THE ROLE OF DIFFERENT 5-HT RECEPTOR SUBTYPES IN MODULATING NOCICEPTION IN THE RAT.

by

Nada El-Yassir

University of Edinburgh

Doctor of Philosophy,

1988

I certify that a substantial part of the work presented here is entirely my own. The investigation into the action of serotonin receptor agonists in lamina I was undertaken in collaboration with Dr. P.J. Hope. Some of the material discussed in this thesis has been presented at meetings and / or published and reprints are included in an appendix to the thesis.

Nada El-Yassir

ACKNOWLEDGEMENTS

This work was sponsored by a scholarship from the faculty of veterinary Medicine, University of Edinburgh. My thanks go primarily to my supervisor, Dr. S.M. Fleetwood-Walker, for the fruitful discussions and for her continued interest and advice throughout this project. Thanks also to Dr. P.J. Hope for invaluable help and support and to Dr. R. Mitchell for the provision of appropriate compounds and for his valuable comments. I would also like to express my appreciation of the technical assisstance I have received from Mrs. H. Anderson and the staff of the photographic, electrical and mechanical workshops, as well as the staff of the Wellcome Animal Research Unit. Last but not least my sincere gratitude goes to my father, who has been a source of inspiration to me and to my family and friends who have provided me with support throughout the past three years.

"...Men ought to know that from the brain, and from the brain only, arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, griefs and tears... These things that we suffer all come from the brain, when it is not healthy..."

attributed to Hippocrates

"... Pleasures and pains in excess are the greatest of the soul's diseases."

Plato

"... An excess of intensity of tangible qualities destroys the animal itself."

Aristotle

CONTENTS

ABSTRACT

Several lines of evidence have implicated a role for the descending serotonergic system in the modulation of somatosensory transmission and in analgesia. It is now known that serotonin (5-HT) has more than one type of receptor. The aim of the present experiments was to investigate the involvement of different 5-HT receptor sites in antinociception and analgesia. By employing agonists and antagonists selective for different types of 5-HT receptors in ionophoretic experiments, it was shown that 5-HT_1 and not 5-HT_2 receptors were responsible for mediating the heterogeneous effects of ionophoretically applied 5-HT on rat dorsal horn neurones. Two different 5-HT_1 receptor subtypes were found to mediate qualitatively different effects, often on the same cell. Whereas the 5-HT_{1B} site appeared to mediate the selective antinociceptive effect of 5-HT, non-selective effects of ionophoretically applied 5-HT, appeared to be exerted through the 5-HT_{1A} receptor site. Focal brainstem stimulation experiments have demonstrated that both the selective antinociceptive and non-selective inhibitory effects of stimulation in the medullary serotonergic nucleus raphe magnus on dorsal horn neurones are mediated through a 5-HT_1-type receptor. Thus a 5-HT_1 and not a 5-HT_2 receptor antagonist could readily reverse the effects of brainstem stimulation. A pilot behavioural study investigated the analgesic potential of two different 5-HT_1 receptor subtype agonists and preliminary evidence appears to confirm the involvement of the 5-HT_{1B} receptor type in behavioural analgesia. The findings of the present study are discussed in relation to previous reports in the literature.

INTRODUCTION

I General Introduction

A. Theories of Pain Mechanisms

Sherrington(1906), described as "noxious" all types of stimuli or events whose intensity is capable of damaging tissue. The detection and signalling of these noxious events is referred to as nociception, a process which may evoke pain in the conscious animal. The scientific community concerned with the study of pain has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such a damage" (International Association for the Study of Pain (IASP), Pain, 1979, 6, 50). Pain, unpleasant as it is, serves a vital and adaptive role in the animal, by acting as a warning system against potentially damaging situations. The importance of pain is clearly illustrated by the serious handicap which people without it suffer (e.g. Sweet, 1981). Pain possesses a highly subjective nature, it is perhaps not surprising therefore that this nature has been the subject of considerable philosophical and scientific debate.

The 19 th century witnessed serious discussion about the nature and mechanisms of pain between advocates of the "specificity theory" on the one hand, and the "intensity theory" on the other. The specificity theory holds that pain is a specific modality which has sense organs in the periphery, specialised to detect noxious stimulation, and its own central neuronal pathway which signals the presence of such stimulation. This theory derives from Muller's "Law of Specific Sensory Energies" which states that the quality of our sensation depends upon the peripheral and central pathway stimulated,

rather than on the stimulus itself (Muller, 1842). Goldscheider (1884), described "temperature points" in the skin that were not pain sensitive. Von Frey (1896), later found that stimulating certain points in the skin with fine probes (Von Frey hairs) produced a "painful sensation with no preceding or accompanying pressure sensation". Based on these observations, Von Frey concluded that "pain is the result of exciting special organs".

The intensity theory argues against pain being a separate modality. Pain, according to this theory, results from over-stimultion of non-specific structures and / or the central summation of inputs. Thus very intense sound, light or pressure can be painful. The earliest advocate of this theory was Goldscheider (1884). He mapped areas of the skin, using a pin as a probe, for points where touch is perceived; these he called "pressure points". He based his theory upon the observation that pain could be elicited by intensely stimulating these pressure points.

The gate control theory of Melzack and Wall (1965), tried to incorporate concepts of specialisation as well as certain aspects of the intensity theory. The gate-theory proposed that the substantia gelatinosa (the SG, an area of the spinal dorsal horn, densely packed with interneurones) functions as a gate which controls the afferent signalling patterns before they reach cells which transmit information directly to supraspinal sites. The activity of this "gate" is in turn dependent upon the balance of activity in larger, generally non-nociceptive A fibres, and that in fine, generally nociceptive Aδ- and C- fibres. This theory has been given support by demonstrations that activation of large fibres can indeed inhibit nociceptive responses of dorsal horn neurones (e.g. Cervero, Iggo and

Ogawa, 1976; Gregor and Zimmermann, 1973), suggesting that this phenomenon could represent the basis of analgesia obtained in humans by transcutaneous or dorsal root stimulation (Nathan and Wall, 1974; Wall and Sweet, 1967).

Interesting as it is, the gate control theory as originally conceived, is no longer a satisfactory experimental model for pain mechanisms (Nathan, 1976). Its main weakness is that it neglected known facts about peripheral nerve fibres. Aδ- and C-fibres do not form a functionally homogeneous group and some can be maximally activated by innocuous cutaneous stimulation (e.g. Beck, Handwerker and Zimmermann, 1974; Bessou and Perl, 1969; Burgess and Perl, 1967; 1973; Iggo, 1960; Perl, 1968). Furthermore, the gate theory does not accommodate the population of lamina I (the most superficial layer of the dorsal horn) neurones, reported to transmit purely nociceptive information directly to supraspinal structures (see below). Evidently, elements of both the specificity and the intensity theories are correct, but neither fully accounts for the state of our knowledge about pain mechanisms and pathways which may be summarised as follows :

1) there are specialised peripheral receptors for detecting information on harmful enviroments.

2) there are neurones which receive afferent input from these receptors and which can transmit this information to supraspinal centres.

3) the nociceptive afferent input is subject to segmental as well as descending modulation from brain centres.

B. Cutaneous Receptors

Cutaneous receptors are the sensory units which transmit information about the environment centrally. It was Adrian (1931) and Zotterman (1939) who first demonstrated that noxious stimulation evoked activity in unmyelinated C- fibres and in slowly-conducting myelinated Aδ-fibres. The involvement of thin myelinated and unmyelinated fibres in nociception and pain now seems certain (e.g. Dubner, 1985). Thus experiments involving stimulation of cutaneous nerves in conscious man have demonstrated that although a sharp pain could be elicited from selective stimulation of A- fibres, prolonged painful sensation resulted only when stimulus intensity was sufficient to excite C- fibres (Torebjork and Hallin, 1973). Electrophysiological and anatomical studies of cutaneous sensation have resulted in the description of several different types of receptors in the skin which may be broadly grouped into three main classes; mechanoreceptors, thermoreceptors and nociceptors.

Mechanoreceptors appear to be mainly concerned with signalling displacement of the skin (for a review see Burgess and Perl, 1973; Iggo, 1966; 1974a). Thermoreceptors are apparently selectively concerned with transmitting information on skin temperature and may therefore be involved in temperature control (for reviews see Hensel, 1973; 1974; Hensel and Iggo, 1971). Thermoreceptors and mechanoreceptors differ from the third class of cutaneous receptors, the nociceptors, in that they are low threshold sensory units responding to relatively low levels of stimulation. Experiments where the level of stimulation was increased above threshold, but without being overtly damaging, demonstrated that the response pattern of these units did not differentiate between noxious

and innocuous stimuli (Bessou and Perl, 1969; Perl, 1968). Nociceptors, on the other hand, appear to respond to potentially damaging stimuli or to chemicals released in inflammatory responses (for review, see Burgess and Perl, 1973; Dubner and Bennett, 1983; Iggo, 1974a; Price and Dubner, 1977). Some nociceptors (mechanical nociceptors) appear only to respond to high threshold mechanical stimulation and seem to be generally insensitive to thermal stimulation or chemical irritants, though some may be weakly activated by innocuous mechanical stimulation. Most mechanical nociceptors have thin myelinated fibres of the Aδ- type, though some have unmyelinated C- fibres (Burgess and Perl, 1973; Iggo, 1960). Mechanothermal nociceptors, on the other hand, apparently respond to both noxious heat or cold, as well as damaging mechanical stimuli. Some mechanothermal nociceptors are in addition, excited by chemical irritants applied to the skin and have been termed polymodal nociceptors (Beitel and Dubner, 1976a, b; Bessou and Perl, 1969; Kumazawa and Perl, 1977; Price and Dubner, 1977). Afferent fibres from mechanothermal nociceptors are usually unmyelinated, though some are of Aδ- calibre (e.g. Beck et al, 1974; Iggo, 1959).

From the above, it can be concluded that there is a certain degree of specialisation of sense organs for signalling the presence of damaging stimuli. It is important to consider whether this specialisation in the periphery is preserved centrally in the form of specialised pathways or modes of signalling nociceptive information.

C. Classification of Dorsal Horn Neurones

The heterogeneous population of dorsal horn neurones can be conveniently classified according to their responses to natural

cutaneous stimulation (see for example Dubner and Bennett, 1983; Handwerker, Iggo and Zimmermann, 1975; Iggo, 1974b; Menetrey, Giesler and Besson, 1977) :

1) Non-nociceptive Cells also known as class 1 cells (Iggo, 1974b). These cells appear to respond to stimulation of low threshold mechanoreceptors. It seems unlikely that these cells play a direct role in nociception, since they are maximally activated by innocuous stimulation. These cells however generally have small, well defined peripheral receptive fields which might indicate that they are involved in stimulus localisation. Non-nociceptive cells do not seem to form a homogeneous group as the mechanoreceptive primary afferent input type can vary from one cell to another.

2) Multireceptive Cells (Mokha, McMillan and Iggo, 1981) also known as wide dynamic range (WDR) (Mendell, 1966) or class 2 neurones (Iggo, 1974b). These cells appear to be excited by innocuous mechanical stimulation, but also by noxious heat and mechanical stimulation. The graded response pattern to increasing levels of stimulation seems quite characteristic of WDR cells (Handwerker et al., 1975; Mendell, 1966; Menetrey et al., 1977). The receptive field of multireceptive cells is generally described as relatively large with the centre responding to both tactile and noxious stimulation, surrounded by an ill-defined larger area which seems to respond only to noxious stimulation. The large, often fragmented receptive fields of these cells appear to include, in addition to cutaneous inputs, inputs from the muscle and viscera (e.g. Foreman, Schmidt and Willis, 1977; Hancock,Foreman and Willis, 1975; Pomeranz, Wall and Weber, 1968). Many multireceptive cells seem to be part of ascending somatosensory tracts carrying information to supraspinal

levels which would indicate a role for this group of cells in nociception, although what exactly that role may be is still debated (Le Bars and Chitour, 1983; Perl, 1984; Price and Dubner, 1977; Wall, 1980). It is worth noting here that the response properties of cells are not absolute and can be subject to modulation from supraspinal structures. thus it appears that the removal of descending inhibition and barbiturate anaesthesia may unmask wide dynamic range properties in previously non-nociceptive cells (Brown, 1971; Collins and Ren, 1987; Price and Mayer, 1975; Wall, 1967). Nevertheless, the existence of multireceptive cells in the normal dorsal horn seems likely since they have been observed by different workers, working on different species with different anaesthetics and types of preparations (e.g. spinal, intact or decerebrate animals).

3) Nocispecific Cells also known as nociceptive specific (Price and Dubner, 1977) or class 3 cells (Iggo, 1974b). They were first described by Kolmodin and Skoglund (1960). These cells do not appear to respond to light mechanical stimulation of the skin, or to hair movement, but respond vigorously to high intensity thermal and mechanical stimulation as well as to electrical stimulation of Aδ- and C- fibres (Cervero et al., 1976; Christensen and Perl, 1970; Menetrey et al., 1977). Nocispecific cells normally have small peripheral receptive fields, although there appear to be some cells of this type which have large receptive fields (Giesler, Yezierski, Gerhart and Willis, 1981b). Nocispecific cells seem to be excellent candidates for signalling intensity and for localisation of the noxious stimulus and are likely to be an important part of the nociceptive system (see Price and Dubner, 1977 for a review).

In conclusion, it appears that aspects of the specialisation displayed by sense organs in the periphery may be preserved in the dorsal horn through a class of nocispecific neurones and non-nociceptive neurones. The specific role of different classes of neurones in nociception and pain is yet to be determined. Perhaps recording from awake animals at the single neuronal level can help us relate response characteristics of cells to animal behaviour and thus provide us with better understanding as to the function of these different classes.

D. Laminar Organisation and Cutaneous Afferent Input to the Dorsal Horn

Rexed (1952; 1954; 1964), based his cytoarchitectonic scheme which divided the spinal gray matter into nine different layers, upon the examination of 100 μm thick Nissl-stained sections of cat spinal cord. The dorsal horn, according to this scheme extends roughly from lamina I to lamina VI. Rexed's scheme has proved very useful and has therefore been widely adopted. The scheme does however contain an inherent weakness in that it fails to provide information on dendritic and synaptic distribution necessary for understanding spinal cord function. Anatomical studies, using Golgi and horseradish peroxidase (HRP) intracellular staining methods (see Brown, 1981; Scheibel and Scheibel, 1968; 1969) have addressed this gap in our knowledge and have demonstrated the dendritic organisation of cells as well as terminal arborization of afferent input to the dorsal horn in the various laminae of Rexed. Other studies investigated the physiology of cells in different laminae. It soon became clear that laminar boundaries were difficult to define in

terms of either detailed morphology or physiology. Characteristic differences have however been demonstrated between neurones in laminae I and II and those in deeper laminae III-V.

Lamina I

This is the most dorsal and thinnest layer, known as the marginal zone, and cells of origin of several tracts ascending directly to the brain have been demonstrated to lie in this region (Brown, 1981; Brown, Fyffe, Noble, Rose and Snow, 1980; Carstens and Trevino, 1978; Ferrington, Sorkin and Willis, 1987; Giesler, Menetrey and Basbaum, 1979; Hylden, Hayashi, Bennett and Dubner, 1985a; Hylden, Hayashi Dubner and Bennett, 1986a; Willis, Kenshalo and Leonard, 1979). Its boundary with the white matter is indistinct and cells belonging to this layer may lie far out in the white matter (Rexed, 1964). Anatomical and electrophysiological studies agree that direct primary afferent input to lamina I arrives apparently solely from small diameter fibres (Cervero and Iggo, 1980; Cruz, Lima and Coimbra, 1987; Gobel, Falls and Humphrey, 1981; Kumazawa and Perl, 1978; Kumazawa, Perl, Burgess and Whitehorn, 1975; LaMotte, 1977; Ralston and Ralston, 1979). Intracellular HRP staining has allowed the anatomical description of terminal arborization of physiologically characterised primary afferents. Using this technique, Light and Perl (1977; 1979b) have shown that Aδ-axons of mechanical nociceptors terminate preferentially in lamina I (but also in lamina V). An anatomical study, however has shown that lamina I also receives input from unmyelinated C- fibres (Gobel et al., 1981). This finding has been supported by observations that stimulating peripheral nerves at intensities sufficient to evoke C-fibre discharge, induces a constant latency C-fibre response in lamina I

cells (Fitzgerald and Wall, 1980). Lamina I receives input not only from cutaneous, but also from muscle and visceral afferent fibres (Cervero and Tattersall 1987; Craig and Kniffki,1985).

Lamina I appears to contain a relatively high proportion of nocispecific cells (Cervero et al., 1976) in addition to multireceptive and non-nociceptive cells (Ferrington et al., 1987; Hylden, et al., 1986b; Woolf and Fitzgerald, 1983). Combined morphological and functional studies have revealed that electrophysiological recordings in lamina I are biased towards the large Waldeyer cells (Perl, 1984; Woolf and Fitzgerald, 1983). Caution must be exercised therefore, in making the extrapolation that properties of lamina I cells are equivalent to those of Waldeyer cells, since an anatomical study has shown that Waldeyer neurones constitute only 5% of the total population of neurones in lamina I (Lima and Coimbra, 1983).

In conclusion, the marginal zone has been proposed to play a specific role in nociception on the basis that it receives input from small diameter afferent fibres and that it contains a high proportion of nocispecific cells, some of which ascend to supraspinal levels. It is clear however that lamina I cells display a heterogeneity both in morphology and in physiology which is inconsistent with a single functional role for this lamina.

