of DA, whilst the action of DA was mimicked by a D_2-agonist, RU24213 (Euvrard et al. 1984), but not by a D_1- agonist, SKF38393 (Setler et al. 1978). This study (results section E(i) and (ii)) implied that a D_2 DA-receptor mediated the selective antinocicepetive action of ionophoretically-applied DA. Within the duration of this study, a parallel behavioural action of DA, via a D_2- receptor at the spinal level, was confirmed

by Barasi and Duggal (1985b). They reported that intrathecal administration of the D_2 DA-agonist, LY171555, led to a significant increase in the nociceptive tail-flick latency of lightly anaesthetised rats, whilst the D_1 DAagonist, SKF38393, had no effect.

C. Involvement of catecholaminergic tracts in descending inhibition.

Descending noradrenergic or dopaminergic projections to the dorsal horn did not appear to exert tonic inhibitory influences on the neurones tested. Prolonged ionophoresis of a selective α_2-antagonist, RX781094 (Chapleo et al. 1981), or a selective D_2 DA-antagonist, sulpiride (Seeman, 1981), did not alter the levels of any of the evoked responses tested, in the preparation described (methods section A(ii)). Pretreatments with intrathecally-administered $D_{1/2}$ DA-receptor antagonist, cis-fluphenthixol, did not affect the baseline tail-flick or hot-plate responses (Jensen and Yaksh, 1984), therefore a tonic dopaminergic modulation of these responses did not appear to be exerted in behavioural studies, although the highly selective D_2 DA-receptor antagonist sulpiride was not tested. However, it has been reported that intrathecally-administered noradrenergic antagonists produced a reduced nociceptive threshold, implying the release of nociceptive reponses from an inhibitory noradrenergic influence (Sagen and Proudfit, 1984). The relative potencies of the intrathecally-

administered antagonists in producing hyperalgesia correlated with their relative affinities for α_2-adrenoreceptors (yohimbine > phentolamine > WB4101 > prazosin). Thus the tonic noradrenergic influence exerted on both spinally (tail-flick reflex) - and supraspinally (hot-plate test) - mediated responses seemed to also involve an α_2-receptor (Sagen and Proudfit, 1984). In contrast, Soja and Sinclair (1983) concluded that NA was not involved in the mediation of, but rather appeared to reduce, tonic descending inhibition. In animals pharmacologically depleted of monoamines, it was found that the degree of tonic descending inhibition (as determined by comparing the nociceptive responses of dorsal horn neurones in the intact and reversible spinal cold-blocked state) was greater than in control preparations. The degree of tonic descending inhibiton was reduced after intravenous-administration of NA uptake blockers, nisoxetine or desipramine. These seemingly opposing findings; the absence of tonic modulatory actions mediated by NA reported in the present study, the reduction by NA of other modulatory influences suggested in that of Soja and Sinclair (1983) and the presence of tonic inhibitory noradrenergic influences demonstrated in behavioural studies (Sagen and Proudfit, 1984); may be due to the different preparations used. In the present study and that of Soja and Sinclair (1983) animals were anaesthetised (with α-chloralose), whilst in the studies

of Sagen and Proudfit (1984) the animals were conscious. In the latter, behavioural study the animals may have been inadvertently subjected to some stress.

Experimentally-induced stress has been shown to cause analgesia (introduction section E). Stress-induced analgesia, evoked by footshock or vaginal stimulation has been reported to be dependent on NA and 5-HT at the spinal level (Steinman, Kamisaruk, Yaksh and Tyce, 1983; Watkins, Johannessen, Kinscheck and Mayer, 1984). Thus, inhibitory noradrenergic influences at the spinal level, absent in the anaesthetised preparation, may have been activated in the behavioural studies. This suggestion is also supported by the report of Tyce and Yaksh (1981), in which high intensity somatic stimulation was found to evoke the release of NA (and 5-HT) into the spinal cord superfusate. Furthermore the bulbospinal noradrenergic cell groups have been implicated by Takagi, Shiomi, Kuraishi, Fukui and Ueda (1979), who reported that an increase in metabolites of noradrenaline in response to noxious cutaneous stimulation, measured at the spinal level, was not attentuated by lesions at more rostral levels of the brain stem.

D. Putative origins of descending catecholaminergic tracts to the dorsal horn:

As described in the introduction (section G(ii)), the supraspinal origins of the noradrenergic innervation of the dorsal horn are unclear, although

particular noradrenergic cell groups in the brain stem, appear to be more strongly implicated.

The region of the lateral reticular nucleus (LRN, which is in close proximity to the area of the A1 cell group) has been proposed as a source of tonic descending inhibition by Hall, Duggan, Morton and Johnson (1982) and Morton, Johnson and Duggan (1983). Bilateral electrolytic lesions of this region were reported to result in a significant increase in the nociceptive response of dorsal horn neurones. Extensive lesions of more rostral sites failed to reproduce this effect, therefore lesions of the region of the LRN (and not fibres of passage) were proposed to release tonic descending inhibitory influences (Hall et al. 1982; Morton et al. 1983). Tonically active, putative CA-containing cells have been recorded in this region (Fleetwood-Walker et al. 1983a). Bragin and Durayin (1985) reported that lesions of this area attenuated the reduction in tail-flick latency induced by cold-water swims in rats and such lesions also result in a reduction of NA levels in the spinal cord (Fleetwood-Walker and Coote, 1981; Bragin and Durayin, 1985). Support for the concept that this region may potentially be involved in a phasically active inhibition of spinal nociception has also come from electrophysiological studies. Electrical stimulation in the region of the LRN, inhibited the nociceptive tail-flick reflex in lightly anaesthetised rats (Gebhart and Ossipov, 1984) or the nociceptive

responses of dorsal horn neurones of cats (Morton et al. 1983). The stimulus-evoked inhibition of the tailflick was attenuated by intrathecally-administered yohimbine, an α_2-antagonist, but not by an α_1-antagonist, prazosin (Gebhart and Ossipov, 1984) and therefore appeared to be mediated by a α_2-adrenoreceptor at the spinal level. It is possible, however, that pathways from the LRN to other NA cell group or axons of passage may also have been affected and the A1 cell group is not necessarily involved. In a similar behavioural study, Jones and Gebhart (1984) report that electrical stimulation in the region of the locus coeruleus/ subcoeruleus may also evoke antinociceptive effects, mediated by an α_2-receptor at the spinal level. However, other electrophysiological studies have revealed either mixed excitatory or inhibitory, non-selective effects on dorsal horn neurones, evoked from the region of the locus coeruleus (Hodge, Apkarian, Steven, Vogelsang and Wisnicki, 1981; Mokha, McMillan and Iggo, 1983). There is also evidence that NA originating from supraspinal sites may mediate antinociceptive effects, evoked from the raphe region (Kuraishi, Harada, Satch and Takagi, 1979; Sagen and Proudfit, 1981; Jensen and Yaksh, 1984b). This is discussed in the following section.

DA antagonists have not been systematically tested at the spinal level in behavioural experiments. Nevertheless some involvement of DA in stress-induced analgesia has been suggested, although unfortunately,

intrathecally-administered antagonists have not been tested. Therefore dopaminergic function in stress-induced analgesia at the spinal level has yet to be defined (Jensen and Smith, 1982b; Snow, Tucker and Dewey, 1982). The A11 DA cell group has been directly implicated to be involved in spinal antinociceptive mechanisms, by the present study. An ipsilateral projection from the A11 DA cell group to the spinal cord is strongly supported by anatomical studies (Hokfelt et al. 1979; Skagerberg et al. 1982, see introduction section H(i)). Swanson, Sawchenko, Berod, Hartman, Helle and Vanorden (1981) and Hokfelt et al. (1979) reported an extensive paraventricular group, consisting of more than 500 cells on each side using immunocytochemistry. However, ALFA-histofluorescent studies have indicated a smaller catecholaminergic group of about 200 cells on each side (Skagerberg and Lindvall, 1985). There is evidence to support the view that the ALFA-histochemical technique may not be sensitive enough to visualize all catecholaminergic cells in this area. Lidbrink, Jonsson and Fuxe (1974) reported a group of paraventricular cells, in reserpine treated animals, which were not visualized in untreated animals and were presumed to be dopaminergic. However some non-specific immunocytochemical staining by tyrosine hydroxylase antibody cannot be completely ruled out in immunocytochemical studies.

Early anatomical studies, using a microfluoremetric

technique, reported to be able to distinguish between NA and DA at the cellular level, suggested the presence of NA-containing neurones in the dorsal A11 cell group (Bjorklund and Nobin, 1973). Subsequent immunocytochemical studies did not confirm this finding and reported all the cells of the A11 group to be dopaminergic (Swanson and Hartman, 1975; Hokfelt et al. 1979). In the present study, the electrically-evoked selective antinociceptive effects elicited from the region of the A11 cell group were clearly not mediated by a α-noradrenergic action, at the spinal level (see results section F(ii)).

The spinal projection from the A11 DA cell group has been reported to be predominantly ipsilateral (Skagerberg et al, 1982; Skagerberg and Lindvall, 1985). This finding is supported in the present study in which stimulus-evoked antinociceptive effects from the region of the A11 DA cell group were only elicited when the dorsal horn neurone being tested was ipsilateral to the supraspinal stimulating electrodes.

Although the lesions study of Commissiong et al. (1978) proposed a projection from the A9 DA cell group to the spinal cord, subsequent anatomical and electrophysiological studies and the present study (results sections F(iii)) indicate that such a direct dopaminergic link is unlikely and that the A9 group does not exert a direct antinociceptive role at the spinal level. Skagerberg et al. (1982) and Hokfelt et al. (1979), in anatomical studies, did not find any evidence for A9 DA

cells projecting to the spinal cord. In agreement with the present study, electrical stimulation in the region of the A9 DA cell group, using similar stimulation parameters, by Barasi and Duggal (1985a) failed to elicit any spinal antinociceptive effects.

E. Selective control of nociceptive transmission.

The studies described in this thesis provide strong evidence for a selective antinociceptive role for NA and DA at the spinal level. The All DA cell group has been strongly suggested to be the primary supraspinal origin of the dopaminergic innervation of the spinal cord, having a selective antinociceptive function, whilst a number of candidate cell groups may be proposed as the supraspinal origin(s) of spinal NA. The supraspinal origins of these catecholamine systems and their spinal antinociceptive actions, are indicative of potential participation in descending inhibition. Early physiological studies demonstrated that descending pathways influenced spinal afferent transmission (Wall, 1967; Pompieano, 1973; Satoh and Takgi, 1971) but did not provide evidence for a specific control of nociceptive input. Head and Holmes in (1911) had postulated that somatosensory input inhibited nociceptive pathways on the basis of clinical observations, in which damage to somatosensory pathways resulted in the development of chronic pain states. Melzack and Wall (1965) cited similar clinical observations and neurophysiological data

to support a detailed neural mechanism to explain the variability of pain in their 'Gate Control Theory'. Both Head and Holmes (1911) and Melzack and Wall (1965) primarily emphasised physiological interactions between different somatosensory modalities, by which nociceptive transmission may be altered. It has now become clear that a great variety of inhibitory influences are exerted within the dorsal horn, often specifically on the nociceptive responses of multireceptive dorsal horn neurones.

Nociceptive responses of many multireceptive dorsal horn neurones can be significantly inhibited by, for example, noxious cutaneous stimulation applied to regions outside the receptive field over the entire body surface. These effects have been called diffuse noxious inhibitory controls, DNIC (Le Bars, Dickenson and Besson, 1979a; 1979b). DNIC is not thought to involve propriospinal mechanisms because the effect is not demonstrable in spinal animals (Le Bars et al. 1979b), but is thought to be mediated by supraspinally-originating descending systems. The NRM-serotonergic system has been proposed to mediate, at least in part, DNIC (Dickenson, Le Bars and Besson, 1980a; Dickenson, Rivot, Chaouch, Besson and Le Bars, 1981; Chitour, Dickenson and Le Bars, 1982).

It has been recently reported that electrical stimulation of the NRM (and the closely associated nucleus reticularis paragigantocellularis, NRPG) increased the efflux of endogenous 5-HT and NA from the spinal cord

(Hammond, Tyce and Yaksh, 1985). These stimulation sites were coincident with brain stem sites from which electrical stimulation evoked analgesia and that this stimulus-produced analgesia (SPA) could be antagonised by intrathecally-administered noradrenergic antagonists (Kuraishi et al. 1979; Sagen and Proudfit, 1981; Jensen and Yaksh, 1984b) as well as serotonergic antagonists (Satoh, Akaike, Nakazama and Takagi, 1980; Schmauss, Hammond, Ochi and Yaksh, 1983). Therefore DNIC (and other modes of activation of endogenous antinociception) may involve noradrenergic, as well as serotonergic transmission, mediated from the region around the NRM. However the involvement of NA in SPA evoked from the regions of the NRM and NRPG seems not due directly to stimulation of NA spinal neurones because noradrenergic perikarya are not contained within these areas (Westlund et al. 1983). Activation of noradrenergic fibres of passage is also unlikely because intrathecal-administration of NA antagonists also attenuates antinociception produced by microinjection of glutamate into regions of NRM and NRPG, held to selectively activate only cell bodies (Jensen and Yaksh, 1984b). Furthermore behavioural analgesia elicited by microinjections of morphine into the NRPG is also blocked by the NA antagoinst, phenoxybenzamine, at the spinal level (Kuraishi et al. 1979). Thus a relay between the region of the NRM and NRPG and separate bulbospinal noradrenergic cell groups may be involved in stimulus- and morphine-produced analgesia, as well as DNIC.

The phenomenon of DNIC prompted Le Bars et al. (1979b) to propose that the activation of such systems may result in a contrast between excitatory information arising from one population of nociceptive neurones against DNIC-mediated silence of the remaining neuronal pool.

In microneuronography experiments in which the electrode was switched alternately between stimulating and recording there is claimed to be a correspondence between the evoked 'elementary sensation' (i.e. pain, pressure, tap) and the largest recordable unit at the same location. The nature of elementary sensations and the correspondence with properties of identified recorded afferents led Ochoa and Torebjork (1983) to postulate that the former derives from the latter, i.e., that the stimulation activates only a single fibre and that this gives rise to a specific localized sensation. However, Wall and McMahon (1985b) criticised this procedure in the belief that even the lower stimulation currents used (1-2 μA) could probably evoke activity in a number of surrounding fibres due to current spread (Ranck, 1975). Clearly, natural cutaneous stimulation evokes activity in a multitude of afferent fibres, which may result in the activation of various modulatory systems, which in turn may integrate, filter and relay specific somatosensory information.

It may be thought that excitation of multireceptive neurones, many of which project directly to supraspinal

sites, may lead to sensory confusion and thus some investigators have attributed only nocispecific neurones as capable of transmitting definitively nociceptive information.

A number of lines of evidence, however, support the hypothesis that multireceptive neurones are involved in ascending nociceptive pathways. DNIC exerted on multireceptive dorsal horn neurones has been related to the counter-irritation phenomenon, the paradoxical pain-relieving effects of pain elicited from distant body areas (Kane and Taub, 1975). The inhibition of nociceptive responses of multireceptive (and nocispecific) neurones can be correlated to a reduction of behavioural nociceptive responses in animals. Another strategy used, has been to combine psychophysical measurements of pain with electrophysiological techniques. Price and Mayer (1975) reported that in the monkey, the electrical thresholds of the axons of nocispecific neurones, projecting along the ventrolateral quadrant (VLQ), were higher than those of multireceptive neurones. Using similar stimulation parameters, electrical stimulation of the VLQ in man, proposed to activate only multireceptive neurones was reported to evoke aversive painful sensations by patients (Mayer, Price and Becker, 1975).

These results do not, however, obviate a nociceptive function for nocispecific neurones, a population of which

have been described, located in lamina I of the dorsal horn (introduction section D(ii)). Some lamina I neurones possibly receive monosynaptic nociceptive afferent inputs (Gobel et al. 1980) and may also contribute significantly to ascending tracts (introduction section D(ii) and D(iii)). It may be proposed, for example, that nocispecific neurones in lamina I may in turn activate descending influences, known to modulate multireceptive dorsal horn neurones and thus modulate ascending nociceptive transmission from these neurones. NA and DA have been shown, for example, to mediate such actions.

The multireceptive neurones tested in the present study all had restricted unilateral receptive fields which remained topographically constant over the duration of the recording period (up to 2 hours). As described in the introduction (section D(iii)) a number of multireceptive dorsal horn neurones, with different physiological characteristics, form ascending nociceptive tracts. In particular, it has been shown that the STT may be divided into lateral and medial components with distinct physiological differences. It has been generally proposed that the lateral thalamus may be involved in the sensory-discriminative aspects of pain, while the medial thalamic region may be involved in the motivational, affective and arousal aspects of pain (e.g Melzack and Casey, 1968; Price and Dubner, 1977). The localized receptive fields of the L-STT have been

proposed to reflect the discriminative role of the lateral thalamus. However, this view may be simplistic, considering for example that the nucleus submedius receives a dense projection from lamina I cells which (Craig and Burton, 1981) may subserve an important, but as yet undefined role in nociception; thereby not easily fitting into a simple mediolateral separation of function within the thalamus. Investigations into possible differential modulatory influences on these different subclasses of dorsal horn neurones may reveal more about their functional role in nociception.

One common characteristic of many identified and unidentified multireceptive dorsal horn neurones is the finding that they are subject to descending inhibitory influences, whether phasic or tonic in nature, although the neurochemical basis of many of these effects are as yet unclear. The action of descending influence on nocispecific neurones is less clear, although the presence of powerful tonic inhibition does not appear to be ubiquitous (Cervero et al. 1979; Dickenson et al. 1980b). The predominant nociceptive input to laminae III-V multireceptive dorsal horn neurones, offers multiple interneuronal targets on which descending and segmental inhibitions may potentially be exerted. This view is supported by the present study, in which a presynaptic locus of action for the mediation of the selective antinociceptive effects, produced by NA and DA, is proposed. The variety of modulatory influences

exerted on dorsal horn neurones may summate to allow only specific somatosensory information to be transmitted to supraspinal sites and thus prevent sensory confusion from afferent inputs through multireceptive neurones. Such influences include tonic effects from the region of the LRN and phasic actions evoked by somatic stimuli or even psychological stimuli. Not only is the modality of information important but also the selection of supraspinal sites to which that information is transmitted. It is clear that many multireceptive neurones, just like nocispecific neurones, project to supraspinal sites, are thought to be intimately involved in nociception (introduction section D(iii)).

The pharmacologically distinct selective antinociceptive actions of DA and NA raise the question as to why multiple inhibitory influences are exerted on dorsal horn neurones. As described previously, one possible function may be to filter and amplify specific somatosensory information before it is projected to supraspinal sites. Another role, that has been proposed for descending inhibitory influences, is the attenuation of nociceptive information being transmitted through the dorsal horn. Awareness of a nociceptive stimulus may evoke withdrawal responses to prevent injury, therefore an analgesic system ought not to be activated trivially (Terman, Shavit, Lewis, Cannon and Liebeskind, 1984). Severe nociception may, however, disrupt escape behaviour, then analgesia may be an appropriate adaptive

response, which may be mediated by different pathways depending on the challenge presented. It is unlikely that these systems exert parallel modulatory influences subserving wholly the same role. Interestingly, the finding that intrathecally-administered DA inhibited the nociceptive tail-flick reflex in spinal rats, but not rats with intact neuraxes, suggested that the spinal action of DA may itself be subject to descending inhibitory influences (Jensen and Smith, 1984a). This was supported by the findings that bilateral lesions of the DLF or intrathecal infusion of serotonergic or noradrenergic antagonist, unmasked an antinociceptive action of the tail-flick reflex, in rats with otherwise intact neuraxes (Jensen and Smith, 1983b; Jensen et al. 1984). It may be argued that a weak dopaminergic action was only demonstrated when the more influential 5-HT and NA spinal actions are blocked. However, the demonstration that intrathecally-administered DA inhibits the hot-plate or acetic acid-writhing responses are evidence of a potent analgesic role. Both the hot-plate and tail-flick tests involve nociceptive thermal cutaneous stimulation and the failure to evoke a hot-plate response in spinalized animals indicates the necessity of a supraspinal link for the hot-plate responses. Thus it appears possible that supraspinally- and spinally-mediated nociceptive responses may be under differential control by the monomines, in the intact animal.

It has been postulated that morphine predominantly exerts its profile of analgesic behavioural changes indirectly by activating descending inhibitory pathways in the brainstem (Takagi, Satoh, Doi, Kawasaki and Akaike, 1976) and NA and 5-HT have been proposed as possible mediators of this action. epletion of NA (using intrathecal 6-OHDA) at the spinal level attenuates the inhibitory action of morphine (administered either systemically or intracerebrally) on the tail-pinch, but not the hot-plate or tail-flick tests. However, depletion of 5-HT (using intrathecal 5,6-DHT) attentuated the action of morphine on the hot-plate, but not the tail-pinch or tail-flick tests (Kuraishi, Harada, Aratani, Satoh and Takagi, 1983). Thus it was concluded that the extent of involvment of descending NA or 5-HT inhibitory systems in morphine-produced analgesia differed, depending on the noxious stimulus used. It may be hypothesised therefore that different nociceptive challenges may possibly evoke appropriate antinociceptive systems which modulate different motor responses depending on the nature of the cutaneous stimulus presented.