Lamina II

This area of the dorsal horn, also known as the substantia gelatinosa (SG) of Rolando (1824) is packed with an enormous number of small cells (Cervero and Iggo, 1980). Because of their small size, lamina II cells have proved elusive to study and only

allowed a closer investigation of the properties of these cells. Intracellular HRP and extracellular studies have demonstrated that SG neurones are powerfully affected by C-fibre afferent input (Kumazawa and Perl, 1976; 1978). This finding is supported by anatomical studies which have described C-fibre terminals in this lamina (e.g Light and Perl, 1979a; Ralston and Ralston, 1979), although inputs to the SG from Aδ- mechanical nociceptive, as well as from hair follicles Aδ- fibres (Light and Perl, 1979b) have also been described. Some SG neurones are thought to receive monosynaptic nociceptive inputs, since electrical stimulation of peripheral nerves and dorsal roots at intensities sufficient to activate C-fibres, evoke responses of very constant latency in intracellularly (Steedman, Molony and Iggo, 1985) as well as extracellularly recorded (Fitzgerald and Wall, 1980) SG neurones.

On the basis of anatomical differences, neurones in the SG have been divided into two main groups (Cajal, 1909; Gobel, 1975) wnich do appear to receive differential inputs (Bennett, Abdelmoumene, Hayashi and Dubner, 1980). Stalked cells located in the dorsal or outer part of the SG (lamina II) have been reported to receive both nociceptive
o
and non-nociceptive inputs and whose dendrites travel dorsally into lamina I. Islet cells, located more ventrally in the inner SG (lamina IIi), on the other hand, have been reported to receive a predominantly non-nociceptive input and to arborize within lamina II.

Although some cells in the SG have been shown to project to supraspinal levels (Giesler, Cannon, Urca and Liebeskind, 1978; Willis, Leonard and Kenshalo, 1978), a large number of SG cells appear to send intraspinal, segmental projections (Szenthaigoti, 1964). In addition, the SG receives input from dendrites of some cells whose somata lie in laminae I-IV (see Brown, 1981 for a

review). This anatomical organisation suggests a segmental modulatory role for some lamina II neurones.

Laminae III-VI

Primary afferent to this region has been demonstrated to include Aβ- (Brown,Rose and Snow, 1977; 1978; Brown, 1981) and Aδ- (Light and Perl, 1979b) fibres from low threshold mechanoreceptors. Some collaterals from mechanical nociceptive Aδ- fibres have also been described to terminate in lamina V (Light and Perl, 1979b). Anatomical studies have described cells at the origin of several ascending tracts to lie in these laminae (see below) and electrophysiological experiments have described mainly multireceptive cells though many non-nociceptive and a few nocispecific cells were also reported (Handwerker et al., 1975; Menetrey et al., 1977; Price and Dubner, 1977; Wall, 1967; Willis, Trevino, Coulter and Maunz, 1974).

The most superficial laminae I and II therefore receive projections, some of them direct, from fine cutaneous primary afferent fibres, including nociceptive fibres. Deeper laminae, on the other hand, receive input mainly from non-nociceptive primary afferent fibres. The fact that some lamina I cells respond to innocuous stimulation and non-nociceptive fibre activation, while many lamina III-VI neurones respond to noxious stimulation and activation of nociceptive primary afferent fibres suggests a relay mechanism is in operation in the dorsal horn. Since the SG receives both nociceptive and non-nociceptive primary afferent terminations and is also innervated by dendrites from cells whose somata lie in laminae I-IV, it is possible that some SG neurones function in such a relay system.

E. Somatosensory Pathways Ascending from the Dorsal Horn

Information from receptors in the skin is transmitted to the dorsal horn where it is processed before being transmitted to supraspinal regions. Intrinsic to the dorsal horn are the cells of origin of several distinct ascending tracts which send their axons through the white matter. In effect, these projection cells are the ascending output cells of the dorsal horn. A number of ascending somatosensory tracts can signal nociceptive information and it may well be that different tracts may play a discrete role in different aspects of pain. The most important tracts will be briefly described below :

The Spinothalamic Tract (STT)

Anatomical and electrophysiological studies have revealed at least three components to this tract; one projecting only to the lateral thalamus, another projecting only to the medial thalamus and yet another projecting to both medial and lateral parts of the thalamus (Applebaum, Leonard, Kenshalo, Martin and Willis, 1979; Carstens and Trevino, 1978; Giesler et al., 1981b; Kevetter and Willis, 1984). The STT projection has been shown to travel through the ventral quadrant (Kerr, 1975; Willis et al., 1979; Willis, 1981) and its cells of origin are usually multireceptive, though some are nocispecific (Giesler et al., 1981b; Willis et al., 1974; Price, Hayes, Ruda and Dubner, 1978). Retrograde HRP transport studies have revealed the distribution in the dorsal horn of neurones giving rise to the STT in cat (Carstens and Trevino, 1978), rat (Giesler et al., 1979) and monkey (Willis et al., 1979). STT cells appear to be located mainly in laminae I-IV and V in both rat and monkey, whereas in cat, STT cells tend to lie in deeper laminae VII and VIII of the ventral horn.

The Spinomesencephalic Tract (SMT)

Electrophysiological and retrograde HRP transport studies have demonstrated a projection to the midbrain, originating mainly in superficial lamina I of the dorsal horn, but also in deeper laminae V-VIII in rat (Menetrey, Chaouch and Besson, 1980; Menetrey, Chaouch, Binder and Besson, 1982; Swett, McMahon and Wall, 1985) and cat (Wiberg and Blomqvist, 1984; Yezierski and Schwartz, 1986). The terminal sites for the SMT projection include lateral PAG, nucleus cuneiformis and the parabrachial nucleus (Cechetto, Standaert and Saper, 1985; Hylden et al., 1985a; Menetrey et al., 1980; 1982; Wiberg and Blomqvist, 1984). Many anatomical and electrophysiological tracing studies have demonstrated that several ascending SMT axons give rise to collaterals which may terminate in more than one mesencephalic site (Hylden et al., 1986b; Yezierski and Schwartz, 1986), in medullary sites (McMahon and Wall, 1983; Pechura and Liu, 1986) or the thalamus (Hylden et al., 1985a; 1986b; Liu, 1986; Yezierski and Schwartz, 1986; Yezierski, Sorkin and Willis, 1987). Furthermore, SMT axons can run in either the ventral quadrant or in the DLF in both rat and cat (McMahon and Wall, 1983; Menetrey et al., 1980; 1982; Yezierski and Schwartz, 1986; Zemlan, Leonard, Kow and Pfaff, 1978), though SMT cells originating in lamina I appear to send their axons preferentially through the DLF (Hylden, Hayashi and Bennett, 1986a; McMahon and Wall, 1985). A few studies have investigated the response properties of SMT cells and have revealed another distinction between lamina I SMT cells and those originating in deeper laminae. Although the majority of SMT cells display multireceptive properties (Yezierski and Schwartz, 1986), the majority of lamina I SMT cells were found to be nocispecific (Hylden

et al., 1986b). Moreover, the receptive fields of lamina I SMT cells appear to be more restricted than the complex large receptive field described for SMT cells which lie mainly in deeper laminae (Hylden et al., 1986b; Yezierski and Schwartz, 1986).

In conclusion, the SMT seems to be a complex pathway containing a neuronal population which is heterogeneous with respect to its site of termination and origin as well as its response properties. Cells of the SMT seem to have many similarities with those of the STT, indeed some cells have been shown to project to both midbrain and thalamus (see above), such a complex projection may reflect a complex function.

The Spinoreticular Tract (SRT)

The projection from the spinal cord to the reticular formation appears to run through the ventrolateral quadrant and SRT neurones located mainly in laminae V and VIII of the dorsal horn were found to be retrogradely labelled from the ponto-medullary reticular formation (Abols and Basbaum, 1981; Kevetter,Haber, Yezierski, Chung, Martin and Willis, 1982) which in turn projects to the thalamus (Bowsher, 1975). Some SRT neurones have been described to send collaterals to the thalamus (Kevetter and Willis, 1983; Peschanski and Besson, 1984). Electrophysiological recordings from SRT neurones in monkey, cat and rat have revealed that many are nociceptive (Fields, Clanton and Anderson, 1977b; Fields, Wagner and Anderson, 1975; Haber, Moore and Willis, 1982; Maunz, Pitts and Peterson, 1978; Menetrey et al., 1980).

The Spinocervical Tract (SCT)

Intracellular recording and staining techniques have demonstrated that the cells of this tract are found chiefly in dorsal

horn laminae III and IV (Brown et al., 1980; Brown, 1981; Craig, 1976; 1978; Hongo; Jankowska and Lundberg, 1968). The axons of SCT cells appear to travel through the DLF terminating in the lateral cervical nucleus, which in turn projects to the contralateral thalamus (Berkley, 1980; Blomqvist, Flink, Bowsher, Griph and Westman, 1978; Boivie, 1980; Craig and Burton, 1979). Electrophysiological studies have revealed that very few SCT cells are nocispecific, while many appear to be non-nociceptive although the majority of SCT cells are multireceptive (Brown, 1981; Brown and Franz, 1969; Cervero, Iggo and Molony, 1977).

The Post Synaptic Dorsal Column System (PSDC)

Studies involving retrograde transport or intracellular staining with HRP have shown the cells of this tract to be positioned mainly in laminae III-V of the dorsal horn and that PSDC cells send their axons through the dorsal column to terminate in the dorsal column nuclei (Bennett, Seltzer, Lu, Nishikawa and Dubner, 1983; Brown and Fyffe, 1981; Rustioni and Kaufman, 1977). The properties of PSDC cells are similar to those of SCT cells, both types of cells share the same location in the dorsal horn. Moreover, like SCT cells, the majority of PSDC cells are apparently multireceptive, though a small population is non-nociceptive and some neurones have been described as nocispecific (Angaut-Petit, 1975a). Anatomical and electrophysiological studies have revealed differences in morphology, receptive field location and response characteristics between SCT and PSDC cells (Angaut-Petit, 1975b; Brown and Fyffe, 1981; Brown, Noble and Riddell, 1986; Noble and Riddell, 1988).

In conclusion, cells of ascending tracts described in this section have been shown to receive both nociceptive and non

nociceptive inputs and to send their axons to supraspinal sites. The activity of ascending tract cells appears to be under considerable modulation from descending systems.

F. Descending Modulation of Dorsal Horn Neuronal Activity

The study of descending control over transmission of nociceptive information is of major importance in understanding the endogenous mechanisms of antinociception, since such research may provide the basis for novel methods of pain treatment in both animal and human. Three major approaches have been used to investigate descending influences :

1) Transecting or reversibly blocking the spinal cord

That tonic descending influences were acting at the level of the spinal cord was first realised by Sherrington and Sowton (1915) who demonstrated that transection of the spinal cord, in the decerebrate animal, reduced stretch reflexes and enhanced flexor reflexes. Later, several investigators demonstrated that tonic input from supraspinal sites has influence on modality and amplitude of neuronal responses as well as the extent of peripheral excitatory receptive fields (e.g. Brown, 1971; Besson, Guilbaud and Le Bars, 1975; Cervero et al., 1976; Duggan, Griersmith and Johnson, 1981; Handwerker et al., 1975; Wall, 1967). Recent investigations have revealed that the lateral reticular nucleus in the brainstem is a likely source of origin of tonic descending inhibition in the anaesthetised cat (Hall, Duggan, Morton and Johnson, 1982; Morton, Duggan and Zhao, 1984).

2) Focal electrical stimulation of brain structures

Electrical stimulation of certain sites in the brain was found

to produce selective behavioural analgesia (defined as selective inhibition of behavioural nociceptive responses without effect on motivational, motor, or emotional responses (Mayer and Price, 1976)) in several animal species (Giesler and Liebeskind, 1976; Liebeskind, Guilbaud, Besson and Oliveras, 1973; Mayer and Liebeskind, 1974; Mayer, Wolfe, Akil, Carder and Liebeskind, 1971; Oliveras, Woda, Guilbaud and Besson, 1974; Reynolds, 1969; Roberts and Rees, 1986; Soper, 1976) and to cause pain relief in humans (Adams, 1976; Richardson and Akil, 1977; Young and Chambi, 1987). This phenomenon has been given the name : stimulation-produced analgesia (SPA). The mesencephalic region known as the periaqueductal gray (PAG), seems to be one of the important sites involved in SPA (Mayer et al., 1971; Soper, 1976; Yeung, Yaksh and Rudy, 1977). Indeed, stimulating this region has been used clinically to induce pain relief in humans (Adams, 1976; Richardson and Akil, 1977). Evidence suggests that the ultimate result of brainstem stimulation is the inhibition of nociceptive transmission at the level of the dorsal horn. Thus stimulation of the PAG has been shown to elicit behavioural analgesia in the awake animal (Liebeskind et al. 1973; Hayes, Price, Ruda and Dubner, 1979; Oliveras, Besson, Guilbaud and Liebeskind 1974) and to inhibit the responses of dorsal horn neurones to noxious stimulation (Carstens, Yokata and Zimmermann, 1979; Carstens, Klumpp and Zimmermann, 1980; Gerhart, Yezierski, Wilcox and Willis, 1984; Hayes et al., 1979).

3) Pharmacological intervention

Similarities exist between SPA and opiate analgesia (OA) induced by systemic administration or brainstem microinjection of opiates. It is now known that at least three different types of opiate receptors (μ, δ and κ) exist in the central nervous system. Morphine, a μ opiate receptor agonist, and naloxone, a good μ but

weak κ and δ opiate receptor antagonist (Kosterlitz and Paterson, 1985), have been used in the studies reported below in exclusion of other and / or more selective compounds to investigate OA. Negative results from such studies where the involvement of the σ and κ sites is not adequately assessed therefore have to be regarded with caution. Effective sites for OA and SPA induction overlap considerably (Yeung et al., 1977) and both SPA and OA seem to be mediated through a pathway which descends through the DLF (Barton, Basbaum and Fields, 1980; Basbaum, Marley, O'Keefe and Clanton, 1977). More importantly however, there appears to be cross tolerance between the effects of brain stimulation and morphine administration (Mayer and Hayes, 1975). Moreover, systemically administered naloxone has been shown to attenuate the effects of PAG stimulation (Akil, Mayer and Liebeskind, 1976). Similarly, naloxone injection into the PAG reverses analgesia induced by systemically administered morphine (Yeung and Rudy, 1980). The anatomical substrates for SPA and OA however may not be identical, since the most effective loci for inducing the two types of phenomena can be different (Gebhart, 1982; Yeung et al., 1977). Moreover, SPA evoked from certain areas in the brainstem (such as the dorsal PAG) are not subject to antagonism by naloxone (Cannon, Prieto, Lee and Liebeskind, 1982; Yaksh, Yeung and Rudy, 1976). It would seem therefore that both opioid but perhaps also non-opioid substrates are involved in SPA. The existence of endogenous antinociceptive, analgesic systems initiated the search for the natural mode of activation of such systems. It was soon discovered that selective analgesia can be induced in the experimental animal model by exposure to stress conditions (Hayes, Bennett, Newlon and Mayer, 1978; Lewis, Terman, Nelson and Liebeskind, 1984). Emergency conditions such as attack by a predator, or severe injury may require the animal's total concentration on escape and survival tactics. In such circumstances, stress-induced analgesia may operate to shut off normal nociceptive and pain reactions and as such may be a useful adaptive mechanism. It is now agreed that stress- induced analgesia involves both opioid

(Madden, Akil, Patrick and Barchas, 1977; Watkins, Cobelli and Mayer, 1982) and non-opioid mechanisms (Hayes et al., 1978; Watkins et al., 1982). Both opioid and non-opioid dependent stress-induced analgesia were further shown to be interrupted by lesions in the DLF (Lewis, Terman, Watkins, Mayer and Liebeskind, 1983; Watkins et al., 1982). Non-opioid mechanisms have also been reported to be involved in the phenomenon of diffuse-noxious-inhibitory controls, DNIC (Le Bars, Dickenson and Besson, 1979a;b). In the anaesthetised rat (Le Bars et al., 1979a) and cat (Morton, Maisch and Zimmermann, 1987), all evoked activity of a multireceptive dorsal horn neurone is inhibited by applying noxious stimuli to an area outside the neurone's receptive field. DNIC may be one of the neuronal substrates for electroacupuncture and counter irritation phenomena. Both methods have been used clinically to relieve pain in humans and seem to depend on eliciting peripheral pain, just bearable to patients in a site distant from the ailing locus (for discussion see Le Bars et al., 1979b; Le Bars, Calvino, Villanueva and Cadden, 1984).

Interest in non-opioid systems was triggered by reports suggesting their possible involvement in intrinsic analgesic pathways. In addition, problems of dependence and tolerance, often accompanying opiate pain therapy, intensified the search for non-opioid therapeutic compounds which may not display such side effects. The monoamines are candidates for the mediation of SPA, OA, stress-induced analgesia and DNIC. Correspondingly, much attention has been paid to understanding their role in antinociception and analgesia.

Serotonin (5-HT), an indoleamine, is a phylogenetically ancient compound found centrally in many primitive animals. In the central nervous system of various vertebrates, 5-HT-containing cell bodies

abound in midline raphe structures. This is in contrast to the catecholamines which tend to be concentrated in more lateral regions of the brain stem (Parent, Poitras and Dube, 1984). There is a large body of evidence pointing in favour of the involvement of 5-HT in descending modulation of nociception and in analgesia (for reviews see Le Bars, 1988; Messing and Lyttle, 1977; Roberts, 1984). In the following sections this evidence will be presented and briefly discussed.