It has been proposed that multiple ascending nociceptive tracts may subserve different aspects involved in the perception of pain, similarly multiple descending antinociceptive systems may subserve different roles. In response to a noxious stimulus it is not only

the somatosensory system that is activated; for example, cardiovascular and motor systems also respond reflexly to actual or potential injury to the animal.

Electrical stimulation and lesion studies indicate that both the A1 and the NRM regions are also involved in cardiovascular control mechanisms (Coote and Macleod, 1974; Blessing, West and Chalmers, 1981; Kuhn, Wolf, Lovenberg, 1980). It has been reported that microinjection of NA into the dorsal raphe nucleus results in a pressor response which seems at least partly due to the excitation of 5-HT neurones in that area (Robinson, 1984). Thus nociceptive inputs which evoke descending noradrenergic mechanisms (Takagi et al. 1979; Tyce and Yaksh, 1981) may also elicit centrally-mediated cardiovascular responses, perhaps to ready the animal to undertake adaptive or aversive behavioural action. Further support for the idea that antinociceptive systems are not functionally separate, but integrate with other homeostatic systems is given in studies dealing with spontaneously hypertensive rats. It has been shown that the nociceptive thresholds of rats with systolic blood pressures over 180 mmHg are significantly higher than rats with lower blood pressures (140 mmHg, Zamir and Segal, 1979; Dickenson, 1984) and these findings have been confirmed in hypertensive humans (Zemir and Shuber, 1980).

Different antinociceptive systems may not only integrate central neural mechanisms. Analgesia in

response to stressors (such as footshock or immobilization) partly represents an adaptive response and the participation of hormonal mechanisms is also thought to be important. Hypophysectomy and interference with adrenal function can attenuate some forms of stress-induced analgesia (MacLennan, Drugan, Hyson, Maiser, Madden and Barchas, 1982; Lewis, Chudler, Cannon and Liebeskind, 1981). Stress (e.g. electric footshock) has been shown to cause the release of pitutary and opioid hormones (Termen et al. 1984). Millan, Tsang, Przewlocki, Hollt and Herz (1981) also reported that immunoreactive dynorphin levels increased in the spinal cord of rats in response to footshock. It may be envisaged that this increase in spinal dynorphin in turn may enhance release of descending noradrenaline, in the manner proposed by Fleetwood-Walker et al. (1985) and described in discussion section B, to mediate the antinociceptive effects of K-receptor agonists. The cells of both the A1 and A11 catecholamine cell groups are thought to project extensively to the hypothalamic neuorsecretory paraventricular and supraoptic, nuclei (Sawchenko and Swanson, 1982; Lindvall, Bjorklund and Skagerberg, 1984) and may therefore be involved in the integration of a number of neurohumoral mechanisms evoked by noxious and other afferent inputs.

The supraspinal origins of the spinal antinociceptive agents, such as DA and NA described in the present study may be activated on reception of different

afferent inputs transmitted through a variety of relay nuclei. Thus the activation of analgesic mechanisms may be crucially dependant on the coding and decoding of nociceptive information via as yet ill-defined pathways. The reactivity of these systems may not only be modulated by ascending afferent information via the spinal cord. Even cerebral cortical regions receiving nociceptive information (Berkley and Palmer, 1974) may in turn exert profound consequences on, for example, diencephalic nociceptive systems (Albe-Fessard, Berkley, Kruger, Ralston and Willis, 1985).

Current treatments for severe pain involve either surgical destruction of neural tissue or the use of addictive opiate drugs. Investigation as to how engoenous antinociceptive systems may be activated especially those which may be opioid-independent, may reveal alternative methods for the relief of pain, in which the problems of tissue destruction and addiction may be avoided.

In summary, the present study has described selective antinociceptive actions produced by ionophoretically-applied NA and DA on multireceptive dorsal horn neurones. These results support an analgesic roles for these two CAs proposed in behavioural studies. Many of the dorsal horn neurones tested had long ascending axons and were therefore presumably involved in the transmission of nociceptive information to supraspinal sites. The A11 DA cell group, in the rat,

was demonstrated to be a source of selective antinociception, evoked by focal electrical stimulation and mediated by DA in the vicinity of the multireceptive dorsal horn neurones being tested. The demonstration of separate monoaminergic antinociceptive systems now raises the important question as to how these systems are activated and interact in the intact animal.

Some of the results presented in this thesis have been published in the following journals:

FLEETWOOD-WALKER, S.M., HOPE, P.J., IGGO, A., MITCHELL, R. and MOLONY, V. (1983). The effects of ionphoretically applied noradrenaline on the cutaneous sensory responses of identified dorsal horn neurones. J. Physiol., 342, 63-64P.

FLEETWOOD-WALKER, S.M., HOPE, P.J., MITCHELL, R. and MOLONY, V. (1983). Investigatioon of interactions between descending noradrenaline-mediated and local GABA- and glycine-mediated influences on spinocervical tract neurones of the cat. J. Physiol., 346, 1983, 49P.

FLEETWOOD-WALKER, S.M., HOPE, P.J. and MOLONY, V. (1984). Dopamine inhibits the responses of multireceptive dorsal horn neurones to noxious, but not innocuous, cutaneous stimulation in the rat and cat. J. Physiol., 354, 95P.

FLEETWOOD-WALKER, S.M., HOPE, P.J., IGGO, A., MITCHELL, R. and MOLONY, V. (1984). An α_2 receptor mediates the selective antinociceptive effect of noradrenaline on identified dorsal horn neurones. Pain, suppl.2, 8278.

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FLEETWOOD-WALKER, S.M. and HOPE, P.J. (1985). A selective antinociceptive effect of the diencephalic dopamine-containing cell group, A11, on dorsal horn neurones in the rat. J. Physiol., 364, 47P.

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As shown overleaf:

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The effects of ionophoretically applied noradrenaline on the cutaneous sensory responses of identified dorsal horn neurones in the cat

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The behavioural analgesia produced by intrathecal infusion of noradrenaline (NA) (Kuraishi, Harada & Takagi, 1979) suggests that noradrenergic systems may be important in modulating sensory transmission in the dorsal horn. Reports of the effects of ionophoretically applied NA on the cutaneous sensory responses of dorsal horn neurones (Headley, Duggan & Griersmith, 1978; Belcher, Ryall & Schaffner, 1978) have provided conflicting results on the selectivity of these effects. The present series of experiments was carried out in order to investigate the actions of NA on the responses of identified dorsal horn neurones with long ascending projections.

Extracellular recordings were made from a total of thirty-eight identified neurones in lumbar cord segments L6 and L7 of sixteen cats anaesthetized with α-chloralose (60–70 mg/kg) and paralysed with gallamine triethiodide. The multibarrelled glass electrodes used contained: DL-homocysteic acid, 0·1 M, pH 8·0–8·5 (DLH); (−)noradrenaline bitartrate, 0·5 M, pH 4·5–5·0; Pontamine Sky Blue (2% in 0·5 M-Na acetate); and 1 M-NaCl for current balancing. Twenty-eight spinocervical tract (SCT) neurones were identified by antidromic activation from the dorsolateral funiculus of cervical cord according to the criteria of Brown & Franz (1969). The excitatory responses of these neurones to stimulation of cutaneous receptive fields by innocuous (motorized brush) or noxious (radiant heat to 50 °C) stimuli, and to ionophoretically applied DLH were investigated. The effects of NA on these responses were studied in twenty-one SCT neurones. In six neurones excited by innocuous cutaneous stimuli only, NA had no discernible effect until ionophoretic currents exceeded 40 nA, then all responses began to be depressed. In thirteen out of fifteen multireceptive neurones excited by both innocuous and noxious stimuli, NA produced a markedly selective inhibition of noxious responses at low currents (50% inhibition at 0–39 nA), which had little or no effect on innocuous responses, DLH-evoked activity or spontaneous activity. In a small sample of neurones (ten) identified as post-synaptic dorsal column (PSDC) cells, five were excited only by innocuous stimulation and NA had no effect on the responses of four of these until currents exceeded 40 nA. The remaining five were multi-receptive and three showed selective inhibition by NA of noxious responses at low currents (0–30 nA).

The results are consistent with noradrenergic systems providing a selective inhibition of nociceptive transmission in these dorsal horn neurones with long ascending projections. This effect appears to be indirect, since DLH- and innocuous stimulus-evoked activity is relatively unaffected.

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Investigation of interactions between descending noradrenaline-mediated and local GABA- and glycine-mediated influences on spinocervical tract neurones of the cat

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Neurones in the spinal dorsal horn with long ascending projections, including spinocervical tract (SCT) neurones, are subject to a modality-selective inhibition by noradrenaline (NA) that is probably indirect (Fleetwood-Walker, Hope, Iggo, Mitchell & Molony, 1983) and some dorsal horn neurones are inhibited by afferents through mechanisms involving GABA and glycine (Game & Lodge, 1975). The present experiments were designed to study interactions between NA and GABA or glycine effects on SCT neurones.

Extracellular recordings were made from seventeen multireceptive SCT neurones in lumbar segments L6/7 of cats anaesthetised with chloralose (60–70 mg/kg) and paralysed with gallamine. Drugs were administered by standard ionophoretic techniques. Bicuculline methiodide and strychnine sulphate produced marked enhancement of the spontaneous activity of the neurones and of the excitatory responses to D,L-homocysteic acid (DLH) and to both noxious and innocuous cutaneous stimuli. DLH-evoked activity was enhanced by $312 \pm 99\%$ with bicuculline and $133 \pm 41\%$ with strychnine (mean ± S.E.M.). Marked selective inhibitions of nociceptive responses by NA were still observed in the presence of bicuculline or strychnine.

The effects of inhibitory amino acids on DLH-evoked activity were also investigated. GABA and glycine produced marked inhibitions with a rapid onset and offset in the six cells tested. The $GABA_B$ receptor agonist, baclofen, produced moderate inhibitions, with a slower time course of action, in three of these six cells and marked inhibitions in the other three. GABA, glycine and baclofen all produced unselective inhibition of the responses to cutaneous stimuli and to DLH (cf. Davies, 1981).

These results are consistent with the existence of prominent GABA and also glycine inputs directly on to SCT cells, and suggest that they may have a tonic inhibitory influence. The marked selective inhibition of nociceptive responses by NA, still prominent in the presence of bicuculline and strychnine, suggests that this effect is independent of local inhibitions by GABA and glycine.

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Dopamine inhibits the response of multireceptive dorsal horn neurones to noxious, but not innocuous, cutaneous stimulation in the rat and cat

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The spinal dorsal horn of both rat and cat has been shown to receive a restricted dopaminergic innervation from supraspinal levels (Skagerberg, Bjorklund, Lindvall & Schmidt, 1982; Fleetwood-Walker & Coote, 1981). Intrathecal application of dopamine and its agonists in the rat selectively elevate the threshold to noxious stimulation in behavioural tests (Jensen & Smith, 1982). In the present study we investigate the action of ionophoretically applied dopamine on the responses of multireceptive dorsal horn neurones.

Extracellular recordings were made in lumbar segments using multibarrelled glass electrodes containing D,L-homocysteic acid (DLH), dopamine (DA) HCl, sulpiride (a selective D2-receptor antagonist) and 1 M-NaCl for automatic current balancing. In cats anaesthetized with α-chloralose (60–70 mg/kg) and paralysed with gallamine, recordings were made from fifteen spinocervical tract (SCT) neurones as described previously. In experiments with rats anaesthetized with α-chloralose (35 mg/kg) and urethane (700 mg/kg), a population of multireceptive neurones was investigated.

In eleven SCT neurones in the cat, DA selectively reduced the response to noxious heat (skin temperature raised from 30 to 48 °C), whereas the response to innocuous mechanical stimulation (motorized brush) and to DLH were unaffected. The response recovered between 10 and 15 min after the end of DA ejection. Sulpiride reversed the effects of DA, which is consistent with the idea that a specific DA receptor is involved. Two of the fifteen SCT neurones responded with a selective increase in the responses to noxious stimulation and the remaining two neurones were unaffected by DA. All of the multireceptive neurones in the rat showed a selective inhibition of responses to noxious stimuli with DA ejected in their vicinity.

Cahusac & Hill (1983) tested a population of multireceptive neurones in the caudal trigeminal nucleus and found a general depression of responses to both noxious and innocuous stimuli with ionophoresed DA, in contrast to the present results, which are consistent with a spinal antinociceptive role for an endogenous DA system.

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S278

AN α_2 RECEPTOR MEDIATES THE SELECTIVE ANTINOCICEPTIVE EFFECT OF NORADRENALINE ON IDENTIFIED DORSAL HORN NEURONES.

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Aims: Somatosensory neurones in the dorsal horn with long, ascending projections, include those of the spinocervical tract (SCT), which frequently respond to both innocuous and noxious cutaneous stimuli. Noradrenaline (NA) produces a powerful behavioural analgesia on intrathecal perfusion, without reducing innocuous sensorimotor function. We therefore investigated the effects of NA on the different responses of individual SCT neurones.

Methods: 44 multireceptive SCT neurones were identified in laminae III-V of L6/7 dorsal horn of cats anaesthetised with chloralose and paralysed with gallamine. Drugs were applied by microionophoresis and their effects were examined on the responses of these neurones to innocuous (brush) and noxious (radiant heat) stimuli to the receptive fields and also to d,l homocysteic acid (DLH).

Results: In 40 neurones NA caused a prominent selective inhibition of nociceptive responses, probably at some indirect site, since DLH excitation was unaffected. The effect of NA was either acutely reversed by, or its potency was greatly reduced by α_2, but not α_1, antagonists. In 2 cells tested with yohimbine and 5 cells with RX 781094, clear antagonism was always seen. Prazosin and WB 4101, however, in 5 cases never produced any antagonism of NA effects, even at high currents.

Conclusions: These results were supported by further experiments using selective agonists (clonidine, phenylephrine and isoprenaline), and may explain, at the neuronal level, the greater analgesic potency of α_2 rather than α_1, or β agonists, upon intrathecal perfusion.

21. Effects of opioid peptide agonists selective for μ, δ and κ receptors on identified dorsal horn neurons

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The intrathecal infusion of opioid receptor agonists reduces behavioural responses to cutaneous noxious thermal stimuli. Compounds selective for μ- and δ-opioid receptors are clearly effective (Schmauss & Yaksh 1984) but a role for κ receptors is less well established (Han & Xie 1984). The dynorphins, which are present in the dorsal horn, are selective ligands for the κ receptor (Corbett *et al.* 1982). The effects of a dynorphin and those of μ- and δ-selective enkephalin analogues DAGO and DADL, are compared here on the cutaneous sensory responses of single identified dorsal horn neurons whose axons ascend towards supraspinal regions (spinocervical tract, SCT neurons).

Extracellular recordings were made by using multi-barrelled glass electrodes in lumbar cord segments L6/7 of cats anaesthetized with chloralose (60 mg kg^{-1}) and paralysed. Solutions of dynorphin$_{1-13}$ (DYNO), (5 mM), [D-Ala$_2$, MePhe^4, Gly-ol^5] enkephalin (DAGO), (20 mM) and [D-Ala$_2$, D-Leu^5] enkephalin (DADL), (20 mM), all at pH 4.5–5.0, were used for iontophoresis. In 14 out of 16 multireceptive SCT neurons tested with DYNO, a selective reduction was seen in the response to noxious thermal stimulation, but not to innocuous brush or to iontophoretically applied D,L-homocysteic acid. This antinociceptive effect (probably exerted at an indirect site) was not reproduced by DAGO (5/5 cells) or by DADL (6/7 cells), even at currents in excess of 100 nA. These results suggest that a κ receptor is involved in the selective antinociceptive effect of DYNO on ascending somatosensory neurons. Duggan *et al.* reported inhibitory effects of the less selective (but δ/μ preferring) analogue [Met] enkephalinamide when applied either close to, or dorsal to the somata of unidentified lamina IV neurons (see Duggan & North 1984). However, δ/μ receptors seem uninvolved here, at any accessible site, whereas κ sites may have an important antinociceptive role. The effect of DYNO was antagonized by iontophoretic application of naloxone and also the specific α_2 adrenoreceptor antagonist, RX 781094, suggesting a novel mechanism for the mediation of segmental antinociceptive effects through activation of the terminals of descending tracts.

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A selective antinociceptive effect of the diencephalic dopamine-containing cell group, A11, on dorsal horn neurones in the rat

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The dorsal horn of the spinal cord receives a restricted dopaminergic innervation (Fleetwood-Walker & Coote, 1981) from supraspinal levels, of which a major source is the A11 cell group in the diencephalon (Skagerberg, Bjorklund, Lindvall & Schmidt, 1982). Dopamine (DA) has a selective antinociceptive action when ionophoresed in the vicinity of dorsal-horn neurones (Fleetwood-Walker, Hope & Molony, 1984). Behavioural studies have shown that intravenously or intrathecally administered DA or DA agonists also have an antinociceptive effect (Barasi & Duggal, 1984; Jensen, Schroder & Smith 1984). This communication reports the effect of electrically stimulating in the area of the A11 cell group on the responses of multireceptive neurones in the dorsal horn.

Extracellular recordings were made in the lumbar spinal segments of rats anaesthetized with intravenous α-chloralose (35 mg/kg) and urethane (700 mg/kg). Multibarrel glass electrodes contained D,L-homocysteic acid (DLH), DA HCl, sulpiride and 1 M-NaCl for automatic current balancing. Bipolar or monopolar stainless-steel electrodes were used to electrically stimulate (10–100 μA, 33–100 Hz) the region of the A11 group whilst testing the evoked responses of the dorsal-horn neurones. Stimulation sites were subsequently identified histologically. The responses of sixteen neurones to noxious cutaneous stimulation (pinch), ionophoresed DLH and innocuous cutaneous stimulation (motorized brush) were tested.

The electrical stimulation caused a selective inhibition of the response to noxious cutaneous stimulation in sixteen neurones tested, whereas the responses to innocuous cutaneous stimulation or DLH were unaffected. Recovery was obtained 5–10 min after stimulation ceased. This selective effect was invariably reversed when sulpiride (a selective D_2-DA receptor antagonist) was ionophoresed in the vicinity of twelve neurones tested. Stimulation 0·5–1·0 mm outside the region encompassing the A11 group was ineffective.

These results are the first indication that the A11 cell group is probably the source of the selective antinociceptive action expressed by DA at the level of the spinal cord.

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An α_2 Receptor Mediates the Selective Inhibition by Noradrenaline of Nociceptive Responses of Identified Dorsal Horn Neurones

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Extracellular recordings were made of 59 neurones with long, ascending projections (spinocervical tract (SCT) and dorsal column postsynaptic (DCPS) neurones) in the lumbar dorsal horn of anaesthetized and paralyzed cats. All showed prominent excitatory responses to innocuous stimuli, applied to their cutaneous receptive fields on the ipsilateral hindlimb. The majority of the population investigated (83%) was multireceptive, being activated by noxious as well as innocuous cutaneous stimuli. Drug effects were examined on a regular cycle of responses to these cutaneous stimuli and also to DL-homocysteic acid (DLH).

In 49 multireceptive SCT and DCPS neurones, ionophoretically-applied L-noradrenaline (NA) produced a potent selective inhibition of the nociceptive responses (to heat or pinch) in 40 out of 44 SCT and 3 out of 5 DCPS neurones, with no statistically significant change in the responses to innocuous brush or DLH, or in spontaneous activity. NA had no effect on the majority of cells (8 out of 11) that responded only to innocuous stimuli.

In 19 SCT neurones that showed NA selectivity, the α_2 selective agonists clonidine (in 12 out of 15) and metaraminol (in 2 out of 3) mimicked this selective effect, whereas, the α_1 agonist, phenylephrine and the β agonist, isoprenaline did not. Furthermore, the α_2 antagonists, yohimbine and idazoxan (RX781094), either reversed or reduced the potency of the NA-elicited inhibition of nociceptive responses in all 7 SCT neurones tested. These results are discussed in relation to other evidence for spinal antinociceptive effects of noradrenergic systems acting at a spinal level and the possible involvement of an α_2 receptor in such effects.