II The Anatomy of the Descending Serotonin-containing System

A. Distribution of Serotonin-Containing Cell Bodies

Establishing the involvement of 5-HT in antinociception necessitates the demonstration of a neuroanatomical substrate for such an involvement. Anatomical studies have shown that this condition is indeed met. Virtually all the serotonin-containing fibres in the spinal cord arrive from supraspinal structures, since most 5-HT-containing terminals disappear upon more rostral transection (Carlsson, Falck and Hillarp, 1964; Dahlstrom and Fuxe, 1965; Newton, Maley and Hamill, 1986; Oliveras, Bourgoin, Hery, Besson and Hamon, 1977) and the spinal cord 5-HT content is decreased by 95% (Hadjiconstantinou, Panulas, Lackovic and Neff, 1984). Some 5-HT-containing terminals however, continue to persist and the spinal c content is not entirely lost several days post transection . This observation has called into question the negative finding of several groups which have reported the absence of 5-HT-containing cell bodies from the spinal cord (Carlsson et al., 1964; Dahlstrom and Fuxe, 1964; Kojima, Takeuchi, Goto and Sano, 1983). It would seem possible that a minor proportion of 5-HT innervation is not supraspinal in

origin. Indeed, the existence of spinally intrinsic 5-HT- containing cell bodies has now been demonstrated in both rat (Bjorklund, Falck and Stenevi, 1970; Newton et al., 1986) and monkey (LaMotte, Johns and deLanerolle,1982). These spinal serotonin-containing cells are few in number and often oriented along the longitudinal axis of the cord, thus they may have been missed by other groups which looked at transverse sections of the cord. These cells lie in lamina X ventral to the central canal in monkey (LaMotte et al., 1982) and in autonomic regions of the cord as well as in laminae VII and X dorsal to the central canal in rat (Newton et al., 1986).

The distribution of serotonin in the nervous system of rat was first described by an elegant study of Dahlstrom and Fuxe (1964). Using fluorescence histochemistry (involving the reaction of indoleamines with formaldehyde to produce intensely fluorescent compounds), they were able to discern that 5-HT- containing cell bodies were clustered into specific loci. These groups were designated B1-B9 and correspond roughly to the raphe nuclei of the brainstem (see Fig.1a). Later histochemical studies, immunocytochemical studies (where a marker, conjugated to a specific antibody to 5-HT, shows up the 5-HT containing axons and terminals and if axonal transport is blocked, cell bodies as well) and autoradiographic studies (involving the exposure of a photographic emulsion overlying tissue radiolabelled with 5-HT, since this method depends on the uptake of radiolabelled 5-HT into cell bodies and its transport into axons, it may not reliably reflect where 5-HT release may occur) have confirmed these original findings in rat (Bowker, Westlund and Coulter, 1981a; Bowker, Westlund, Sullivan and Coulter, 1982; Chan-Palay, 1977; Steinbusch, 1981; Takeuchi, Kimura and Sano, 1982), rabbit (Felten and Cummings, 1979; Howe, Moon and Dampney, 1983), cat (Poitras and Parent, 1978; Takeuchi et al., 1982; Wiklund, Leger and Persson. 1981), opossum (Crutcher and Humbertson, 1978) and primate (Jacobowitz and MacLean, 1978; Sladek, Garver and Cummings, 1982).

B. Distribution of 5-HT-containing Terminals in the Spinal Cord

In their fluorescence histochemical study, Dahlstrom and Fuxe

(1965) described the distribution of 5-HT-containing terminals throughout the spinal cord of rat and cat. They observed that these terminals were concentrated around the intermediolateral cell column at thoracic levels, around the central canal and in the ventral horn at all levels of the spinal cord. The superficial dorsal horn was found to receive a moderate number of 5-HT-containing terminals. Different techniques such as immunocytochemistry, autoradiography and microdissection coupled with biochemical detection in cat (Hylden, Ruda, Hayashi and Dubner, 1985b; Oliveras et al., 1977; Segu and Calas,1978), rabbit (Zivin, Reid, Saavedra and Kopin, 1975), rat (Bowker et al., 1982; Oliveras et al., 1977; Zivin et al., 1975) and monkey (LaMotte and deLanerolle, 1983) have revealed a similar distribution pattern for 5-HT-containing terminals. The highest concentration of terminals seems to be in the intermediolateral column, the ventral horn and the central canal. In the dorsal horn, the concentration of terminals seems to be high in superficial laminae I and II, sparse in lamina III and moderate in lateral laminae IV and V. It appears therefore that 5-HT-containing terminals are concentrated where cells of origin of ascending somatosensory tracts are known to lie within the dorsal horn. Indeed, identified SMT (Hylden, Hayashi, Ruda and Dubner, 1986c) and PSDC neurones (Nishikawa, Bennett, Ruda, Lu and Dubner, 1983) have been shown to receive direct serotonin-containing contacts on both their somata and their dendrites.

In the white matter however, results vary. Assay techniques have found that the concentration of 5-HT in the white matter is only one half that of the grey matter (Hadjiconstantinou et al., 1984; Oliveras et al., 1977; Zivin et al., 1975). Segu and Calas (1978), using an autoradiographic technique, observed that while both

ventral funiculi contained many 5-HT fibres, the dorsl funiculi were almost devoid of 5-HT innervation. There are studies however, which convincingly demonstrate 5-HT axons in the dorsolateral funiculus (DLF)(Basbaum, Zahs, Lord and Lakos, 1988; Carlsson et al., 1964; Hylden, et al., 1985b). It seems from these studies that the concentration of 5-HT axons in the DLF is lower than that in the ventral funiculi and that the visualisation of axons in the DLF therefore probably requires more sensitive detection methods.

C. Organisation of the Descending Serotonin-containing Projection to the Spinal Cord

The topographic distribution of 5-HT-containing terminals in the spinal cord suggests the possibility that innervation of different parts of the spinal cord may originate in separate sites in the brain stem. This indeed was found to be the case. Early fluorescence histochemistry and lesion experiments indicated that the serotonin-containing fibres terminating in the ventral horn of the spinal cord descended through the medial ventral funiculus (VF) and through the ventrolateral funiculus (VLF). Serotonin-containing terminals in the intermediolateral column and in the dorsal horn on the other hand, appear to descend through the DLF (Dahlstrom and Fuxe, 1965).

In an elegant study, using a double labelling technique, Bowker and his colleagues studied the projection to the spinal cord originating from different raphe nuclei and the proportion of this projection which was serotonin-containing (Bowker et al., 1981a; Bowker, Westlund and Coulter, 1981b; Bowker et al., 1982; Bowker, Reddy, Fung, Chan and Barnes, 1987). They used retrograde transport of HRP to demonstrate the raphe-spinal projection to different levels of the cord coupled with immunocytochemistry to show 5-HT-containing cell

bodies in the brain stem. Their studies revealed that nearly all the serotonin-containing projection to all levels of the spinal cord originated from the medullary raphe groups; nucleus raphe obscurus (NRO), nucleus raphe pallidus (NRPa) and nucleus raphe magnus (NRM) as well as the reticular formation just dorsal to the pyramidal tract in the nucleus reticularis paragigantocellularis (NRPG) and more laterally between the pyramid and the facial nucleus, in the nucleus reticularis paragigantocellularis lateralis (NRPGL) - see Fig. 1b. This is in agreement with Dahlstrom and Fuxe's earlier observation (Dahlstrom and Fuxe, 1965). Although pontine and midbrain raphe nuclei were also shown to project to the spinal cord, the projection was either sparse (as in the case of B5 or nucleus raphe pontis), or restricted to cervical levels (as in the case of B7 and B9) (Bowker et al., 1981a; 1981b; 1982).

Nearly three quarters of all serotonin-containing cells in the medullary raphe groups and adjacent reticular formation in rat (Bowker et al., 1981a; 1982) and nearly one half in cat (Bowker et al., 1987) were found to descend to the spinal cord. Of all neurones which send projecting axons from these medullary regions, Bowker and coworkers found that nearly 90% in rat and 70-80% in cat contained 5-HT immunoreactivity (Bowker et al., 1981a; 1982; 1987). In contrast, Skagerberg and Bjorklund (1985) using retrogradely transported True Blue coupled with fluorescence histochemistry, found the non-serotonin-containing projecting component from medullary raphe nuclei to be larger than that reported by Bowker and his colleagues. It is possible that the different techniques employed in the two studies were responsible for the discrepancy in results. It is possible for example that True Blue may be more efficiently transported than HRP.

On the other hand, it is also possible that the fluorescent reaction product used by Skagerberg and Bjorklund may not have been intensive enough to enable them to visualise all the 5-HT containing cell bodies.

The route taken by the serotonin-containing raphe-spinal pathway was also investigated . Cord lesion studies revealed that the 5-HT containing cell bodies in NRM and ventral nucleus reticularis paragigantocellularis (NRPG) send axons to the lumbosacral cord through the DLF. On the other hand, the serotonin-containing fibres from NRPa and NRO travels through the VF (Bowker et al., 1982). Another component of the raphe-spinal projection travels from NRM and lateral reticular formation through the VF, though this projection was found to be non-serotonin-containing (Bowker et al., 1982). The differential route of projection to the spinal cord from different raphe nuclei and the adjacent reticular formation has been confirmed by many other studies (Basbaum, Clanton and Fields, 1978; Basbaum and Fields, 1979; Basbaum, Ralston and Ralston, 1986; Goode, Humbertson and Martin, 1980; Holstege and Kuypers, 1982; Leichnetz, Watkins, Griffin, Murfin and Mayer, 1978; Watkins, Griffin, Leichnetz and Mayer, 1980; Zemlan, Behbehani and Beckstead, 1984).

In addition, supraspinal 5-HT-containing cell groups contribute differentially to different laminae of the spinal cord in cat (Basbaum et al., 1978; Bobillier, Seguin, Petitjean, Salvert, Touret and Jouvet, 1976; Holstege and Kuypers, 1982; Light, 1985), opossum (Goode et al., 1980) rat (Zemlan et al., 1984) and monkey (Basbaum et al., 1986). Serotonin-containing terminals in the superficial and deeper dorsal horn seem to originate from the nucleus raphe magnus and the lateral reticular formation, while the intermediolateral column

receives axons from NRM and lateral reticular formation as well as from NRO and NRPa (Basbaum et al., 1978; Bobillier et al., 1976; Goode et al., 1980; Holstege and Kuypers, 1982). Ventral horn laminae IX and X also receive some terminals from NRM but a more dense projection from NRPa and NRO (Basbaum et al., 1978; Holstege and Kuypers, 1982; Martin, Cabana, Humbertson, Laxson and Panneton 1981).

Interestingly, many raphe-spinal fibres have been shown to collateralize extensively in rat (Cavada, Huisman and Kuypers, 1984; Huisman, Kuypers and Verburgh, 1981), opossum (Martin et al., 1981), cat (Huisman, Kuypers and Verburgh, 1982; Light and Kavookjian, 1985) and monkey (Huisman et al., 1982). Indeed, recent studies have shown that some raphe-spinal neurones which project through the DLF and terminate in superficial laminae I and II_o send collaterals to deeper laminae V-VII and in some cases to lamina X as well (Light, 1985; Light and Kavookjian, 1985). Such branching may allow a single raphe-spinal neurone to modulate activity at more than one level of the spinal cord. In addition to dorsoventral collateralization, raphe-spinal neurones have been shown to collateralize in the rostrocaudal direction. By injecting one dye at the level of the cervical cord, while injecting a different dye at more caudal levels, it has been calculated that 40% of all NRM neurones which project to cervical cord send collaterals to more caudal levels of the spinal cord in rat, opossum and monkey (Huisman et al., 1981; 1982; Martin et al., 1981). In cat this proportion is larger reaching 55-60%. Thus, a large proportion of raphe-spinal neurones in the NRM distribute collaterals throughout the length of the spinal cord, while a different subgroup projects to restricted parts of the cord.

It is possible tnat this may reflect a division of the raphe-spinal projection into a diffuse and a focused components, though the function of the two types of projection has yet to be investigated. In conclusion, it seems that a major part of the serotonin-containing projection to the dorsal horn of the spinal cord originates from the NRM as well as the adjacent reticular formation and courses through the DLF. The projection to the intermediolateral column also descends through the DLF and probably arises mainly from NRM. The serotonin-containing pathway to the ventral horn, on the other hand, appears to originate mainly in NRO and NRPa and to course through the VF and VLF.

NRM therefore appears to be an important site of origin for 5-HT-containing terminals in the dorsal horn. This finding aroused interest in this region of the medulla and initiated studies into its morphology and physiology. Thus, anatomical and electrophysiological studies on the fibre calibre of the raphe-spinal projection revealed that there were both myelinated and unmyelinated axons descending from NRM (Basbaum et al., 1986; Chiang and Pan, 1985; Light, 1985; Wessendorf, Proudfit and Anderson, 1981; West and Wolstencroft, 1977). The serotonin-containing component of the projection to the spinal cord from NRM however appears to be predominantly unmyelinated (Basbaum et al., 1988; Dahlstrom and Fuxe, 1965), although the presence of a component of small diameter myelinated fibres has also been demonstrated (Ruda and Gobel, 1980; Wessendorf et al., 1981). The heterogeneity of NRM cells extends to their neurochemistry. NRM cells do not contain only 5-HT; a quarter(Bowker et al., 1987) to one half (Skagerberg and Bjorklund, 1985) of all NRM cells projecting to the spinal cord do not contain 5-HT. There is evidence moreover, for

the coexistence of TRH (Harkness and Brownfield, 1986; Johannsson, Hokfelt, Pernow, Jeffcoate, White, Steinbusch, Verhofstad, Emson and Spindel, 1981; Towle, Breese, Mueller, Hunt and Lauder, 1986), Substance P (Bowker, Westlund, Sullivan, Wilber and Coulter, 1983; Chan-Palay, Jonsson and Palay, 1978; Hokfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow and Goldstein, 1978; Johansson et al., 1981; Pelletier, Steinbusch and Verhofstad, 1981; Singer, Sperk, Placheta and Leeman, 1979; Towle et al., 1986), enkephalin (Leger, Charnay, Dubois and Jouvet, 1986), GABA (Millhorn, Hokfelt, Seroogy, Oertel, Vernofstad and Wu, 1987a) and proctolin-like peptide (Holets, Hokfelt, Ude, Eckert, Penzlin, Verhofstad and Visser, 1987) with 5-HT within NRM cells. The co-localisation of TRH (Appel, Wessendorf and Elde, 1987; Harkness and Brownfield, 1986; Helke, Sayson, Keeler and Charlton, 1986; Hokfelt, Fuxe, Johansson, Jeffcoate and White, 1975; Towle et-al., 1986) substance P (Helke, Neil, Massari and Loewy, 1982; Singer et al., 1979) and proctolin-like peptide (Holets et al., 1987) with 5-HT in terminals in the spinal cord however seems to be restricted to the ventral horn and to the intermediolateral column. The co-localised enkephalin and 5-HT on the other hand, seem to be distributed mainly in dorsal horn terminals (Tashiro, Satoda, Takahashi, Matsushima and Mizuno, 1988). In addition, enkephalin and somatostatin have been shown to coexist in NRM cells projecting to the spinal cord (Millhorn, Seroogy, Hokfelt, Schmued, Terenius, Buchan and Brown, 1987b).

As well as sending a descending projection to the spinal cord, NRM also appears to send ascending efferents to the mesencephalon (Bobillier et al., 1976; Bowker, 1986; Brodal, Taber and Walberg, 1960a). It seems that virtually all NRM cells giving rise to the

ascending projection do not contain 5-HT and are larger in size than that giving rise to the descending projection, most of which is 5-HT containing (Bowker et al., 1981a; 1982; Bowker, 1986). Moreover, the localisation of the ascending and descending systems has been shown to be different. Although there was found to be an overlap in the rostrocaudal orientation, the group of cells giving rise to the ascending efferents was ventral and medial to that giving rise to the descending projection to the spinal cord (Bowker, 1986). Afferent connections to the NRM arrive from various areas including the spinal cord, the periaqueductal gray and nucleus cuneiformis (in the midbrain), nucleus reticularis ventralis, nucleus reticularis magnocellularis / paragigantocellularis and nucleus reticularis gigantocellularis (in the medulla) (Abols and Basbaum, 1981; Brodal, Walberg and Taber, 1960b; Fardin, Oliveras and Besson, 1984; Gallager and Pert, 1978). The nucleus raphe magnus would therefore appear to be strategically placed to receive information and to relay it to higher centres as well as to the spinal cord.