INTRODUCTION

A characteristic feature of neurones in the dorsal horn of the lumbar spinal cord, that respond to cutaneous sensory stimuli is that they are subject to powerful supraspinal modulation. In particular, many of the large neurones in laminae IV and V excited by both noxious and innocuous stimuli, are subject to a descending inhibition that can selectively reduce their nociceptive responses[4,7,10,18,44]. Supraspinal noradrenergic tracts that terminate in the dorsal horn could contribute to this effect. These appear to originate entirely from the brainstem[12]. It is unclear, however, whether the different NA cell groups in the brainstem preferentially innervate specific laminae of the dorsal horn. A role for the noradrenergic system in modulating sensory transmission in the dorsal horn is suggested by a reduced behavioural response to noxious cutaneous stimuli on intrathecal perfusion of NA[23,32] and by previous ionophoretic studies on unidentified dorsal horn neurones[2,20,36]. Belcher et al.[2] found, in the cat, that NA produced a general inhibition of both nociceptive and DLH-evoked activity in multireceptive cells, but had no effect on cells responding only to innocuous stimuli. On the other hand, Headley et al.[20] reported, also in the cat, that NA caused a selective reduction of the nociceptive, but not non-nociceptive responses of multireceptive neurones. This selectivity was not observed by Satoh et al.[36] when studying rabbit dorsal horn neurones.

The present experiments were carried out to analyze in detail the effects of ionophoretically-applied NA on the sensory responses of defined populations of dorsal horn neurones; spinocervical tract (SCT)

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and dorsal column postsynaptic (DCPS) neurones. Both groups have long ascending projections and are probably involved in the transmission of cutaneous noxious and innocuous information to supraspinal levels[1,5,6,10,34].

Preliminary reports of part of this work have been made in abstract form[15,16].

METHODS

Experiments were carried out on 35 adult cats, anaesthetized with intravenous α-chloralose (60–70 mg/kg) after induction with halothane. Animals were paralyzed with gallamine triethiodide (Flaxedil, 15 mg/kg) and artificially respired with room air following bilateral pneumothorax. The effects of gallamine were allowed to wear off periodically to allow monitoring of the state of anaesthesia. Additional doses of chloralose (30 mg/kg) were given when required.

Laminectomies were carried out to expose the upper cervical cord (from C1 to C5) for antidromic stimulation of the axons of SCT and DCPS cells and the lumbar cord (segments L3–S1) for recording. The animal was then transferred to a spinal frame and the head was fixed in a head holder by blunt ear bars, bars at the orbits and under the upper jaw. The vertebral column was rigidly held by means of clamps, each attached to a vertebral spine at the sacral, upper lumbar and thoracic levels. The pelvis was fixed by pins on the iliac crests and the foot attached to a wooden block by means of plaster. Skin flaps were sewn to the frame to form pools filled with liquid paraffin. The condition of the animal was controlled by maintaining the end-tidal CO_2 at 3.5–4.0% and the rectal and spinal cord pool temperatures at 37–38 °C. Femoral arterial blood pressure and cardiac activity were monitored continuously.

Electrophysiological recording and ionophoresis

Extracellular recordings of SCT and DCPS neurones were made in lumbar segments L6–7, from the central barrel of a 5- or 7-barrelled electrode constructed from 1.5 mm external diameter, filamented glass tubing. The electrodes had a tip side of 3.5–5.0 μm and a DC resistance of 4–8 MΩ. The bandwidth of the recording amplifier was 100 Hz–7 kHz. The other barrels contained, respectively, 1 M NaCl (pH 4.0–4.5) for automatic current balancing and independent current controls, Pontamine Sky Blue (2% in 0.5 M NaAc) for marking the recording sites and various selections of the following drugs (all at 0.1 M, pH 4.0–4.5, unless otherwise indicated): DL-homocysteic acid (DLH, pH 8.0–8.5); 0.1 or 0.5 M D- or L-noradrenaline bitartrate; clonidine HCl; metaraminol HCl; phenylephrine HCl; isoprenaline HCl; 0.005 M yohimbine HCl; idazoxan HCl (RX 781094); 0.05 M WB4101 HCl and 0.0015 M prazosin HCl. Drugs were applied with cathodal currents (DLH, with anodal) between 10–80 nA using a Neurophore BH2 ionophoresis system (Medical Systems Co.). Retaining currents of 10–15 nA were applied to minimize drug leakage. The positions of some of the recording sites were marked with dye spots (50 μA min), and were restricted to laminae III, IV and V of the dorsal horn (see Fig. 1).

Identification of the neurones

Neurones were identified by the standard criteria[25] of collision of antidromic and orthodromic action potentials (see Fig. 2), together with fast following frequency (up to 300 Hz), constant latencies (± 0.1 ms) and distinct threshold for activation. Antidromic stimuli could be applied through an array of paired silver ball stimulating electrodes, at appropriate sites on the ipsilateral dorsal/dorsolateral surface of the exposed upper cervical spinal cord. These electrodes were placed on the dorsolateral funiculus at C3 and C1 and also on the dorsal columns at C4–5. Restricted surgical ablation of the dorsal columns was made at C4, rostral to the relevant stimulation site, in order to prevent mis-identification from crossing fibres. The parameters of the search stimuli used were: monophasic square wave pulses, 1 Hz, 0.5 ms pulse width, 600–700 mV amplitude. Spinocervical tract (SCT) neurones were identified in accordance with the criteria of Brown et al.[8]. All SCT neurones described, were activated from the C3 electrode, but not the C1 electrode. None of these neurones was activated antidromically from the dorsal column electrode (see Fig. 2). The small sample of neurones identified as belonging to the DCPS system[1,6], were activated antidromically only from the C4/5 dorsal column electrode (see Fig. 1).

Quantification of the cutaneous sensory responses

Neurones were excited by controlled cutaneous

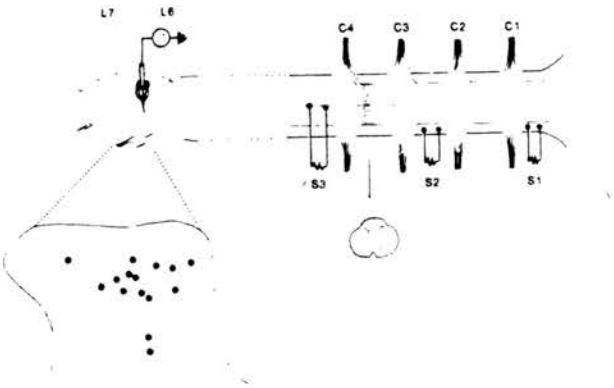


Fig. 1. Stimulating/recording sites. Extracellular recordings were made using multibarrelled electrodes in the lumbar cord of anaesthetized and paralyzed cats. Identification of neurones was by standard antidromic criteria, from pairs of silver ball stimulating electrodes at various positions on the cervical spinal cord. S1, dorsolateral funiculus at C1; S2, dorsolateral funiculus at C3; S3, dorsal columns at C4. A discrete surgical lesion (hatching) was made across the dorsal columns at C3/4 to prevent misidentification of neurones from crossing fibers. The positions of dye marks corresponding to 15 of the neurones investigated are shown in the inset diagram of a representative section of the dorsal horn (they were all identified as SCT cells). Approximate locations of the laminae (according to Rexed) are shown and all neurones lie within laminae III–V.

noxious and innocuous stimuli applied to their receptive fields on the ipsilateral foot. Innocuous stimuli were provided by a motorized, rotating brush and noxious stimuli by a thermistor controlled radiant heat lamp (giving a surface temperature ramp between 30–48 °C). These were applied to adjacent areas within the receptive field to give reproducible responses. A regular cycle of responses to these stimuli was set up and also to ionophoretically-applied DLH (as a directly acting excitant). The neurones could invariably be excited by DLH (10–70 nA). Control responses were always repeated at least in duplicate, as were many of the test responses. Test sequence results were only considered acceptable when duplicate responses varied by less than 10–15%. The magnitudes of the various responses were matched for the number of action potentials per response and were always submaximal. Action potentials of the antidromically activated neurones could be clearly discriminated from other field potentials and were continually monitored throughout drug applications for any non-specific distortion.

The cycle of stimuli was repeated every 3 min, with most drugs being ejected for 1 min before the start of a cycle (see Fig. 3). The possibility of differences due to the order in which the responses were tested, was controlled by pseudo-random ordering of the various stimuli. Ionophoretic currents were increased in a stepwise manner, between each cycle of responses. This allowed the construction of cumulative current–effect curves for each type of response, on the same neurone (see Fig. 4). After any test involving drugs, the responses were allowed to recover fully (up to 40 min) before further study. Continuous records of firing rate (over 400–2000 ms bin width) were plotted,

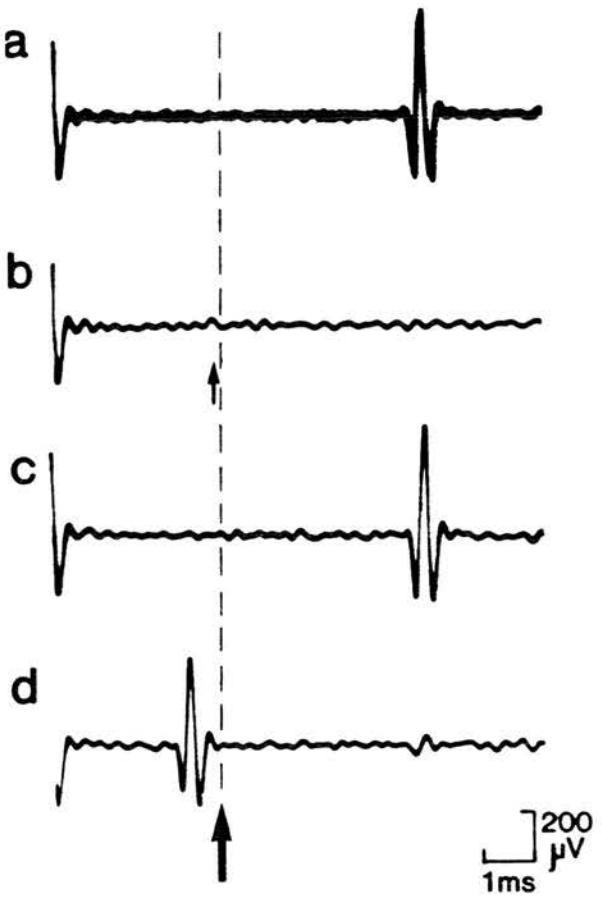


Fig. 2. Antidromic identification of an SCT neurone by collision testing; spontaneous action potentials were used to trigger the antidromic stimulus (the usual timing of which is shown by the dotted vertical line and large arrow, after a delay of 3.2 ms). (a) shows 6 superimposed sweeps of an antidromic action potential, recorded at the cell body. In (b) the delay until triggering the antidromic stimulus (here shown by the small arrow) is reduced below the critical period for this cell and collision has occurred. (c) shows the reappearance of the antidromic action potential when the original stimulus delay is restored. (d) shows cancellation when a second spontaneous potential has occurred within the critical period.

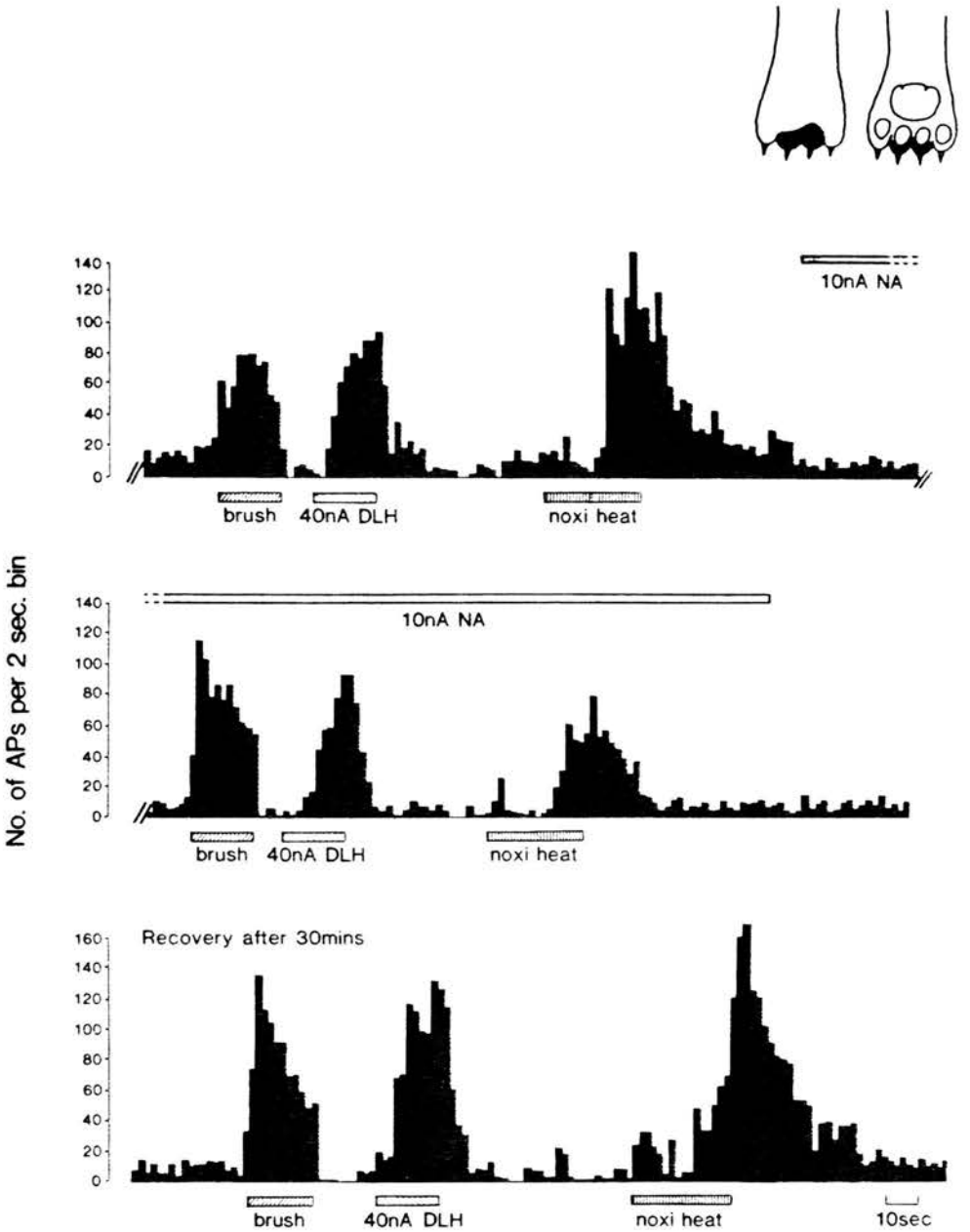


Fig. 3. Continuous records of firing rate of a neurone plotted as the number of action potentials per 2 s bin against time. Prominent excitatory responses to brush, DLH and noxious heat stimuli can be seen on the top row. Typical effects of ionophoretically applied NA can be seen (middle row), where NA has caused a selective inhibition of the response to noxious heat with no prominent change in the other responses. Effects were maximal after about 1 min of ionophoresis. Recovery, which can be seen in the bottom row, was delayed, up to 25 min. (1 M NaCl, passed at similar currents had no effect, data not shown.)

together with the analogue signals from the stimulators and Neurophore, and then stored on floppy disc (Cromemco system III). Data was analyzed 'on'- and 'off'-line by integrating the numbers of stimulus-induced action potentials in selected epochs. The integrated responses were normalized and expressed graphically, to permit comparisons of the potency of a particular drug on the different types of activity.

RESULTS

(1) Characteristics and receptive fields of the neurones

The properties and locations of the SCT neurones were very similar to those reported previously[5,10]. Neurones could be driven antidromically from the C3 electrode, usually at frequencies up to about 300 Hz and with latencies constant to within ± 0.1 ms. The calculated conduction velocity of these neurones was high, with little variance through the population (53.5–2.7 m/s; n = 26). The cutaneous receptive fields of the neurones were all on the ipsilateral hind paw and toes and remained topographically constant throughout the recording. All the neurones encountered were excited by the innocuous cutaneous stimuli and about half of them were additionally excited by the noxious stimuli. Attention was concentrated here on the modality-convergent (multireceptive) neurones. Nociceptive responses were generally most intense at the centre of the innocuous receptive field and were never elicited from outside its perimeter. Inhibitory components of the responses were rarely observed in these neurones and were not analyzed in detail.

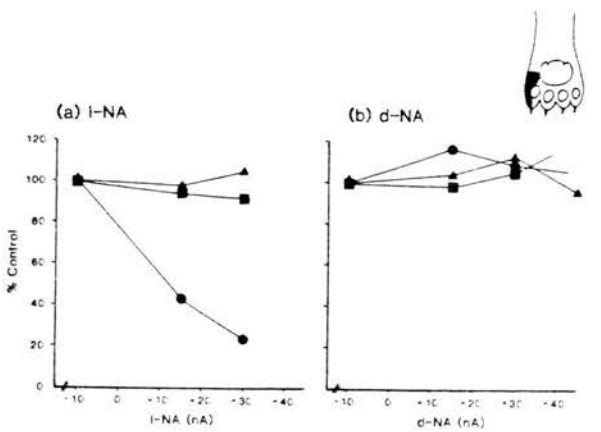


Fig. 4. Typical effects of NA on an SCT neurone. Each response has been integrated and expressed as a percentage of the control response. (a) Cumulative application of the L-isomer of NA produced a potent selective inhibition of the responses to noxious heat (circles) at currents giving little or no reduction (< 20%) in the brush (triangles) or DLH-evoked activity (squares), or spontaneous activity (not shown). (b) shows that this effect is not mimicked by the D-isomer of NA. There is no effect on any of the responses, indicating a receptor-like stereospecific requirement of the effect. D-NA bitartrate was prepared by resolution from the racemic mixture, using the selective crystallisation method of Tullar et al.[41] (1948). Melting point analyses on samples of the product confirmed its identity.

The DCPS neurones recorded displayed properties similar to those described previously[1,6,34] and showed parameters of antidromic activation generally similar to the SCT cells. The conduction velocity of these neurones was 39.6 ± 1.9 ms (n = 9). The receptive fields were on the ipsilateral hind paw and toes, as for the SCT neurones, but they sometimes showed boundary changes over a time scale of about an hour.

(1a) Effects of ionophoretically-applied noradrenaline

(i) General. In the present experiments, NA failed to cause any change of rapid onset (i.e. with a latency of < 20 s) in either the spontaneous or the DLH-evoked activity of the neurones. This is consistent with there being little direct noradrenergic innervation of SCT or DCPS neurones. Indeed, the modulatory effects observed (see below), required about 1 min of ionophoresis before reaching a steady-state for that current. Recovery was prolonged, often taking 25–30 min to complete.

(ii) Multireceptive neurones. When NA was applied ionophoretically to neurones responding in turn, to brush, DLH and noxious heat stimuli, the dominant effect observed in 40 of 41 SCT and 3 of 5 DCPS neurones, was a selective inhibition of the response to noxious heat, with little or no effect on the other evoked or spontaneous activity (see Fig. 3). This effect was seen both on cells with very low and also higher threshold innocuous receptive inputs, corresponding to the subtypes II and III of multireceptive SCT neurones described by Brown[5]. In each case, the ionophoretic current was increased in a stepwise progression whilst repeating the cycle of stimuli. The cumulative current–response curves which can then be constructed (although probably non-linear with respect to concentration at the synapse), enable a direct and reliable comparison of the relative sensitivity of different responses to applied substances (see Fig. 4). Selective inhibition by NA of nociceptive responses was observed irrespective of the order of application of the stimuli, or of the magnitude of the heat response compared to the other responses. Duplicate observations of control responses and NA effects were very similar when either the stimulus-cycles were repeated, or the cycle sequence was altered. The nociceptive responses were very much more sensitive to NA than the other responses.

Low currents of NA (generally only 10–20 nA), were required to produce prominent inhibition of nociceptive responses. Occasionally, even removal of the retaining current was sufficient to produce an effect. In contrast, the other responses were usually unaffected (less than 15–20% change, up to currents in excess of 40 nA (Fig. 4)). At higher currents, some generalized depression of activity became apparent. A quantitative estimate of the degree of selectivity is shown in Table I for 16 SCT neurones. From the cumulative current–response curves, estimates were made in each case of the current which should correspond to 50% inhibition of the nociceptive response. At this current, the percentage of each other type of activity remaining was calculated. The means ± S.E.M. (n = 16) of these values are shown in the table, and clearly all remain very close to control values, being not significantly different from control values ($P > 0.05$) by the sign test R statistic or by Student's t-test on the raw data. At currents of NA estimated to give a mean 50% reduction in the noxious heat response (14.6 ± 6.1 nA), there was no significant change in either brush-, DLH-evoked, or spontaneous activity. Similar effects were seen irrespective of the NA concentration used in the electrode, (slightly lower currents being required with 0.5 M than with 0.1 M NA). Ionophoresis of 1 M NaCl at similar currents, had no effects on any of the responses. All the effects described, were obtained with the bitartrate salt of L-NA and in several further trials, similar effects were seen with the hydrochloride salt. The selective effects of L-NA bitartrate were not reproduced by D-NA bitartrate, even at more than 5-fold greater currents (see Fig. 4). This marked stereoselectivity is consistent with the selective antinociceptive effect of NA being a receptor-mediated process.