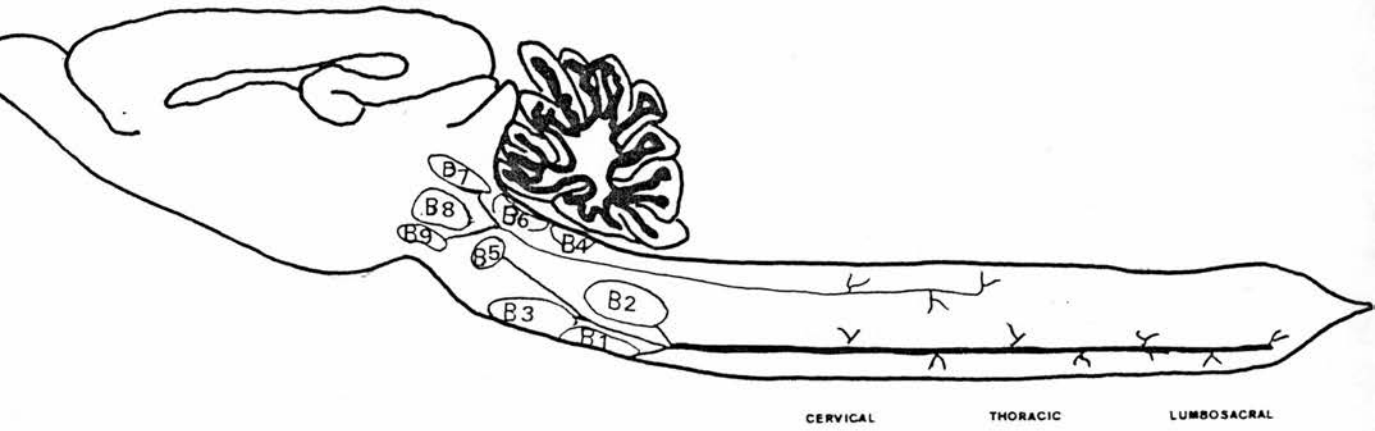
Figure 1.

The origin of the serotonergic raphe-spinal projection

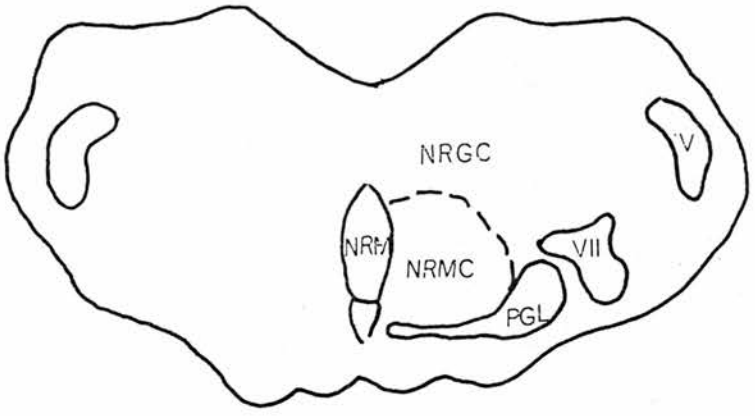
a) The figure portrays the descending serotonergic projection to the spinal cord. Midbrain cell groups B7 and B9 seem to provide 5-HT input only to cervical levels. The major serotonergic input to all levels of the spinal cord originates in the medullary cell groups B1-B3 (roughly equivalent to nucleus raphe pallidus, nucleus raphe obscurus, nucleus raphe magnus (NRM) and part of reticular formation adjacent to NRM) and B5. Cell groups B4, B6 and B8 do not appear to send a serotonergic input to the spinal cord. (Adapted from Bowker et al., 1982).

b) A schematic drawing of a cross section through the medulla showing the organisation of the reticular formation surrounding NRM in cat and rat. Serotonergic cell bodies lie in NRM and nucleus reticularis paragigantocellularis lateralis (PGL) in both cat and rat. The nucleus reticularis magnocellularis (NRMC) in the cat is equivalent to the nucleus reticularis paragigantocellularis (NRPG) in the rat and lies just adjacent to the NRM. The nucleus reticularis gigantocellularis (NRGC), on the other hand, lies dorsal to the NRM in both rat and cat. V = spinal trigeminal tract, VII = facial nucleus. (Adapted from Basbaum et al., 1983).

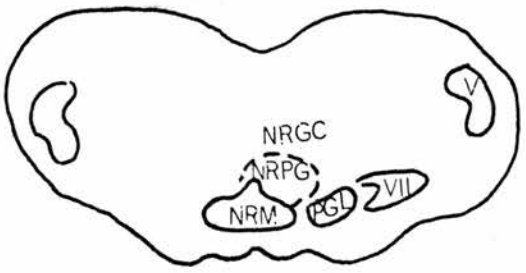
a)


B7
B8
B9
B6
B4
B5
B2
B3
B1
CERVICAL
THORACIC
LUMBOSACRAL

b)


NRGC
V
NRM
NRMC
VII
PGL

CAT


V
NRGC
NRPG
NRM
PGL
VII

RAT

III The Involvement of the Serotonin-containing Raphe-Spinal System in Analgesia

In this section the data implying the involvement of the NRM, and specifically its serotonin-containing component, in antinociception and analgesia will be discussed.

A. Stimulation-Produced Analgesia

Several studies have demonstrated a connection between PAG, an important site for inducing SPA (see above) and NRM (Abols and Basbaum, 1981; Fardin et al., 1984; Fields and Anderson, 1978; Gallager and Pert, 1978; Shah and Dostrovsky, 1980). Electrophysiological studies have further shown this connection to be excitatory, since stimulating the PAG excites many NRM cells (Behbehani, 1982; Pomperoy and Behbehani, 1979), including raphe-spinal neurones (Fields and Anderson, 1978; Lovick, West and Wolstencroft, 1978; Pomperoy and Behbehani, 1979; Willcockson, Gerhart, Cargill and Willis, 1983). Moreover, activating PAG cell bodies with glutamate (which is thought to excite cell bodies and not axons of passage which are likely to be activated in addition during electrical stimulation), was also found to increase firing of NRM cells, to inhibit their responses to peripheral noxious stimulation and to result in behavioural analgesia (Behbehani and Fields, 1979). The neurochemical nature of the PAG-NRM connection has been investigated and NRM was found to receive a neurotensin input from the ventrolateral PAG (Beitz, 1982a), a compound which when injected into the NRM (and adjacent reticular formation) causes antinociception (Fang, Moreau and Fields, 1987). NRM has also been shown to receive a serotonin-containing input from the PAG (Beitz, 1982a). Ionophoretically applied 5-HT excites many NRM cells

(Llewelyn, Azami and Roberts, 1983), but inhibits raphe-spinal neuronal responses to PAG stimulation. Moreover, 5-HT microinjected into the NRM can also cause antinociception (Llewelyn, et al., 1983). In addition to its inputs from the PAG, NRM has been shown to receive an enkephalinergic input from midbrain nucleus cuneiformis and parabrachial nucleus (both areas have been shown to be termination sites for SMT cells in the dorsal horn - see above) as well as from the medullary noradrenergic A5 cell group and from NRPG (Beitz, 1982b). It would seem therefore that several brainstem regions may influence NRM activity through neurochemically diverse inputs. The precise conditions which may bring each putative activating pathway into play remain to be discerned.

The periaqueductal gray sends only a sparse (but maybe important) connection to the spinal cord, (Kuypers and Maisky, 1975; Mantyh and Peschanski, 1982), and thus the idea that the action of PAG stimulation on dorsal horn neurones is mediated through a medullary connection, namely the NRM and adjacent reticular formation, gained credence (Basbaum and Fields, 1978; Fields and Basbaum, 1978). Indeed, it appears that NRM is necessary for inducing SPA from the ventral PAG as assessed by the tail flick test. Thus lesions in the NRM (Abbot and Melzack, 1983; Behbehani and Fields, 1979; Prieto, Cannon and Liebeskind , 1983), or in the DLF, through which the raphe-spinal projection to the dorsal horn travels, (Basbaum et al., 1977) can reduce or even abolish this analgesia in rat. It is noteworthy however that lesioning the NRM failed to reduce PAG -induced SPA as assessed with the formalin writhing test (Abbot and Melzack, 1983). Other findings also question the importance of

NRM in antinociception, hence inactivation of NRM by lesions (Morton et al., 1984) or by microinjection of local anaesthetic (Gebhart, Sandkuhler, Thalhammer and Zimmermann, 1983a) was found to minimally reduce the inhibitory effect of PAG stimulation on dorsal horn neurones in cat.

Stimulating the NRM itself can inhibit nociceptive reflexes in cat (Dostrovsky, Hu, Sessle and Sumino, 1982; Oliveras, Guilbaud and Besson, 1979; Oliveras, Redjemi, Guilbaud, and Besson, 1975) and produce powerful analgesia against noxious thermal or mechanical stimulation in rat (Barbaro, Hammond and Fields, 1985; Sandkuhler and Gebhart, 1984a; Zorman, Hentall, Adams and Fields, 1981; Zorman, Belcher, Adams and Fields, 1982). Evidence suggests that the analgesic effects of NRM stimulation are mediated through the DLF, lesions of which block this SPA (Basbaum et al., 1977). The analgesic effect of NRM stimulation is thought to be mediated, at least partially, through an action in the dorsal horn. Thus stimulating NRM inhibits responses of dorsal horn neurones to noxious stimulation in cat (Belcher, Ryall and Schaffner, 1978; Chapman, Amons and Foreman, 1985; Duggan and Griersmith, 1979; Edeson and Ryall, 1983; Fields, Basbaum, Clanton and Anderson, 1977a; Gebhart, Sandkuhler, Thalhammer and Zimmermann, 1983b; Gray and Dostrovsky, 1983; Guilbaud, Oliveras, Giesler and Besson, 1977; McCreery, Bloedel and Hames, 1979; Miletic, Hoffert, Ruda, Dubner and Shigenaga, 1984), monkey (Gerhart, Wilcox, Chung and Willis, 1981; Willis, Haber and Martin, 1977; Yezierski, Wilcox and Willis, 1982) and rat (Rivot, Chaouch and Besson, 1980). Although NRM stimulation produced predominantly inhibitory effects, excitations were occasionally noted (Belcher, et al., 1978; Dubuisson and Wall, 1980; Gray and

Dostrovsky, 1983; McCreery, et al., 1979; Yezierski, et al., 1982). The inhibitory effect of NRM stimulation was found to be selective for the nociceptive response in some studies (Duggan and Griersmith, 1979; Fields, et al., 1977a; Gerhart, et al., 1981; Guilbaud, et al., 1977), but non-selective in others (Belcher, et al., 1978; Chapman, et al., 1985; Gray and Dostrovsky, 1983; Kajander, Ebner and Bloedel, 1984; McCreery, et al., 1979; Miletic, et al., 1984; Willis, et al., 1977). Results from studies which assessed the effects of NRM stimulation on responses evoked by peripheral nerve stimulation are in accord with the view that the effects of NRM are non-selective in that both A- and C- fibre responses are affected. There does seem however, to be selectivity in the degree of inhibition exerted on different inputs. Correspondingly, NRM stimulation inhibits responses to Aβ - fibre stimulation (Beall, Martin, Applebaum and Willis, 1976; Gebhart, et al., 1983b; Gerhart, et al., 1981; Rivot, et al., 1980; Willis, et al., 1977), although inhibition of the Aδ- response is reported to be more pronounced (Beall, et al., 1976; Gerhart, et al., 1981; Rivot, et al., 1980; Willis, et al., 1977) and that of the C-response even more so (Chapman, et al., 1985; Gerhart et al., 1981; Rivot, et al., 1980). From the above, it seems possible that NRM stimulation exerts both selective and non-selective effects in the dorsal horn. The serotonergic component of the raphe-spinal system has been suggested to be specifically involved in mediating SPA. Thus stimulating NRM has been shown to increase 5-HT metabolism (Bourgoin, Oliveras, Bruxelle, Hamon and Besson, 1980; Rivot, Chiang and Besson, 1982; Rivot, Ory-Lavollee and Chiang, 1983; Suzuki and Taguchi, 1986) and release (Sorkin, Steinman, Hughes, Willis and

McAdoo, 1988) into the spinal dorsal horn of rat, cat and monkey.

Furthermore, systemically administered 5-HT antagonists have been shown to attenuate SPA resulting from stimulation of the midbrain (Hayes, Newlon, Rosecrans and Mayer, 1977) and NRM (Satoh, Akaike, Nakazawa and Takagi, 1980) and to antagonise the inhibitory effect of PAG stimulation on responses of dorsal horn neurones to C-fibre stimulation in cat (Carstens, Fraunhoffer and Zimmermann, 1981a). Foong and co workers have nowever demonstrated that although systemically-applied methysergide reduced inhibition by PAG stimulation of dorsal horn neuronal responses to C-fibre stimulation, ionophoretically-applied methysergide failed to do so (Foong, Terman and Duggan, 1985). This observation stresses the disadvantage of using systemic administration of compounds as a method of assessing the neurochemical nature of descending systems, since a drug introduced into the vascular system may interact (perhaps non specifically) at more than one site. Depletion of 5-HT with the neurotoxin pCPA has also been shown to attenuate SPA from PAG and periventricular structures in the midbrain (Akil and Liebeskind, 1975; Akil and Mayer, 1972) which can be restored with the 5-HT precursor, 5-hydroxytryptophan (5-HTP)(Akil and Liebeskind, 1975). Moreover, administering 5-HTP on its own can augment the analgesic effects of NRM stimulation and can reverse analgesic tolerance caused by long and repeated stimulation (Oliveras, Hosbuchi, Guilbaud and Besson, 1978). Significantly, increasing 5-HT availability by treating animals (Uzan, Kabouche, Rataud and LeFur, 1980) and man (Johansson and Von knorring, 1979) with 5-HT uptake inhibitors has been shown to increase nociceptive thresholds and result in anlgesia. More importantly, the spinal site of action of the serotonin-containing raphe-spinal neurones in mediating analgesia has been demonstrated by the ability of intrathecally-applied methysergide, a 5-HT antagonist, to attenuate the analgesic effect of stimulating the NRM (Barbaro et al., 1985; Hammond and Yaksh, 1984; Jensen and Yaksh, 1984a) in the tail flick and pinch tests although not in the hot plate test (Jensen and Yaksh, 1984a). The attenuation is not complete however, which points to the involvement of transmitters in addition to 5-HT.

Based on the above results, it appears that the raphe-spinal system is one of the important loci for mediating SPA and that its analgesic action is mediated at least partially through a 5-HT receptor in the spinal cord.

B. Opioid Analgesia (OA)

Opioid analgesia has been investigated by administering agonists and antagonists : (1) systemically and (2) via a microinjection technique. The disadvantage of the first method is that one cannot pin-point the specific areas of the central nervous system activated in OA. The two approaches have however been combined successfully in many studies. Microinjecting morphine into the PAG not only causes powerful analgesia (Gebhart, 1982; Jensen and Yaksh, 1986a; Yaksh, 1979; Yeung, et al., 1977), but excites cells in the NRM (Cheng, Fields and Heinricher, 1986; Fields and Anderson, 1978) and causes a significant increase in the level of 5-HT contained in spinal superfusates (Yaksh and Tyce, 1979). Microinjection of morphine into NRM itself increases 5-HT metabolism in the spinal cord (Suzuki and Taguchi, 1986, Vasko et al., 1984), produces analgesia (Azami, Llewelyn and Roberts, 1982; Dickenson, Oliveras and Besson, 1979; Jensen and Yaksh, 1986a; Llewelyn, et al., 1983; Vasko, Pang and Vogt, 1984) and innibits dorsal horn neuronal responses to noxious stimulation (Thalhammer, Du, Kitanata and Zimmermann, 1983). Furthermore, blocking spinal 5-HT receptors by intrathecally applied 5-HT antagonists attenuates the effect of morphine microinjected into both the PAG (Jensen and Yaksh, 1986b; Yaksh, 1979) and NRM (Jensen and Yaksh, 1986b). Similarly, depletion of spinal cord 5-HT content attenuates the antinociceptive effect of morphine microinjected into NRM (Vasko et al., 1984). The above data suggests that local

injection of morphine in the brainstem activates the descending serotonin-containing system. Data obtained from experiments where morphine was systemically administered provides indirect support for the involvement of the descending serotonin-containing system. It has been demonstrated, for instance, that systemically administered morphine increases 5-HT metabolism in the spinal cord (Suzuki and Taguchi, 1986; Weil-Fuggaza, Godefroy, Coudert and Besson, 1981). Furthermore, lesions of the NRM (Abbot and Melzack, 1982; Azami et al., 1982; Proudfit and Anderson, 1975) or of the DLF (Barton et al., 1980; Basbaum et al., 1977) antagonise some of the analgesic effect of systemically administered morphine. Moreover, naloxone microinjected into NRM attenuates the effect of systemically administered morphine in rat (Azami et al., 1982). Systemically-applied morphine however causes both excitation and inhibition of NRM neuronal activity (Anderson, Basbaum and Fields, 1977; Chiang and Goa, 1986; Chiang and Pan, 1985; Fields and Anderson, 1978; Oleson, Twombly and Liebeskind, 1978). Some data also question the importance of the descending serotonin-containing system in OA. Thus intrathecal administration of 5-HT antagonists (Proudfit and Hammond, 1981) or reversibly blocking NRM with local anaesthetic (Proudfit, 1980) fail to block analgesia produced by systemic morphine. Systemic administration of morphine may however, produce analgesia by interacting at several sites in the central nervous system, some of which may by-pass NRM and/or the 5-HT synapse at the dorsal horn level. If this were the case, interference with the descending serotonin-containing system may not be expected to totally block OA. The bulk of evidence is thus in favour of a role for the descending serotonin-containing system in mediating OA. The raphe-spinal system may be one of several parallel pathways involving different transmitters. The function of replicated pathways is not clear, it

may be that different systems are activated under different conditions.