(iii) Neurones responding to innocuous stimuli only. NA, at currents up to 40–50 nA, had no apparent effect on brush- or DLH-evoked activity, or on the spontaneous activity of 4/6 SCT and 4/4 DCPS neurones which responded only to innocuous cutaneous stimuli, (see Fig. 5). Two SCT neurones showed a weak non-selective inhibition of all activity at currents greater than 40 nA.

(1b) Effects of adrenergic agonists on multireceptive neurones

These experiments were restricted to SCT neurones, because of the greater stability of their receptive fields during long recording periods. Agonists were applied to multireceptive neurones, using the same strategy that had been used with NA to give quantitative comparisons of any differential sensitivity.

(i) α_2-selective agonists. The imidazoline derivative, clonidine, shows a marked selectivity (often in the order of 100-fold), for α_2 compared to α_1 receptors in a variety of systems ranging from ligand-receptor interaction models, to functionally operative receptor systems[38,43,45]. In view of a recent suggestion[28] that there may be distinct subtypes of α_2 receptors activated by imidazoline and phenylethylamine classes of agonist, an example of this latter type was

TABLE I

Summary of effects of NA on responses

The degree of selectivity in a sample of 16 of the multireceptive SCT cell population tested. At currents of NA estimated to give a 50% reduction in the noxious heat response, there was no significant change from control levels in any other type of activity (Students matched paired t-test on raw data). The mean and standard error values are shown, together with the range of observed values (0–39 nA) in the first column.

Ionophoretic current of NA for 50% inhibition of response	*% of control at current giving 50% inhibition of heat response*		
Heat	*Brush*	*DLH*	*Spontaneous*
14.6 ± 3.5 nA (0–39)	101.5 ± 6.1	93.3 ± 5.6	89.3 ± 8.6

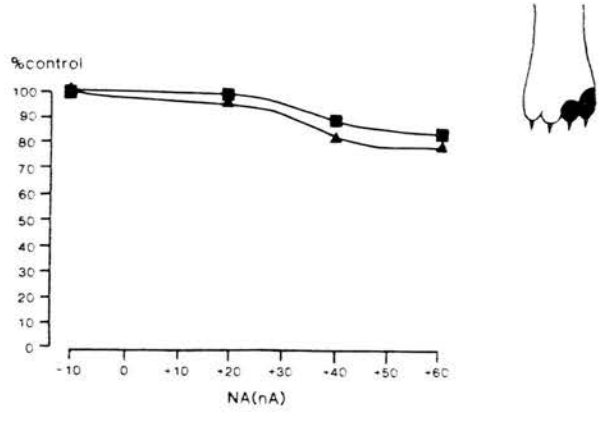


Fig. 5. Typical example of an SCT neurone that responded only to light tactile stimuli (brushing). NA (at currents up to 40–50 nA) had no marked effect (i.e. < 20% change) on these cells. Triangles, brush; squares, DLH-evoked activity.

also tried. The compound, metaraminol, was chosen that in addition to being an inhibitor of NA uptake, appears also to be a potent receptor agonist with several-fold selectivity for α_2 over α_1 receptors[45].

Ionophoretically-applied clonidine (10–60 nA), showed the same selective inhibition of nociceptive responses as NA in 12 out of 15 neurones (80%), with little or no effect on other activity (see Fig. 6 and Table II). The remaining 3 neurones showed little effect even up to 60–80 nA clonidine.

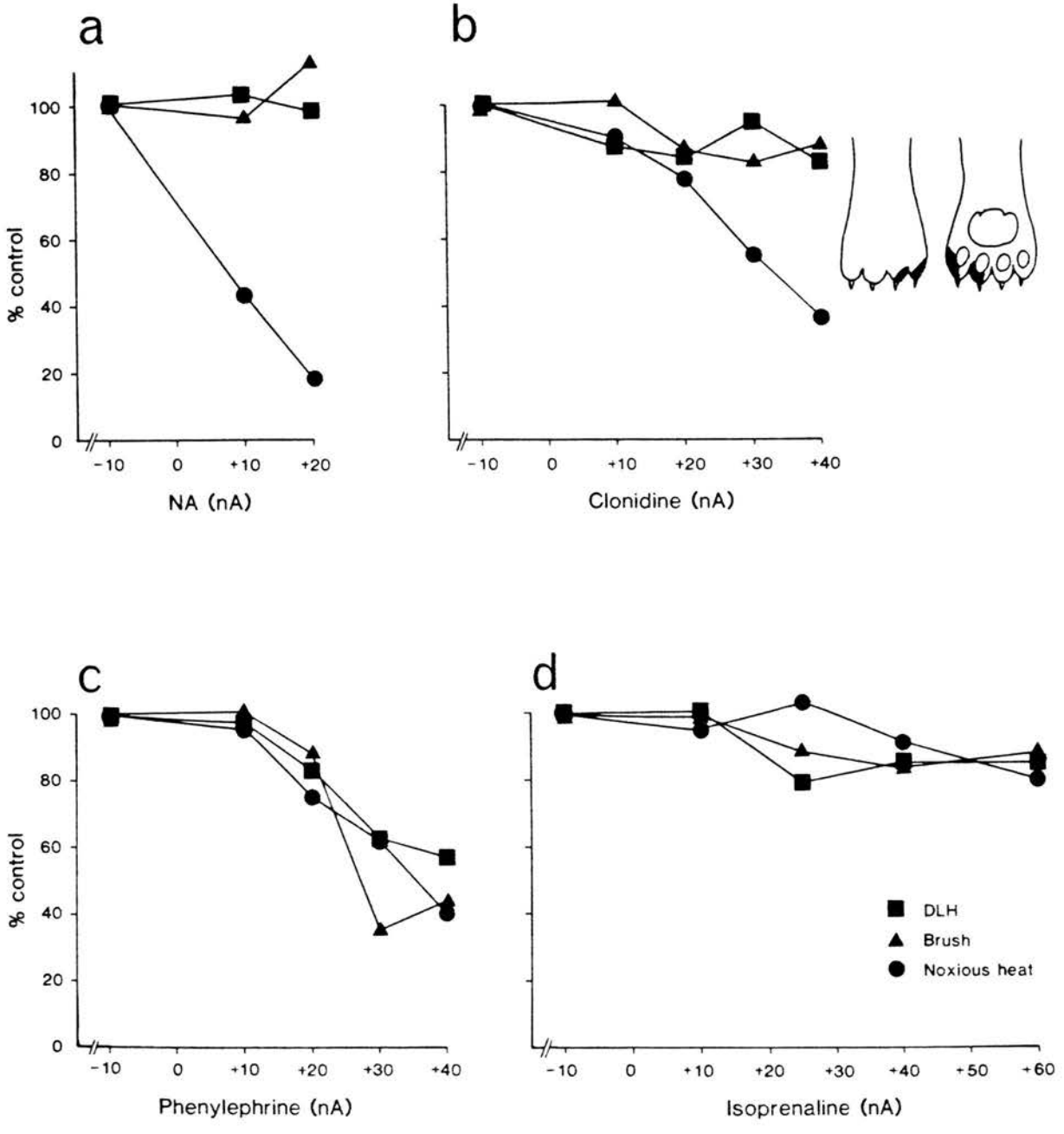


Fig. 6. A neurone in which the selective effect of NA (a), was mimicked by the α_2 agonist, clonidine (b). The α_1 agonist, phenylephrine (c) produced a non-selective depression in all the responses, whilst the β agonist, isoprenaline (d), showed little effect at all. Circles, noxious heat; triangles, brush; squares, DLH-evoked activity.

TABLE II

Effect of agonists

The table summarises the effects of adrenergic receptor agonists, compared with the effect of NA, on the responses of multireceptive SCT neurones. The selective effect of NA appears to be mediated through an α_2-type receptor.

	Total number of cells	*Selective inhibition of noxious responses*	*Unselective inhibition of all responses*	*No marked effect (< 20%)*
NA	28	27	1	0
Clonidine	15	12	0	3
Metaraminol	3	2	0	1
Phenylephrine	11	2	6	3
Isoprenaline	6	0	2	4

Metaraminol was tested on 3 neurones. In two of them it exhibited a clear margin of selectivity, like NA.

(ii) α_1- and β-selective agonists. Phenylephrine, which has a pronounced selectivity for α_1 rather than α_2 adrenoceptors[38,43,45] was tested on 11 cells that showed a selective effect of NA on nociceptive responses. Phenylephrine (10–50 nA) failed to reproduce this selectivity in 9 cells (80%). Six of these neurones showed a non-selective inhibition of all types of response (see Fig. 6 and Table II), while 3 exhibited no changes at all up to 50 nA. In the remaining 2 cells, the response to noxious heat appeared to be slightly more sensitive to inhibition by phenylephrine, but all activity was reduced to a similar extent and there was clearly no distinct margin of selectivity as was observed with NA, clonidine and metaraminol. In view of the generalized inhibition of activity observed in a number of cells, the possibility was investigated that there might be a population of α_1 receptors, not involved in any selective influence on nociceptive inputs, but present directly on SCT cells. However, in a further 5 neurones, driven at a steady rate by DLH, phenylephrine failed to cause any inhibitory effects, of either rapid or delayed onset in 3 of the cells (even up to 80 nA). In the other 2 cells, a modest generalized inhibition was seen at high currents (> 60 nA), but this effect could not be reversed by α_1 antagonists (see below).

The potent and selective β agonist, isoprenaline, was also tested on 6 cells. Isoprenaline has a large margin of selectivity for β rather than α_2 or α_1 receptors[43]. It produced no marked effect here on any response, even up to currents of 60–80 nA. Four neurones showed absolutely no effect, right through the current range (see Fig. 6 and Table II) and the remaining 2 showed only very minor, generalized inhibition at the highest currents.

(1c) Effects of adrenergic antagonists

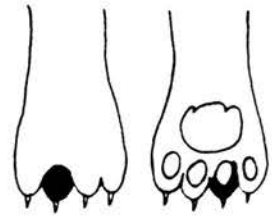
In 7 SCT neurones showing selective inhibition of nociceptive responses by NA, the effect was antagonized by the selective α_2 antagonists[11,24], yohimbine (2 cells) and idazoxan (5 cells). Acute reversal (mean 49%) of the NA effect was seen with idazoxan but not with yohimbine, although the time-course of recovery from NA was markedly shortened by a factor of 2–3. Ionophoresis of antagonists prior to the NA test (for 12–20 min at currents of 20–50 nA) always reduced the NA inhibition, in comparison with a previous control test (see Fig. 7). Under these conditions, idazoxan and yohimbine produced a mean reversal of the NA effect of 82% and 75%, respectively, with no apparent change in control responses. The α_1 selective antagonists, prazosin[9,38] and also WB4101[26,38], never produced any reversal of the NA effect when tested on 5 SCT neurones. Neither acute or chronic application (up to 80 nA for 20 min) produced any effect other than some non-specific spike distortion and excitation at the higher currents used.