Nucleus raphe magnus and the adjacent reticular formation have been proposed to form a single functional unit (Watkins et al., 1980). The medullary reticular formation which surrounds NRM (see Fig. 1b) is composed of the nucleus reticularis paragigantocellularis (NRPG) which is just lateral to NRM in the rat, which appears to have its equivalent in the cat as the nucleus reticularis magnocellularis (NRMC) and nucleus reticularis gigantocellularis (NRGC) which lies adjacent and ventral to NRM in both rat and cat (Basbaum, Moss and Glazer, 1983). Many similarities exist between the three medullary groups which appear to be anatomically interconnected (Abols and Basbaum, 1981; Beitz, 1982b; Gallager and Pert, 1978) and it may be that all three sites are involved in antinociception and analgesia. Indeed, all three parts of the medulla receive a projection from PAG (Abols and Basbaum, 1981; Fardin et al., 1984; Gallager and Pert, 1978). Moreover, it was demonstrated that the antinociceptive effect of PAG stimulation on dorsal horn neurones and on nociceptive reflexes was not blocked by microinjection of local anaesthetic (Gebhart, et al., 1983a) or by lesion (Behbehani and Fields, 1979) until the area affected included both NRM and adjacent NRPG. Similarly, in order to block totally the analgesic effect of midbrain stimulation it was found to be necessary to interrupt both NRM and NRPG simultaneously, either by electrolytic lesion (Prieto, et al., 1983) or by microinjection of local anaesthetic (Sandkuhler and Gebhart, 1984b). It has also been shown that electrically stimulating NRPG / NRMC (Akaike, Shibata, Satoh and Takagi, 1978; Barbaro, et al., 1985; Dostrovsky, et al., 1982; Gebhart, et al.,

1983a; Satoh, et al., 1980; Zorman, et al., 1981) or NRGC (Zorman, et al., 1981) has similar effects, in rat and cat, to stimulating NRM in that both behavioural analgesia and inhibition of dorsal horn neuronal responses (Gebhart, et al., 1983a; Gray and Dostrovsky, 1983; 1985; Haber, Martin, Chung and Willis, 1980) can be induced. Moreover, there are no large differences between current thresholds in all three sites for the inhibition of rat tail flick (Gebhart, et al., 1983a; Sandkuhler and Gebhart, 1984a) or jaw opening reflex in cat (Dostrovsky, et al., 1982). In addition, behavioural analgesia can be produced by the microinjection of morphine into NRM (see above), NRPG (Akaike et al., 1978; Azami, et al., 1982; Kuraishi, Harada, Satoh and Takagi, 1979; Llewelyn, et al., 1983; Satoh, Akaike, Nakazawa, Masuda and Takagi, 1983a) or NRGC (Akaike, et al., 1978; Kuraishi, et al., 1979). But here the similarities end. Escape behaviour has been shown to result from NRGC stimulation (Casey, 1971). Lesions in the NRGC moreover, do not seem to block SPA, while they do antagonise OA induced by microinjection into PAG (Mohrland, Mc Manus and Gebhart, 1982). There is also reason to consider NRM and NRPG as separate entities, NRPG for instance seems to be much more sensitive than NRM to microinjected morphine (Akaike, et al., 1978; Azami, et al., 1982; Satoh, et al., 1983a) or glutamate (Satoh, Oku and Akaike, 1983b). Moreover, different transmitters appear to mediate the analgesic effects of NRM and NRPG. Whereas intrathecal administration of methysergide, a 5-HT antagonist attenuated the analgesic effect of NRM stimulation, (Barbaro et al., 1985), it did not reverse the effects of NRPG stimulation. In contrast, adrenergic receptor blockers applied in the spinal cord were reported to reverse the analgesic effect of stimulating NRPG

(Barbaro, et al., 1985; Satoh, et al., 1983b), but also NRM (Barbaro, et al., 1985). Adrenergic receptor blockers also attenuate the effect of morphine microinjection into NRPG and NRGC (Kuraishi, et al., 1979). It would seem therefore that the electrical stimulation of NRM activates both serotonin-containing and noradrenline-containing descending pathways. Since NRM does not appear to have noradrenaline-containing neurones (Poitras and Parent, 1978; Westlund, Bowker and Ziegler, 1983) it is likely that the activation of the descending noradrenaline-containing pathway was indirectly mediated through axons of passage. The analgesic pathway through NRM seems to be independent of that through NRPG since, reversibly inactivating NRPG with local anaesthetic has no effect on OA induced by microinjecting NRM with morphine (Azami et al., 1982). Lesions of NRM on the other hand attenuate but do not abolish the analgesic effects of morphine microinjected into NRPG (Azami, et al., 1982) indicating some dependence of the pathway through NRPG on an intact NRM. The consensus of opinion thus has been that differences between the three medullary groups in question merit their classification as separate units.

C. Additional Evidence for the Involvement of 5-HT in Analgesia

In confirmation of a role for 5-HT in antinociception and analgesia, important evidence has been obtained from studies where exogenous 5-HT is applied to the spinal cord either intrathecally or ionophoretically.

1) Intrathecal Studies

Serotonin locally applied to the spinal cord through intrathecal administration has been shown to have similar effects to both NRM

stimulation and local morphine injection in producing behavioural analgesia in rat, cat and rabbit (Minor, Post and Archer, 1985; Schmauss,Hammond, Ochi and Yaksh, 1983; Yaksh and Wilson, 1979). This analgesic action can be attenuated by 5-HT antagonists in a dose-dependent fashion (Schmauss, et al., 1983).

2) Ionophoretic Studies

Several groups employed the technique of ionophoresis in an effort to investigate the role of the serotonergic raphe-spinal system in modulating nociceptive transmission. Serotonin, ionophoretically-applied into the substantia gelatinosa (Griersmith and Duggan, 1980; Headley, Duggan and Griersmith, 1978) (where a number of nociceptive fibres is known to terminate), or near cell bodies of cells under study (Belcher et al., 1978; Jordan, Kenshalo, Martin, Haber and Willis, 1978; Randic and Yu, 1976; Willcockson, Chung, Hori, Lee and Willis, 1984) caused inhibition of the cells' nociceptive responses. Non-nociceptive responses however, were also reported to be inhibited (Belcher et al., 1978; Headley et al., 1978) and cases of excitation were occasionally observed (Belcher et al., 1978; Randic and Yu, 1976). Some reports maintain that non nociceptive responses of most cells are not affected by 5-HT (Belcher et al., 1978; Jordan et al., 1978). Firing evoked by ionophoretically applied excitatory amino acids was found to be either facilitated (Belcher et al., 1978) or inhibited (Jordan and McRea, 1976; Jordan et al., 1978; Willcockson et al., 1984). Similarly, spontaneous activity of dorsal horn neurones has been reported to be predominantly inhibited (Jeftinija, Raspantini, Randic, Yaksh, Go and Larson, 1986; Jordan and McRea, 1976; Jordan,

Kensnalo, Martin, haber and Willis, 1979; Randic and Yu, 1976), though excitation was also noted (Belcher et al., 1978; Headley et al., 1978; Jeftinija et al., 1986; Jordan et al., 1979; Todd and Miller, 1983; Weight and Salmoiraghi, 1966). It seems therefore that ionophoretically applied 5-HT has complex and heterogeneous effects on dorsal horn neurones.

It is clear from the above evidence that both exogenously-applied 5-HT and NRM activation have similar effects at a single cell level, in the dorsal horn and also behaviourally. Complex actions of 5-HT on dorsal horn neurones may reflect the involvement of more than one type of 5-HT receptor in somatosensory processing.

IV Heterogeneous 5-HT Receptors

As early as 1957, two distinct sites for 5-HT were described in the guinea pig ileum (Gaddum and Picarelli, 1957). One site was present in the smooth muscle and was susceptible to blockade by dibenzyline (D-receptor), the other was located in the neuronal tissue and was susceptible to blockade by morphine (M-receptor). This classification has been criticised because on the one hand, dibenzyline is not a specific "D-receptor" blocking agent and on the other, morphine action was found not to be on the "M-receptor" but indirectly on acetylcholine release. The M/D receptor classification was therefore abandoned.

In the central nervous system, classification of 5-HT receptor sub-types has been based on high affinity binding studies with 5-HT agonists and antagonists (for reviews, see Bradley, 1984; Bradley, Engel, Feniuk, Fozard, Humphrey, Middlemiss, Mylecharane, Richardson and Saxena, 1986; Peroutka, 1988) . This could be a very confusing

method of classification as the functional correlates of receptor subtypes are often elusive. Until functional roles for different subtypes are described, therefore, binding studies should be treated with caution (see Humphrey, 1984).

Peroutka and Snyder (1979) using tritiated 5-HT and spiperone, a potent 5-HT antagonist, in ligand binding studies on brain tissue, demonstrated two specific binding sites for 5-HT. One to which 5-HT itself binds with high affinity, the other displaying high affinity for spiperone. The two sites were named 5-HT_1 and 5-HT_2 respectively. Serotonin agonists normally seem to bind with high affinity to 5-HT_1 sites, whilst the 5-HT_2 site seems to have high affinity for 5-HT antagonists (Peroutka and Snyder, 1981). Furthermore, there seems to be a good correlation between the binding affinities of the antagonists to the 5-HT_2 sites and their potencies against some effects of 5-HT both in vivo and in vitro (see for example, Peroutka, Lebovitz and Snyder, 1981). The 5-HT_1 site has been subclassified into 5-HT_{1A} (to which spiperone binds with high affinity) and 5-HT_{1B} sites (which have relatively low affinity for spiperone) (Pedigo, Yamamura and Nelson, 1981). A third subtype, the 5-HT_{1C} site has been identified in the choroid plexus of several animals (Pazos, Hoyer and Palacios, 1984) and the most recently identified 5-HT_{1D} site has been characterised in the bovine brain (Heuring and Peroutka, 1987). In rat spinal cord, both 5-HT_{1A} and 5-HT_{1B} sites are present (Huang and Peroutka, 1987; Mitchell and Riley, 1985; Monroe and Smith, 1983; Pazos and Palacios, 1985). Although the 5-HT_2 site also exists in the spinal cord, it is present at quite low levels (Mitchell and Riley, 1985; Pazos, Cortes and Palacios, 1985). A selective agonist at the 5-HT_{1A} site, ($\pm$) 8-

hydroxy-2-(di-n-propylamino)tetralin,(8-OH-DPAT), recently described (Doods, Kalkman, DeJonge, Thoolen, Wilffert, Timmermans and Van Zweieten, 1985; Gozlan, El Mestikawy, Pichat, Glowinski and Hamon, 1983; Hjorth, Carlsson, Lindberg, Sanchez, Wikstrom, Arvidsson, Hacksell and Nilsson, 1982; Middlemiss and Fozard, 1983), has low affinity for both 5-HT_2 and 5-HT_{1B} sites (Hall, El Mestikawy, Emerit, Pichat, Hamon and Gozlan, 1985; Hamon, Cossery, Spampinato and Gozlan, 1986; Middlemiss and Fozard, 1983). 5-methoxy-3(1,2,3,6-tetrahydropyridin-4yl) 1H indole, (RU 24969), on the other hand, is a high affinity agonist at the 5-HT_{1B} site (Doods et al., 1985; Hoyer, Engel and Kalkman, 1985 a;b; Peroutka, 1986). Although it has little affinity for the 5-HT_2 site (Middlemiss, 1985), it retains moderate activity at the 5-HT_{1A} site (Hamon, et al., 1986; Hoyer, et al., 1985 b; Middlemiss, 1985; Peroutka, 1986; Sills, Wolfe and Frazer, 1984). For the 5-HT_2 site, a potent and selective agonist, (±)1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane hydrochloride, (DOI) has been described (Glennon, 1986; Glennon, Titeler and Young, 1986; Shannon, Battaglia, Glennon and Titeler, 1984). Selective antagonists for the 5-HT_2 sites, such as ketanserin (Leysen, Niemegeers, Van Nueten and Laduron, 1982; Leysen, De Chaffoy, De Courcelles, De Clerck, Niemegeers and Van Neuten, 1984) and for the 5-HT_1 sites such as cyanopindolol (Hamon, et al., 1986; Hoyer, et al., 1985 a; b; Neale, Fallon, Boyar, Wasley, Martin, Stone, Glaeser, Sinton and Williams, 1987; Pazos and Palacios, 1985) are now also available . Although cyanopindolol is active as a β-adrenoreceptor blocker (Edwards and Whitaker-Azmitia, 1987) , it can be used in the present experiments, since the β-receptors have

previously been shown not to play an important role in the regulation of nociception (see for example Fleetwood-Walker, Mitchell, Hope and Molony, 1984). Unfortunately, no specific antagonists have been developed yet to distinguish between different 5-HT_1 receptor subtypes. During the course of this project, 5-HT_3 sites have been identified in the central nervous system (Kilpatrick, Jones and Tyres, 1987), but have not been investigated in the present study. The central function of this site has not yet been determined, but unlike the inhibitory effects of 5-HT_1 agonists, the 5-HT_3 site has been shown to mediate depolarisation or evoke transmitter release from terminals of peripheral neurones (for a review see Bradley et al., 1986; Peroutka, 1988; Richardson and Engel, 1986)

In view of the complex effects of ionophoretically applied 5-HT and NRM stimulation on dorsal horn neuronal activities, our aim was to test whether different types of action of the descending 5-HT system are mediated by different receptors. Using the most selective agonists and antagonists for the 5-HT_{1A}, $_{1B}$ and 5-HT_2 sites, currently available, our objective was to elucidate the role of each receptor type in antinociception.

METHODS

I Electrophysiological Experiments

A. Surgical Procedures

Adult Wistar rats (170-350 g) were anaesthetized with a mixture of α-chloralose (35 mg/kg) and urethane 700 mg/Kg) administered through a cannula in the jugular vein, following initial induction with halothane. Additional doses (1 mg α-chloralose and 20 mg urethane) were given as required to maintain the absence of reflexes such as the corneal and pinch-evoked limb withdrawal reflexes. Blood pressure was monitored via a transducer (4-327-L221-1-B4 M5, Bell & Howell ltd.) connected to a cannula in the carotid artery and a thermostatic blanket maintained core temperature at 37-38 ^{o}C. The rat was placed in a stereotaxic frame and the tail was fixed to a bar with masking tape to keep the spinal cord straight and to aid recording stability. Three pairs of "swan-neck" clamps were used to support the vertebral column. A laminectomy was performed to expose the dorsal spinal cord between T_{13} and L_3 and a pool was formed around this area by tying skin flaps to the spinal support frame. To improve extracellular recording stability, 4% agar, at 37^{o}C was injected under the spinal bone of T_{12}, then poured over the surface of the exposed cord filling the pool. Once the agar had set, it was removed from the laminectomy area, the dura was then retracted and the exposed area was covered with mineral oil at 37^{o}C to prevent dehydration (see Fig. 2a). In brain stimulation experiments, a craniotomy was performed, exposing the appropriate brain area (approximately 20mm^2) which was then covered with mineral oil. To aid spontaneous respiration, the rat was allowed to breathe air

enriched with humidified oxygen which was passed at a rate of 0.1-0.2 litres/minute, through one end of a Y-shaped attachment to its tracheal cannula.

B. Electrophysiological Recordings and Ionophoresis of Drugs

The electrodes used were of a seven-barreled format, constructed from 1.5 mm outer diameter filamented glass tubing (Clark Electromedical Instruments). Tip size was 4.0-4.5 μm, DC resistance of the recording electrode was 5-8 MΩ and the band width of the recording amplifier was 100 Hz-1 KHz (WPI model M-707, W-P Instruments Inc.). Single unit extracellular recordings were made through the central barrel containing 4M NaCl at pH 4.0-4.5, whilst drugs were ionophoresed through the side barrels of the multibarreled electrode. One side barrel contained 1 M NaCl at pH 4.0-4.5 for automatic current balancing. The algebraic sum of currents flowing from the electrode tip was neutralized to within ± 0.1-0.2 nA through the current balancing system employed by the neurophore BH2 ionophoresis system. This was necessary, in order to exclude the possibility that unbalanced movement of charged species might alter neuronal excitability by producing potential changes. To further exclude the possibility of pH or osmotic changes causing alterations in neuronal excitability, the effect of 1M NaCl, at pH 4.0-4.5, ionophoretically-applied at high currents was occasionally assessed. One side-barrel always contained pontamine sky blue(0.2% in 0.5 M sodium acetate) for marking the positions of recording sites in the dorsal horn. The remaining side-barrels contained a combination of the following drugs all dissolved in 0.1 M NaCl and at pH adjusted to 4-4.5 unless otherwise stated : 100 mM D,L-homocysteic acid, DLH, in aqueous solution at pH 8.0-8.5

(Sigma); 100 mM glutamate, Glu, in aqueous solution at pH 8.0-8.5 (Sigma), 2.5 mM serotonin creatinine sulphate, 5-HT,(Sigma); 1.25 mM (±) 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide, 8-OH-DPAT, (Research Biochemicals Inc.); 1.25 mM 5-methoxy-3(1,2,3,6, tetrahydropyridin 4-yl) 1H indole,RU 24969, (Roussel); 1 mM (±)1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane HCl, DOI, (Research Biochemicals Inc.); 1 mM (±) cyanopindolol, in aqueous solution (Sandoz); 1 mM ketanserin tartrate, in aqueous solution (Janssen); 100 mM Idazoxan (RX781094 HCl), RX, in aqueous solution (Reckitt and Colman); 100 mM naloxone HCl, in aqueous solution (ENDO Research Labs). Drugs were ejected with a cathodal current, except for DLH and Glu which were ejected with an anodal current. Retaining currents (10-15 nA) of opposite polarity were applied to each barrel to minimize leakage between tests.