DISCUSSION

The present experiments establish that ionophoretically applied NA selectively inhibits the responses to noxious cutaneous stimulation of SCT and DCPS neurones of the cat, whilst leaving unaffected the responses to innocuous mechanical stimuli, or to an excitant amino acid. These effects indicate the presence of specific adrenergic receptors at sites in the dorsal horn that could mediate similar effects from descending noradrenergic systems. The neurones investigated have been characterized as having long projections, ascending the spinal cord, and if such noradrenergic systems are functionally operative, an important regulation of incoming somatosensory information could be occurring before it reaches supraspinal levels.

Evidence for the site of action of NA

The probable explanation for the selective effect



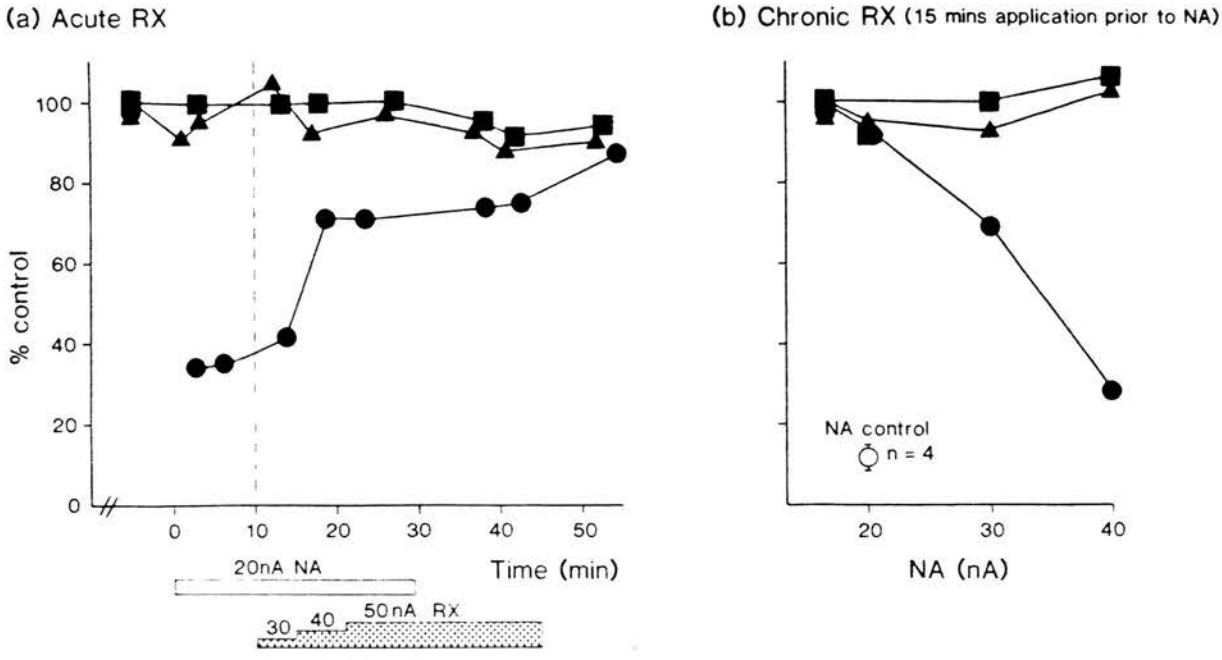


Fig. 7. Antagonism of the selective effect of NA: responses evoked by the various stimuli are shown as follows: ▲, brush; ■, DLH; and ●, noxious heat. a: acute application: an example of an SCT neurone where the selective inhibition of the noxious heat response produced by NA, was rapidly reversed by increasing currents of the α_2 antagonist, idazoxan (RX 781094). b: chronic application: the antagonist was applied at 40 nA for 15 min prior to the NA test whereupon the potency of the NA selective effect was found to be greatly reduced. Control tests, with NA alone, showed that 20 nA of NA gave a 90% reduction in the noxious heat response, but after application of the α_2 antagonist, this degree of inhibition was not seen until a current of more than 40 nA was reached. This antagonist had no effects alone on any of the control responses and similar currents of NaCl did not show any change in the degree of NA selectivity.

of NA is an action at a site remote from the cell being recorded, since a postsynaptic inhibition of the neurone would be expected to cause non-selective inhibition of rapid onset. The receptor sites recognizing NA, however, are likely to be at loci restricted to a specific nociceptive input pathway rather than non-specifically depressing all polysynaptic inputs, since the brush response (which was unaltered by NA) probably activates both polysynaptic and monosynaptic inputs to SCT neurones[5,21].

The neurones investigated here, like a large proportion of the unidentified lamina IV–V neurones described by Headley et al.[20], showed selective effects of NA. However, the margin of selectivity seen here, is more distinct than these authors reported, who often also observed some reduction of the response to innocuous stimuli and sometimes, an inhibition of spontaneous activity. This greater margin of selectivity may be due to different populations of neurones studied, or may simply have been revealed by our use of comparisons through a range of iontophoretic currents. In similar experiments, Belcher et al.[2] found that NA reduced all activity (either spontaneous activity or that evoked by noxious stimuli and

DLH) in unidentified, multireceptive neurones, but little effect was observed on non-nociceptive cells. They interpret their observations as suggesting a different pattern of noradrenergic influence: a direct input onto multireceptive neurones, but not onto non-nociceptive cells. Although on published evidence it is not possible to decide that a substantially different population of neurones may have been examined, both this and another group[36] reporting non-selective inhibition by NA, used high ionophoretic currents of NA (up to 100 nA) at which levels we often observed generalized depression of activity.

The precise receptor site(s) mediating the selective antinociceptive effect of NA here, could be at any point from primary afferent terminals through a number of antecedent neurones in a polysynaptic input. The termination of many fine, unmyelinated Aδ- and C-fibres, appears to be largely confined to the superficial dorsal horn around laminae I and II (a region which has a high density of α_2, but not α_1 receptor sites[13,47]), whilst dendrites of SCT cells are reported to extend dorsally only into lamina III[5]. There is evidence that some primary afferent neurones have specific receptors for NA[14], and ionophoretically applied NA has been reported to increase the threshold of single sural C-fibres to antidromic activation[22], although afferent fibres degenerating after dorsal rhizotomy do not appear to be in close proximity to noradrenergic terminals[35]. There are reports that unidentified cells in the superficial dorsal horn are either excited[40], or hyperpolarized[29] by NA. The latter effect apparently being mediated by an α_2 receptor. The relation of these observations, however, to the selective antinociceptive effect seen here, is difficult to establish. The site of the selective action of NA might be closer to SCT somata than laminae I and II. This is suggested by the observation of Headley et al.[20], that selective effects of NA required lower currents when ejected in laminae IV/V rather than lamina II and supported by the marked potency of NA, ionophoresed in lamina III/IV/V here. It might be possible that selectivity could be produced by a direct noradrenergic input, restricted to dendrites receiving nociceptive inputs. In SCT cells, however, we did not find the concomitant inhibition of spontaneous activity which might be predicted for such a mechanism, although other groups working with heterogeneous populations often did. Furthermore, electron microscopic investigation of the dendritic trees of SCT neurones has not revealed synapses with terminals containing the dense core vesicles characteristic of monoamines[27]. Clearly, confirmation that the NA effect on SCT cells is indirect rather than direct, requires the use of intracellular recording techniques.

Characterization of receptors mediating the effect of NA

The selective inhibition of nociceptive responses by L-NA, but not D-NA (Fig. 4), establishes the stereospecificity typical of receptor-mediated effects. Although this was not observed in an early report of general inhibitory effects on unidentified dorsal horn neurones[15], it is now clear that α_2 receptors show much greater affinity for the L- rather than the D-isomer[43]. The selective effect of NA was mimicked by clonidine and metaraminol, two α_2 receptor-preferring agonists. Antagonism of the NA effect by yohimbine and idazoxan, confirms the involvement of an α_2 receptor. No antagonism was observed with the potent α_1-selective antagonists, WB4101 and prazosin, although these substances, ionophoretically applied, are readily capable of reversing agonist effects at α_1 receptors[3,33].

The lack of selective effects of the α_1 and β agonists phenylephrine and isoprenaline, provides further support for the conclusion that an α_2 site mediates the NA effect. The generalized inhibition produced by phenylephrine in a minority of cells was not reversed by α_1 antagonists, suggesting that α_1 receptors may not be involved.

Functional aspects of the NA effects

Our results predict that α_2 receptors could play a large part in any endogenous spinal antinociceptive effects of NA. Intrathecal application of drugs has been used to investigate a potent spinal effect of NA in producing behavioural changes suggestive of analgesia. In rats and in primates, α_2 but not α_1, or β agonists, produced behaviour consistent with selective inhibition of both propriospinal and supraspinal reflexes to noxious stimulation, apparently without effects on other reflexes or muscle tone[31,46]. Similarly, intrathecal application of α_2 antagonists is reported to produce the inverse effect[30]. Recent experiments on central or systemic administration of adren-

ergic drugs, indicate that there may also be a contribution from an α_1 receptor to antinociceptive effects[19]. Clearly, whilst α_1 receptors do not appear to regulate sensory input to the neurones described here, they might well act on some other component of the behavioural reflex tested.

The selective inhibition by NA of nociceptive input to these dorsal horn neurones, could form the substrate for an endogenous antinociceptive system, regulating not only spinal sensory responses, but also the transfer of information to higher levels. It is important to consider whether such a system is tonically active, or activated only by particular stimuli. Intrinsic behavioural effects of intrathecal α_2 antagonists, might suggest some tonic influence, but there is evidence that noradrenergic systems are unlikely to contribute greatly to the tonic descending inhibition of dorsal horn neurones[37]. Furthermore, in the present experiments, ionophoresis of α_2 antagonists alone, close to SCT neurones, had no direct effect, indicating that SCT cells, at least, are not subject to a tonic regulation by NA. Nevertheless, it is clear that stimuli of appropriate intensity to activate C fibre afferents, elicit increases in both the metabolic turnover of NA in the dorsal horn[39], and the release of NA into intrathecal perfusates[42]. The questions of specificity and relevance of this response, the mechanism by which it is produced and how it relates to the function of other putative antinociceptive systems, clearly need to be answered.

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