C. Quantification of Cutaneous Sensory Responses

The peripheral receptive field of each cell was briefly characterized. Cells were classified as multireceptive (wide dynamic range) when they were excited by innocuous tactile stimuli and had a progressively higher discharge rate to increasing levels of pressure applied by pinching with a pair of rat-toothed forceps. Some of these cells, when tested, also responded to radiant heat. Cells which did not appear to respond to innocuous stimulation and only responded to high levels of mechanical or thermal stimulation, were classified as high threshold only or nocispecific cells. Cells which were excited maximally by innocuous cutaneous stimulation were, on the other hand, classified as low threshold non-nociceptive cells. Innocuous stimulation was provided by a motorized rotating brush (10

seconds duration) whilst the noxious stimulation was usually provided by a quantifiable constant pressure mechanism (10 seconds duration). This mechanism consisted of a pair of serrated forceps connected to a graduated scale, modified from a glass syringe barrel, and was operated manually to constant displacement (see Fig. 2b). The local pressures (equivalant to a loading of approximately 200-700 g) applied to the skin by this mechanism caused a withdrawal reflex in lightly anaesthetized animals and were confirmed to be distinctly noxious but not damaging in humans. In some cases, noxious stimulation was provided by a thermistor- controlled radiant heat lamp, producing a skin temperature ramp from 30 - 48 ^{o}C within 5 seconds (10-15 seconds duration). Noxious stimulation was applied once every 3-4 minutes to avoid damage to the skin. Noxious and innocuous stimuli were applied to adjacent areas within the excitatory receptive field of a cell, on the hind paw or limb, ipsilateral to the recording site. Activity evoked by ionophoretically-applied DLH or Glu (4-100 nA, 10-20 seconds) was also often assessed. The evoked responses were always submaximal and were matched in magnitude, as far as possible, by altering the strength and / or the duration of the different stimuli.

D. Protocol for Ionophoretic Drug Application and Testing of Drug Effects

Regular cycles (2-4 minutes) of control responses to noxious and / or innocuous, and DLH or Glu stimuli were repeated until two or more sets of these responses corresponded closely, varying by less than 20%. Drugs were then ejected for one minute before the start of a test cycle, and the ejected current increased between cycles until a clear response was observed. Antagonists were usually acutely

applied ; the agonist was applied first. On observing a clear effect with the agonist, the antagonist was subsequently applied concurrently with the agonist. In a few cases, however, the antagonist was chronically applied, that is prior to applying the agonist. Care was taken to monitor and discriminate neuronal spikes (Digitimer Spike Processor, model D130) throughout the tests (see Fig. 2c). After testing a neurone with a particular drug, the cell was allowed to recover for up to 100 minutes, before further study was undertaken. To ensure that observed changes in neuronal responses were not caused by the order in which these stimuli were applied, randomization of this order was undertaken during tests.

Continuous records of neuronal firing were collected over 400 ms bin width, together with analogue signals from the stimulators and stored on computer disc (Cromemco System III) and sometimes on FM tape. Data was analyzed "off-line", by integrating the number of stimulus-evoked action potentials in selected epochs corresponding to the duration of the entire response. To permit comparison of the effects of a single drug on the different responses of the same neurone, integrated test responses were normalized and expressed as percentages of control values. Graphs were then constructed plotting the percentage of control values for each of the evoked responses against drug ejection current. To test whether a particular drug had significant effects on any of the evoked responses, results from different cells were pooled. Using each cell as its own control, the total number of spikes (in response to, for example, brush) was analysed pre- and post-drug application. The mean pre- and post-drug responses were then compared using the paired student t-test and statistical significance was accepted at the 5% level.

Figure 2.

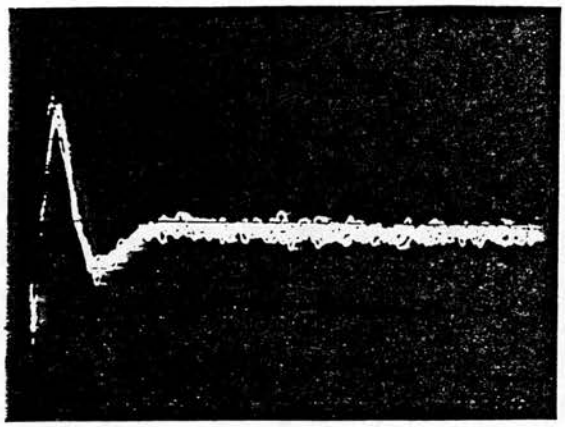
Method of identification and testing of dorsal horn neurones

a) A schematic diagram of the conditions under which electrophysiological recordings were made. The spinal cord was exposed and agar at 37°C was poured over the cord. Once it had solidified, the agar was removed from the surface and after retracting the dura, liquid paraffin at 37°C was poured over the exposed cord. A multibarreled electrode was used both for recording and ionophoresis.

b) The mechanical stimulators : a motorized rotating brush provided the innocuous stimulus and a pinch mechanism (serrated forceps calibrated for constant displacement) provided the noxious mechanical stimulus.

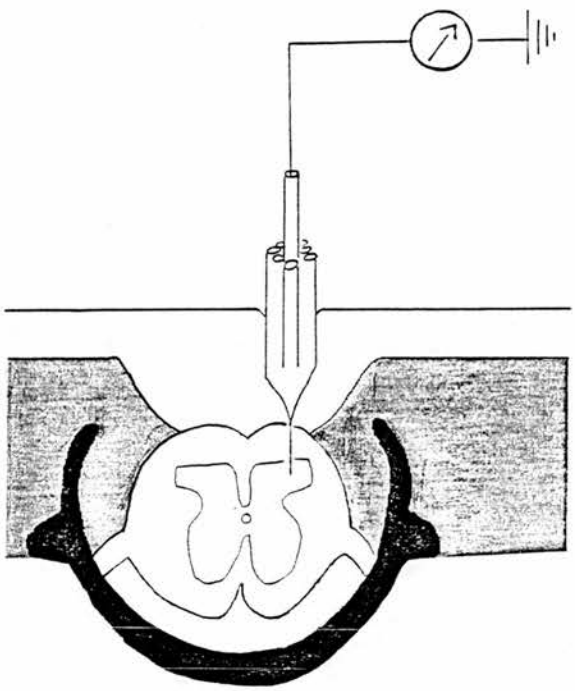
c) During extracellular recording, care was taken to clearly discriminate the signal from the background noise, The figure depicts six oscilloscope traces of the same spike superimposed.

c)



12·5 μv
0·5 ms

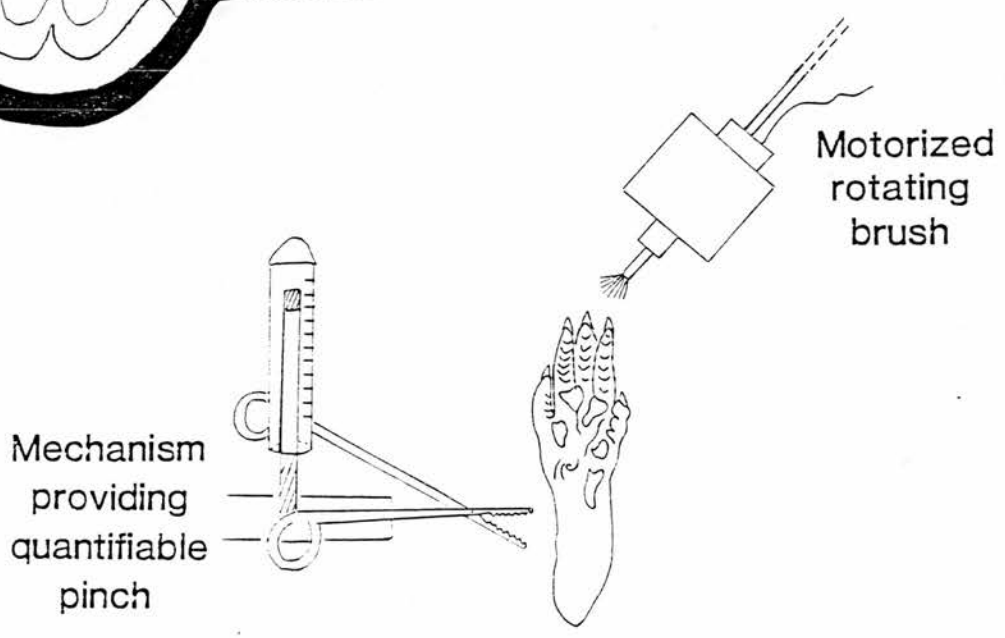
a)



Paraffin

Agar

b)



Motorized rotating brush

Mechanism providing quantifiable pinch

E. Protocol for the Focal Electrical Stimulation in the Region of the Nucleus Raphe Magnus (NRM)

The effects of focal electrical stimulation in the region of the NRM were tested on rat dorsal horn neurones. One of a pair of concentric bipolar stimulating electrodes (outer diameter 0.25 mm, inner diameter 0.1 mm, distance from tip to annulus 0.25 mm, Clark Electromedical Instruments, SNE-100) was stereotaxically placed in the NRM (10.0 mm posterior to Bregma, 0.2 mm lateral, 9 mm ventral) according to Paxinos and Watson rat brain atlas (1986). The other electrode was placed 1-1.5 mm lateral, posterior or anterior to the electrode in NRM. Electrical stimulation was initiated 10 seconds before and maintained during peripheral stimulation. Monophasic square pulses were of 0.4 ms width and 5-300 μA amplitude and were delivered at a frequency of 33-100 Hz. To determine the type of receptor which mediates the effect of brain stem stimulation , the ability of different antagonists to reverse the effects of brain stimulation at the spinal level was assessed. Thus, antagonists were applied either acutely or chronically in the spinal cord in the vicinity of the dorsal horn neurones recorded from.

F. Antidromic Identification of Spinomesencephalic Tract (SMT) Neurones

Dorsal horn neurones projecting to the midbrain were identified using standard antidromic criteria (Lipski, 1981) :

- Collision of antidromic with orthodromic spike
- Response following high frequency stimulation (up to 300 Hz)
- Constant latency and well defined threshold of activation from a particular point of stimulation.

Two concentric bipolar electrodes (same specifications as defined above) were placed bilaterally in the region of the parabrachial nucleus. This area was shown to be a major target of lamina I neurones (Cechetto et al., 1985; Hylden et al., 1985a). Stereotaxic coordinates were as follows : from bregma 9.6 mm caudal, 1-2 mm lateral to the midline, 7 mm ventral (Paxinos and Watson, 1986). The search stimulus delivered at a frequency of 1 Hz, was monophasic and square in shape, 0.4 ms wide and 5-150 μA in amplitude. It has been shown that in a number of cases, SMT neurones send axon collaterals to the thalamus (e.g. Hylden et al., 1986b; Yezierski et al., 1987). According to Hylden (personal communication), about 40% of neurones projecting to the parabrachial nucleus in the rat send axon collaterals to the thalamus. We did not investigate the axon collateralization patterns of our neurones, since it was sufficient, for our purposes, to identify neurones with supraspinal connections. Once a neurone was identified as projecting to the parabrachial nucleus (according to the criteria above), its response properties were briefly characterized before tesing was commenced. The position of the electrode used for antidromic activation was verified at the end of each experiment (see below).

G. Histological Identification of Electrode Placement

The position of brain stimulating electrodes was marked at the end of each experiment by an iron deposit from each electrode (30-60 seconds, 30 μA D.C.). The animal was then killed by an overdose of the anaesthetic and the brain removed and fixed in 10 % formal saline with 1 % potassium ferrocyanide for 2-3 days. The tissue was then sectioned on a cryostat (100 μm), mounted on gelatinised glass slides, left overnight in saturated formaldehyde vapour, and then

stained with neutral red. For determining the position of recording sites in the spinal cord, spinal cord tissue was removed and fixed in 10 % formal saline for 12-18 hours, then processed as above to reveal pontamine sky blue dye marks.

II Behavioural Experiments

A. Protocol for Intrathecal Cannulation

The procedure followed was modified from the original protocol of Yaksh and Rudy (1976). Adult wistar rats (200-250g) were anaesthetized with 2-4 % halothane and mounted on a block of wood which supported the body and tilted the head downwards. A 3 cm midline incision was made caudally from the inter-aural line and the fascia retracted from the skull. A pair of blunt scissors was used to retract the superficial muscles and underlying neck musculature was naturally split down the middle. A curved retractor held the split muscles apart so that the fascial layer connecting the base of the skull to the first spinal vertebra could be easily discerned. Using a scalpel blade, a small incision was then made in the atlanto-occipital membrane and through the membrane layers covering the cisterna magna. The membrane was lifted upwards using a fine pair of forceps and the tip of the sterile cannula was introduced into the incision. The cannula consisted of a 14 cm long piece of polythene tubing of 1.8 mm outer diameter and 0.4 mm inner diameter stretched to twice its original length. To facilitate insertion, the cannula was coated with sterile vaseline. The angle of the catheter was kept parallel to the dorsal surface of the brain stem and the cannula was carefully advanced downwards for approximately 2 cm. The animal's spine was then flexed for the remainder of the insertion, On

encountering any resistance during the insertion procedure, the tubing was withdrawn slightly and then reinserted. Sometimes, the animal's spine was twisted downwards and then upwards before reinserting the catheter. It was found that such a maneouver greatly facilitated cannulation. The tip of the cannula usually extended caudally 7-7.5 cm from the base of the skull and positioned around T12-T13. Once inserted, halothane administration was terminated and a piece of sterile tubing was threaded to lie around the cannula and secured inside the head muscles at the base of the skull with cyanoacrylate . 0.05 ml of Streptopen (0.05 ml contains 12.25 mg procaine penicillin and 12.25 mg dihydrostreptomycin sulphate, Glaxovet) was administered to the open wounds of the head and the head incision then covered by attaching the skin flaps together with cyanoacrylate. A 1.5-2 cm long collar, made of 2 mm diameter tubing was attached to the head end of the cannula and the open end sealed with glue. This collar served to protect the protruding end of the catheter and also made injection procedures easier by making the cannula less flaccid. Each rat was given 0.2 ml of Streptopen and 0.2 ml of the analgesic Temgesic (0.2 ml contains 60 µg of buprenorphine hydrochloride, Reckitt & Colman).

Rats were individually housed and allowed free acess to food and water. They were left to recover for 6-14 days and were checked at least twice daily. Animals displaying signs of distress or motor abnormalities were sacrificed.

B. Injection and Testing Procedures

Rats were acclimatized to experimental conditions for an hour before testing commenced. Two analgesiometric tests were used in

this study. The tail flick test (D'Amour and Smith 1941) and the hot plate test (Woolfe and Mac Donald 1944). In the tail flick test, the rat was restrained and its tail (5-7 cm from the tip) was positioned over a circular slit through which a focussed light bulb heated the tail to 50 ^{o}C in five seconds. The time from the onset of this thermal stimulus to the reflex flick of the tail away from the light source was recorded automatically and termed the tail flick latency (TFL). The trial was terminated after 15 seconds had elapsed (cut-off time) to prevent tissue damage. The mean of three control trials was determined and termed the baseline latency. Tail temperature was monitored throughout testing using a digital temperature guage and noted down just before testing the animal with the tail flick test. Skin temperature was monitored to check whether the applied drugs had any effect on skin blood flow. The hot plate test was performed by placing the rat on a circular metal surface maintained at 55 ^{o}C by a temperature control box. The hot plate latency (HPL) was considered as the time which had elapsed between the placement of the animal on the hot plate and the rats reaction by lifting one of its hind limbs. The cut off time for the hot plate test was 30 seconds. The baseline response latency was calculated as the mean of three control trials. To ensure that observed responses to a drug were not attributable to motor effects of this drug, the rat's motor coordination and balance were assessed by allowing it to walk for 10 seconds on a rod rotating at 6 r.p.m.

Drug doses were calculated as the salt and drugs were dissolved in sterile saline on the day of use. pH was adjusted to as near 7.0 as was possible, though RU 24969 and cyanopindolol would not dissolve

in solutions of pH >5, for this reason, the effect of saline at pH 4 on nociceptive thresholds was also assessed. Each drug was microinjected in a volume of 15 µl and the cannula was then flushed with 10 µl of saline to ensure delivery of the drug to the caudal intrathecal space. Injections were made over 40-60 seconds and testing was carried out immediately following injection and at 5 minutes intervals from then onwards. The antagonist was applied on its own in some cases to determine whether it can alter nociceptive thresholds. In other experiments, cyanopindolol was intrathecally administered 10 minutes prior to agonist application. This time course was chosen because agonist effect was determined to be maximal 10-15 minutes post injection. The following drugs were used :

DRUG	TOTAL DRUG DOSE IN VEHICLE
8-OH-DPAT	3.3-6.6 µg (10-20 nmoles)
RU 24969	14 pg-7.5 µg (37.5 fmoles-20 nmoles)
cyanopindolol	2.9 ug (10 nmoles)

At the end of testing, the rat was sacrificed and a postmortem carried out to determine the position of the cannula verified by injecting a little pontamine sky blue.

C. Statistical Analysis

Examination of the time course of the effect of intrathecally administered saline with one way analysis of variance (ANOVA) indicated that time post-injection had no influence on nociceptive responses. Thus, responses before and after intrathecal saline administration were pooled and compared using paired t-test to reveal that intrathecally applied saline had no effect on nociceptive responses. Since intrathecally applied saline had some inconsistent

effects on nociceptive thresholds, the significance of drug effects were assessed with respect to control responses. ANOVA in conjunction with paired t-test were used to determine the effects of each drug dose over time. The maximum response latency measured during testing with RU 24969 was used to construct the dose-response curve. ANOVA was used to determine the significance of antagonist reversal of agonist effect. Linear regression was used to demonstrate the relationship between tail temperature and nocieptive latencies.

RESULTS

Ionophoretic Experiments

Results were obtained from one hundred and eight cells, of which ninety one were nociceptive and the rest were classified as non-nociceptive. The distribution of fifty two recording sites in the dorsal horn is shown in Fig. 3. Some of the results reported here have been published elsewhere (see appendix).

I Effects of 5-HT Receptor Agonists

A. Effects of Ionophoretically Applied 5-HT

Thirty two cells were tested with 5-HT, seven of which were classified as low threshold only cells (see methods), while the remaining twenty five were nociceptive, having excitatory responses to noxious pinch and in some cases to radiant heat as well. Most of the cells had virtually no spontaneous activity, which may have been an effect of anaesthesia.

1) Nociceptive Cells

The recording sites for sixteen out of the twenty five nociceptive cells tested were distributed in laminae III-VII, whilst the rest were located in lamina I. All but two of the cells tested were classified as wide dynamic range cells. Two cells appeared to be nocispecific (see methods).

i) Cells in Laminae III-VII

Results obtained with this compound are summarised in Table Ia. The effects observed with 5-HT were generally of slow onset, usually requiring at least one minute of ionophoresis and recovery was often prolonged. In 11/16 cases, 5-HT caused a selective inhibition of the

nociceptive response. An example of this type of effect can be seen in Fig. 4a. It was noted that 5-HT ejection currents required to produce a selective inhibition of responses to the noxious input were relatively low (see Table IIa). The mean current causing an inhibition of the pinch response to 60% of control values was 12 ± 4 nA (on for 5 ± 2 minutes). In 8 cases, this current was less than or equal to 10 nA. At these current levels, responses to brush and ionophoretically-applied DLH or Glu were not significantly affected. At higher currents however, responses to DLH or Glu, unlike those to brush, varied considerably. The DLH- or Glu- evoked activity was augmented by 5-HT in five cases and depressed in two. In the remaining four cells this activity remained unaltered. Serotonin produced a non-selective inhibitory effect on all responses tested in two cells. An example of this type of effect can be seen in Fig. 4b. Furthermore, upon increasing 5-HT currents near three cells which had displayed selective inhibition of the nociceptive response at low 5-HT currents, selectivity was abolished and the brush response was inhibited also. The DLH- or Glu-evoked activity, too was inhibited in one of these three cells, but remained near control values in one cell and was augmented in another. These variations in the DLH- or Glu-evoked activity underly the high mean % control values of this response relative to the pinch- and brush- evoked responses, at mean currents of 5-HT employed in tests (see Table IIIa). Serotonin (40-80 nA, on for 21-36 minutes) had no effect on 2/16 cells. Finally, in only one case, 5-HT caused a progressive non- selective excitation of all responses tested. When dopamine was ionophoretically applied near this cell, a selective inhibition of the pinch response was observed. This is in agreement with Fleetwood-Walker, Hope and

Mitchell, 1988, and demonstrated that this cell was not unusual with respect to other modulatory influences.

The effects of 5-HT on the background activity of cells was variable. No effect was observed in the majority of cases (10/16), but most were silent, so there might have been a bias in this observation. Decreases were observed in five cases and increases in one. There seemed to be no clear correlation between the effect of 5-HT on evoked responses and that on the background activity of the cell. Ionophoretically applied 1M NaCl, at pH 4.0-4.5 (40-80 nA) had no effect on neuronal responses, thus discounting the possibility that the effects observed were due to current changes rather than the compound under testing.

ii) Lamina I Cells

Nine lamina I cells were tested. Four were classified as belonging to the spinomesencephalic tract, two of which were classified as nocispecific. The effects of ionophoretically applied 5-HT on this population of neurones were similar to those obtained in the deeper dorsal horn (see Table Ib). Selective inhibitory effects on the nociceptive response whilst sparing the response to innocuous stimulation was observed on five multireceptive cells, one of which was an SMT cell. In contrast, the pinch- and DLH- evoked responses of two nocispecific SMT cells were non selectively inhibited by 5-HT. Two further multireceptive cells, one of which was an SMT cell, were not significantly affected by 5-HT currents of up to 80 nA ; on for 16 minutes. The mean current required to produce clear effects was very low indeed (see Tables II and III b).

The majority of cells (8/9) had virtually no background activity and 5-HT did not alter this. The background activity of one cell

however, was markedly increased with 5-HT.

The predominant effect of 5-HT on nociceptive cells in both lamina I and deeper laminae was therefore a selective inhibitory action on the nociceptive input. A minor population of cells was non-selectively inhibited by 5-HT, but the least frequent effect observed was a non-selective excitation, noted only once in this study. Some neurones were not affected by 5-HT.

2) Non-nociceptive Cells

Seven cells which responded only to innocuous mechanical stimulation were tested with 5-HT and results are summarised in Table IV. In 6/7 of these cells , 5-HT (8-80 nA) failed to affect neuronal responses to brush. In one case, 5-HT significantly inhibited this response. The response to DLH, on the other hand, was almost always affected. Serotonin markedly inhibited the DLH-evoked response of the cell whose brush response was affected, but, in addition, that of three cells whose responses to brush were unaffected. The DLH-evoked response of two cells was excited, and that of a further cell was unaffected. Spontaneous discharge was unaffected by 5-HT in four cells, whilst it was significantly increased in three.

In general therefore, 5-HT failed to affect non-nociceptive responses in most instances. Serotonin did however affect DLH-evoked activity, increasing it in some cases and decreasing it in others.

B. Effects of 8-OH-DPAT : a 5-HT_{1A} Receptor Agonist

Forty cells were tested with this compound, ten of them were non-nociceptive cells whilst the remaining thirty were nociceptive.

1) Nociceptive Cells

The recording sites for twenty four cells tested were

distributed in laminae III-VII of the dorsal horn, whilst the remaining six recording sites were judged to be in lamina I. All cells were classified as multireceptive cells, except for one which appeared to be nocispecific.

i) Cells in Laminae III-VII

The results obtained with this compound are summarised in Table Ia. In the majority of cases, (17/24), 8-OH-DPAT caused a non-selective inhibition of all responses tested. An example of this type of effect is shown in Fig. 5a. From Table IIIa, it is clear that quite low currents of 8-OH-DPAT produced marked inhibition of responses to all stimuli to more or less the same degree. Although the values in this Table are indicated for pinch only, the response of two cells to heat was assessed and it was noted that heat-evoked activity was similarly inhibited. There thus seems to be no differential effect of this compound on the two nociceptive inputs. The background discharge of the majority of these cells (10/17) was unaffected by 8-OH-DPAT, though inhibition was noted in four cases and excitation in three.

The 5-HT_{1A} agonist, 8-OH-DPAT, caused non-selective excitation of all evoked responses and of spontaneous activity in three cells. The increases were observed at currents of 8-OH-DPAT, comparable to those which produced non-selective inhibition in other cells. Upon analysis however, the increases were found to be statistically insignificant. It is not clear from this study whether this excitatory effect of 8-OH-DPAT is a real one or not, since the size of the population which displayed this effect was quite small.

The pinch- and brush-evoked responses of four cells were unaffected by 8-OH-DPAT (mean current; 60 $\pm$ 9 nA, on for 15 $\pm$ 3

minutes). In three of these cells the DLH-evoked and spontaneous activity were also unaffected, but in one cell, both these activities showed an increase with 8-OH-DPAT.

ii) Cells in Lamina I

Six cells were tested with 8-OH-DPAT (see Table Ib), the majority of these cells (5/6) were multireceptive, though one appeared nocispecific. All cells showed a significant non-selective inhibition of their evoked responses including the nocispecific cell (see Fig. 5b). The mean current required to inhibit the pinch response to 60% of control values was quite low (see Table IIIb), being about half the current required to produce the same level of inhibition in the deeper dorsal horn. The background activity of three cells was inhibited while it was unaffected in the remaining three. In general therefore, 8-OH-DPAT non-selectively inhibited nociceptive as well as non-nociceptive and DLH-evoked activity of the majority of neurones tested, both in lamina I and in deeper dorsal horn. A few cells in laminae III-VII seemed to be non-selectively excited by 8-OH-DPAT, though this effect was not observed in lamina I.

2) Non-nociceptive Cells

The effect of ionophoretically-applied 8-OH-DPAT was assessed on ten non-nociceptive cells. The results were quite complex (see Table IV). In five cells, the brush-evoked response was significantly inhibited. In four of these cells, the DLH-evoked activity was also inhibited, in one case, the DLH response was increased. Two cells were non-selectively excited by this compound; both brush- and DLH-evoked, as well as spontaneous activity being affected. In the remaining three cells, the brush-evoked response remained unaltered, whilst the DLH-evoked response was decreased in two cases and

increased in one. The non-nociceptive response as well as DLH-evoked activity of most cells tested were therefore affected by this compound. Inhibitory effects were predominant, although occasional excitatory effects were also observed.

C. Effects of RU 24969 : a 5-HT1B Receptor Agonist

Fifty three cells were tested with this compound, six of which were non-nociceptive, while the remaining forty seven responded to noxious stimulation.

1) Nociceptive Cells

The recording sites for forty two cells tested with this compound were judged to be in laminae III-VII, whilst the rest were located in lamina I. All cells were multireceptive, except for two which appeared to be nocispecific.

i) Cells in Laminae III-VII

The effects observed with RU 24969 were of slow onset, requiring at least one minute of ionophoresis. The results obtained with this compound are summarised in Table Ia. In the majority of cases (27/42 including the two nocispecific cells), RU 24969 caused a selective inhibition of the nociceptive response (to either or both pinch and radiant heat). Ejection currents required to produce this effect were relatively low (see Table IIa). The mean current which produced a 40 % inhibition of the nociceptive response was 11 $\pm$ 2 nA for a duration of 6 $\pm$ 1 minutes. At this current level, responses to noxious pinch (and heat in 3/3 cells) were significantly inhibited while responses to brush and ionophoretically applied DLH or Glu were not significantly altered from control levels. At higher currents however, selectivity in action was abolished and all evoked responses

were inhibited. Moreover, at higher currents, responses to DLH or Glu, unlike those to brush, sometimes varied. In four cases this activity was inhibited and in a further two cases it was augmented. In the remaining cells, responses to DLH or Glu remained stable. Similarly, background activity was affected differently in different cells. In the majority of cases (19/27 cells) it was unaffected, it was however decreased in five cases and increased in three. RU 24969 produced a non-selective inhibitory effect on all responses tested (7/42 cells). The mean current at which the pinch response was inhibited to 60% of control values was 18 ± 6 nA, on for a duration of 8 ± 2 minutes. At this current level, other evoked responses were significantly inhibited, though the brush-evoked response (61 ± 4 % controls) was inhibited to a greater degree than was the DLH- or Glu- evoked response (78 ± 8 % controls). The majority of cells (6/8 cells) inhibited non-selectively by RU 24969 had virtually no background discharge and this 5-HT_{1B} receptor agonist had no affect on this discharge in the remaining cases.

RU 24969 on at 15-50 nA for 7-28 minutes failed to significantly affect the pinch- and brush-evoked activities of eight cells. Although the response to excitatory amino acids remained unaltered in the majority of cases (6/8 cells), it was increased in one case and decreased in another. Similarly, the background discharge was unaffected in 6/8 cases but decreased in two.

ii) Cells in Lamina I

Five multireceptive lamina I cells were tested with this compound and all displayed a selective inhibition of the pinch-evoked activity in response to RU 24969. An example of the characteristic effect of this compound is shown in Fig. 6. The current required to

produce a 40 % inhibition of the pinch response was quite low and comparable to the mean current required to produce the same level of selective inhibition observed on cells in the deeper dorsal horn. In general therefore, RU 24969 had a predominantly selective antinociceptive effect on cells both in marginal and deeper layers. A few cells however were non-selectively inhibited by this compound.

2) Non-nociceptive Cells

Six cells were tested with RU 24969 and it was found to have no effect on three cells (15-70 nA, on for 3-11 minutes) - see Table IV. In a further cell, the brush response was unaffected, while the DLH response was markedly reduced. In two further cells, RU 24969 (10-30 nA, on for 9 minutes) caused a non-selective inhibition of both brush and DLH responses. Four of the low threshold cells had no spontaneous activity. RU 24969 did not affect this activity in one cell, but decreased it in another cell.

D. Effects of DOI : a 5-HT_2 Receptor Agonist

This compound was tested on five cells with a mean maximum current of 74 ± 15 nA, on for 11 ± 2 minutes. In all five cells DOI had no significant effect on either the pinch-evoked response, which remained at 89 ± 5 % of control values, or the brush-evoked response which remained at 90 ± 5 % of control values. In contrast, the response to DLH, assessed in three cells only, was significantly reduced to 19 ± 8 % of control values. An example of this effect of the 5-HT_2 receptor agonist is shown in Fig. 7. The spontaneous activity of three cells was unaffected by this compound, background discharge was however increased by this compound in one case, while it was decreased in another. DOI therefore failed to mimic any of

the effects of 5-HT on nociceptive cells.

II The Effects of Antagonists

Two 5-HT antagonists were employed in this study; ketanserin, a selective 5-HT_2 receptor antagonist and cyanopindolol, a selective 5-HT_1 receptor antagonist Cyanopindolol has little affinity for the 5-HT_2 site, but high affinity for the 5-HT_{1B} site and moderate affinity for the 5-HT_{1A} site (see introduction). Although it cannot be used to distinguish between the 5-HT_{1A} and the 5-HT_{1B} sites, cyanopindolol is useful in determining whether a certain effect of a 5-HT agonist is mediated through a 5-HT_1 site. In a few cases, the selective α_2 adrenoceptor antagonist, Idazoxan (RX) (Chapelo, Doxey, Mayers, and Roach, 1981) was tested to determine whether any of the 5-HT effects in the dorsal horn could have been mediated through an α_2 -type receptor. The antagonists were predominantly tested on responses to 5-HT agonists as these produced a more homogeneous profile of action than 5-HT itself.

A. Cyanopindolol : a 5-HT_1 Receptor Antagonist

Table Va summarises the effects of this compound. Cyanopindolol was tested on 16 cells, and ejected concurrently at currents of 20-80 nA with one or the other of the 5-HT_1 agonists. Cyanopindolol was tested against 5-HT in only two cells and was found to be effective in antagonising the selective inhibitory effect of 5-HT on the pinch-evoked response in one cell whilst there was no obvious effect in another. In 5/6 cases (including two lamina I cells) the effects of the agonist were significantly antagonised by cyanopindolol, in one case only, cyanopindolol failed to reverse the effect of 8-OH-DPAT, the 5-HT_{1A} receptor agonist. This antagonist reversed the non

selective inhibitory effects (see Fig. 8) as well as the non selective excitatory effects of the agonist. The mean maximum current of cyanopindolol producing reversal of the non-selective inhibitory effect of 8-OH-DPAT was 52 ± 10 nA, on for 11 ± 3 minutes. At such current levels, the effect of 8-OH-DPAT on the pinch response was reversed from 54 ± 3 % to 87 ± 7 % of control values and on the brush response from 50 ± 19 % to 93 ± 8 % of control values (n=4). Cyanopindolol was also very successful in reversing the effects of RU 24969, the 5-HT_{1B} receptor agonist, in all cases tested (8/8 cells). In seven of these cells RU 24969 had a selective inhibitory effect on the nociceptive response. The mean maximum current of cyanopindolol employed was 40 ± 8 nA, on for 10 ± 4 minutes. At these current levels the nociceptive response which had been inhibited by the agonist to 52 ± 10 % of controls returned to near control levels 93 ± 6 % (see Fig. 9a), whereas brush- and DLH-evoked activity were not significantly affected. Cyanopindolol ejected on its own (50 ± 13 nA, on for 19 ± 3 minutes) near four cells had no significant effect on either pinch- or brush- evoked activity. The response to DLH however, assessed in two cells was increased in one case and slightly decreased in another (see Table Vb). In one case cyanopindolol caused a slight non-selective excitation of all responses tested.

In summary therefore, cyanopindolol on its own had no significant effect on either nociceptive or non-nociceptive responses. Applied in conjunction with one or the other of the 5-HT_1 agonists employed however, cyanopindolol effectively reversed the different actions of these two compounds.

B. Ketanserin : a 5-HT_2 Receptor Antagonist

This compound was tested on eight cells and ejected at currents of 10-50 nA concurrently with one or the other of the 5-HT_1 agonists. Table Va summarises the results obtained. The ejection currents often could not be increased beyond 30 nA as severe spike reduction occurred. In 7/8 cases, ketanserin failed to antagonise the effects of either agonist (see Fig. 9b), in one case only ketanserin was able to antagonise the selective inhibitory effect of RU 24969 on the pinch-evoked response. Ketanserin ejected on its own (16-50 nA on for 12 ± 3 minutes) near eight cells did not significantly affect any of the responses tested (see Table Vb). Ketanserin did however depress the spontaneous activity of three cells. The overall trend therefore indicates that ketanserin is unable to block the effects of 5-HT_1 agonists.

C. Idazoxan : an α_2 Adrenoceptor Antagonist

This compound was tested on four cells and the results are summarised in Table Va. In all four cells, Idazoxan at 30-60 nA on for upto 10 minutes failed to antagonise the effects of either 5-HT_1 agonist. Idazoxan on its own, at 30-40 nA on for 15-24 minutes, ejected near three cells caused a slight but significant increase in both brush- and DLH-evoked activity. Pinch- and radiant heat-evoked activity were also increased but not significantly (see Table Vb).

III Sequential Drug Testing on Cells

In a few cases, more than one agonist or antagonist was tested on the same nociceptive cell and the direct comparisons that this made possible are reported here.

A. Results with Agonists

Five cells were tested with 5-HT and additionally with one and / or the other of the two 5-HT_1 agonists employed in this study. The effect of 5-HT on three of these cells was selectively antinociceptive. In two of these cases however, increasing 5-HT currents caused this selectivity to be abolished. The selective antinociceptive effect of 5-HT was mimicked by RU 24969 in all three cells, whilst 8-OH-DPAT tested on the two cells which displayed the phenomenon of "abolished selectivity" with 5-HT caused a non-selective inhibitory effect on both cells. Serotonin ejected at relatively low currents, caused non-selective inhibitory effects on two cells, this effect was mimicked by 8-OH-DPAT on these same cells.

Thirteen cells (including two lamina I cells) were tested with both RU 24969 AND 8-OH-DPAT in a random order. In 11/13 cases, RU 24969 caused a selective inhibitory effect on the nociceptive response. In two cells however, increasing ejection current abolished this selectivity. On ten of these cells, 8-OH-DPAT caused a non-selective inhibition of all evoked responses tested. An example of this differential effect of the two agonists on the same cell is shown in Fig. 10. In one of the 11 cells, 8-OH-DPAT had no effect. One cell was unaffected by either agonist, while another showed non selective inhibition in response to both agonists.

The results of this section are therefore consistent with observations presented in sections IA, IB and IC. Two separate actions of 5-HT were mimicked by two different agonists. Whereas the 5-HT_{1B} receptor agonist mimicked the selective antinociceptive effect of 5-HT, the 5-HT_{1A} agonist mimicked the non-selective effect of 5-HT. Moreover, the two separate actions of the two different agonists

seem to be exerted on the same cell.

B. Results with Antagonists

In four cases, ketanserin and cyanopindolol were applied acutely and sequentially, in a random order, together with one or the other of the 5-HT_1 agonists employed here. In all four cases, ketanserin failed to block the non-selective inhibitory effect of 8-OH-DPAT (1/4 cells) or the selective inhibitory effect of RU 24969 on the pinch-evoked response (3/4 cells). Cyanopindolol on the other hand, was successful in reversing the effects of either agonist on all of these same four cells. An example of the differential abilities of these two 5-HT antagonists in reversing the effects of RU 24969 can be seen in Fig. 11. This confirms that the 5-HT_1 and not the 5-HT_2 site is involved in mediating the effects of both RU 24969 and 8-OH-DPAT.

Focal Brainstem Stimulation Experiments

Several areas in and around the NRM were stimulated and the effect of stimulation on the pinch- and brush- and in some cases the DLH-evoked responses was determined. The position of the bipolar stimulating electrodes was marked by an iron deposit at the end of each experiment (see methods).

I Effects of NRM Stimulation on Nociceptive Cells

The results are summarised in Table VIa. Stimulating the brain stem in 12 sites in and around the NRM and NRPGL (see Fig. 12) selectively inhibited the pinch-evoked responses of fourteen multireceptive cells (including one lamina I cell) and Fig. 13 shows an example of this type of effect. The mean stimulating current

required to produce 40 % inhibition of the response to pinch was 54 ± 16 μA, at a stimulating frequency of 100 Hz, though for the majority of cells this current was less than or equal to 30 μA. At such current levels, brush-evoked activity was not significantly altered (see Table VIa). Similarly, DLH-evoked activity, tested on eight cells, was not altered. Inhibition of the nociceptive response developed within twenty seconds of brain stem stimulation and recovery was relatively prolonged requiring 1-6 minutes. The level inhibition of the pinch-evoked response, as Fig. 14a demonstrates, was not related to stimulating current intensity at low levels . It was further noted that the selective inhibitory effect on the nociceptive input can be reproduced using lower stimulating frequencies . Fig. 14b is a cumulative graph for a number of cells which showed this selective response to brain stem stimulation and which were tested with more than one stimulating frequency. In general, at lower frequencies of stimulation, higher current intensity is required to produce the inhibition of the pinch response.

Stimulating the brain stem in six different sites (see Fig. 12) with a mean current of 130 ± 50 μA, at a frequency of 100 Hz, produced non-selective inhibition of brush- and pinch-evoked responses in six cells, including one nocispecific lamina I cell -see Table VIa . This effect was produced at low currents (10-33 μA)in three cells. Stimulation frequencies of 50 and 33 Hz, when employed, were also sufficient to produce this non selective inhibitory effect.

As a control, stimulating the brain stem in 14 sites, commonly far outside the NRM (see Fig. 12), at a frequency of 100 Hz and at an intensity ranging between 100 to 300 μA (mean 178 μA) was undertaken in 23 trials. The number of trials was not equivalent to the number

of cells as often the effect of stimulation of two different sites on the evoked responses of the same cell were assessed. Stimulation in these 14 sites failed to affect pinch- or brush-evoked activity significantly in 15 cells, while spontaneous and DLH-evoked activity did not show marked or consistent changes.

II The Effect of Antagonists

Four antagonists were used in this part of the study; cyanopindolol, ketanserin, Idazoxan, and naloxone (an opiate receptor antagonist). Antagonists were usually applied chronically (by ionophoresis close to the dorsal horn neurone being recorded), before brain stem stimulation commenced, but in a few cases, antagonists were applied acutely. Results are summarised in Table VIb.

A. Results with 5-HT Receptor Antagonists

Cyanopindolol, either chronically or acutely applied, in all six cells tested, caused remarkable antagonism of the effects of brain stem stimulation from five sites in or very close to the NRM (see Fig. 13). In contrast, ketanserin, applied either chronically or acutely, near four of these same cells did not in any case, antagonise the effects of brain stem stimulation. The differential abilities of these two 5-HT antagonists in reversing the effects of NRM stimulation is illustrated in Fig. 15.

B. Results with Idazoxan and Naloxone

Idazoxan, on at 30-40 nA, on for 9-10 minutes, failed to reverse the effects of NRM stimulation on two cells, one of which displayed selective and the other non-selective inhibitory responses to NRM stimulation (Fig. 16). In contrast, stimulation at the adjacent electrode lying on the border between NRM and NRPG (see Fig. 16) had a selective inhibitory effect on the latter cell. This effect was

reversed by Idazoxan ionophoretically applied at 30 nA, for 14 minutes.

Naloxone , on at 15 nA for 5-11 minutes was tested on the effects of stimulation in the same site in NRM on two cells. This opiate antagonist failed to reverse either the selective or the non-selective inhibitory effects of brain stem stimulation.

Behavioural Studies : Pilot Studies with 5-HT Receptor Selective Agonists

Five rats were sham-operated to test for any effect that the operation itself might have had on nociceptive threshold. Five further rats were implanted with cannulae that only reached the cervical level of the cord to test whether the effects of compounds tested were due to their diffusion to supraspinal structures. The remaining twenty rats, upon post mortem examination were confirmed to have cannulae positioned in the lower thoracic and upper lumbar levels of the spinal cord.

There were no significant differences between control responses of sham-operated and test animals (2 tailed t-test, $p>0.05$). Moreover, time post-injection of saline appeared to have no effect on either tail flick (TFL) or hotplate (HPL) latencies. Similarly, no significant differences in TFL or HPL between control animals and animals intrathecally injected with saline (mean TFL of control animals = 8.3 seconds, of saline animals = 8.0 seconds ; mean HPL of control animals = 4.3 seconds, of control animals = 4.1 seconds).

Most experiments involved finding the appropriate dosage range of a particular drug for selective analgesic effects. As a result,

replicate number at a particular dose was low (maximum n=5). It is therefore impossible to make definitive statements from this pilot study and all that can be pointed to would be trends in the data. It is hoped that further experiments may clarify and extend the results presented here. Nociceptive thresholds were allowed to recover (often for longer than 60 mins) to near control values before further testing was undertaken. The two agonists, RU 24969 and 8-OH-DPAT were never tested in the same animal.

I Effects of Intrathecally Applied RU 24969

This drug was tested at different doses in fourteen animals. The changes incurred by all but one dose proved insignificant in the tail flick test and the effects at none of the doses proved significant in the hot plate test. This may not reflect what is actually happening because the small sample size employed here introduces large variability in the data which may mask any significance. The data presented thus must be viewed with caution. Low doses of RU 24969 (14-28 pg) had no effect on HPL and slightly decreased TFL though not significantly, medium doses (150-750 pg) did not appear to alter either TFL or HPL. Using higher doses (1.8, 3.7 and 7.5 µg) did however appear to increase TFL sometimes upto cut off (see fig 17a). This response appeared at 5 mins post-injection, the effect appeared to be maximal at 10 mins and was long lasting at the higher two doses (> 40 mins for recovery). Though the increase in TFL was statistically insignificant at doses of 1.8 and 7.5 µg, it was significant at the 3.7 µg dose (see Fig. 17a). Since both other doses clearly mimicked the effect of 3.7 µg RU 24969, the lack of signficance may be entirely a reflection of the small sample size. At doses of 3.7 and 7.5 µg RU 24969, there was also a transient

increase in HPL (Fig. 17b) which appeared at 10 mins and was maximal at 15 mins post-injection. The increase was however statistically insignificant. Fig. 18a and b shows a dose response curve for the effects of different doses of RU 24969 tested against TFL 10 mins and HPL 15 mins post-injection. The graphs indicate that the effects of RU 24969 are dose dependent.

Pinch applied to the tail in random trials with a pair of rat-toothed forceps did not evoke withdrawal responses in animals treated with 1.8, 3.7 and 7.5 µg of RU 24969. Moreover, this drug , even at the highest doses did not appear to impair the animal's motivational or motor responses. Thus rats treated with this compound responded to light touches on their hindlimbs and their performance on the rotarod was equivalent to that during control trials. Some slight shivering and teeth clicking and splayed hindlimbs were however noted with treated animals especially at the highest doses. On the other hand, intrathecal application of 7.5 and 15 µg doses in rats implanted with cannulae reaching only the cervical cord (n=4) had no significant or apparent effect on either TFL or HPL.

II Effects of Intrathecally Applied 8-OH-DPAT

This drug was tested at two different doses in five animals only. Neither a 3.3 µg nor a 6.6 µg dose had any significant effect on nociceptive thresholds monitored in the two behavioural tests employed here. In the tail flick test, (Fig. 19a) however, it appeared as though 8-OH-DPAT may have acted to reduce TFL at the 3.3 µg dose, though this effect did not appear to be dose-dependent. More tests are needed to clarify this effect of 8-OH-DPAT on TFL. Paradoxically, in the hot plate test (Fig. 19b), the trend of action

of 8-OH-DPAT was to increase HPL, though again more tests are required before definitive statements can be made. Neither TFL nor HPL appeared to be altered in one rat which was injected with 13.2 µg of 8-OH-DPAT at the cervical level of the cord. High doses of 8-OH-DPAT caused some motor in-coordination and impaired performance on the rotarod. Often the hindlimbs were splayed (even at the lower dose of 3.3 µg) and urination and defecation occurred frequently.

III Effects of Intrathecally Applied 5-HT_1 Receptor Antagonist

Cyanopidolol (2.9 µg, n=4) applied intrathecally on its own had no significant effect on either TFL or HPL over time although it did appear to slightly decrease HPL (see Fig. 17a,b) . When cyanopindolol was intrathecally applied 10 mins before the intrathecal application of RU 24969 at doses which appeared to produce antinociception in previous tests (3.7 or 7.5 µg), it blocked this putative antinociceptive effect (n=4), such that there was no statistical difference between control latencies and those with intrathecally applied RU 24969 after pretreatment with cyanopindolol (see fig 17a, b).

IV Observations on Tail Temperature : Possible Drug Effects on Cutaneous Blood Flow

There was no significant difference between control tail temperature and tail temperature after intrathecal injection of saline (2 tailed t-test, $p > 0.1$). The intrathecal injection of RU 24969, on the other hand, raised tail temperature sometimes by 2-3 ^{o}C within two minutes of injection and the effect was noticeable at 5-15 mins. Upon analysis, the increases in temperature were found not to be significant. However, there was marked variation of tail

temperature between rats and it is possible that a bigger sample size may reveal significant effects. We decided to compare the effects of RU 24969 on tail temperature with respect to TFL. As can be seen from Fig. 20a, the relationship between tail temperature and control tail flick latency is linear; the higher the resting tail temperature, the shorter the tail flick latency ($r = -0.93$, $n = 5$). RU 24969 at doses of 3.7 and 7.4 µg measured at 10 mins post-injection (the time of maximum effects), shifted this relationship and TFL was consistently lengthened, regardless of resting tail temperature ($r = -0.87$, $n = 5$).

Similarly, doses of 3.3 and 6.6 µg of 8-OH-DPAT caused a rise in tail temperature which was also found to be insignificant. The linear relationship between tail temperature and control TFL is again demonstrated in Fig. 20b ($r = -0.94$, $n = 3$) and 8-OH-DPAT at the above doses caused a parallel shift of the skin temperature-response curve to the left ($r = -0.93$, $n = 5$).

In conclusion, although TFL was found to be clearly shortened at higher resting skin temperature, a fact long ignored by experimenters, the elevation of TFL in response to RU 24969 is not likely to be substantially due to any change in skin blood flow since it occurred regardless of resting tail temperature. In contrast, 8-OH-DPAT appeared to cause a parallel leftward shift in the linear relationship between tail temperature and TFL directly, indicating a possible interaction between the effects of 8-OH-DPAT on skin temperature control and on TFL. Thus any small reduction in TFL observed with 8-OH-DPAT may be due to an increase in tail temperature which probably reflects an increase in cutaneous blood flow.

Figure 3.

The position of extracellular recording sites in the dorsal horn

A schematic drawing of a cross section of the spinal cord showing the position of fifty two recording sites marked by pontamine sky blue spots at the end of the recording. Most recording sites were in laminae III-VII, although a few appeared to be in the most superficial lamina I.

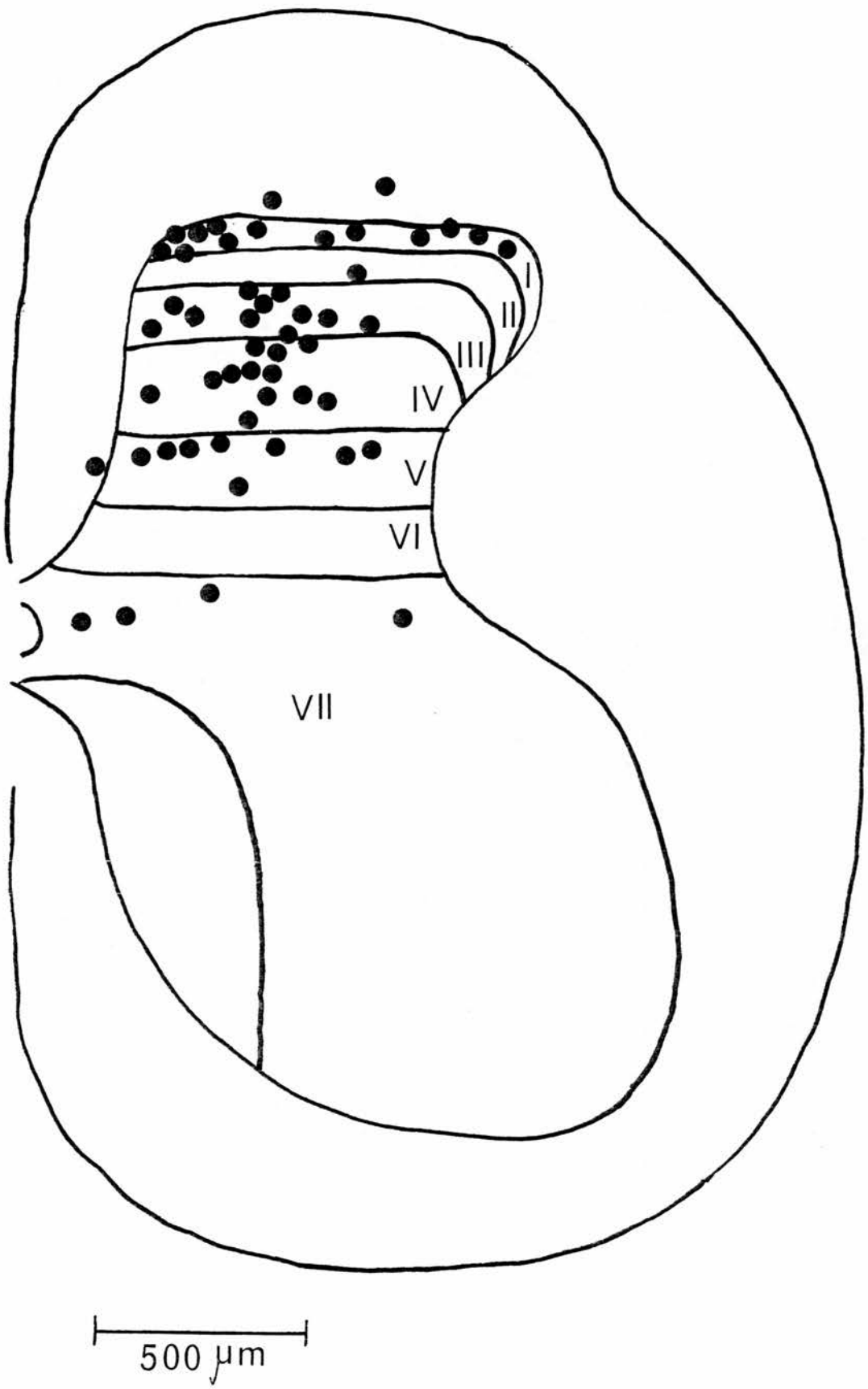
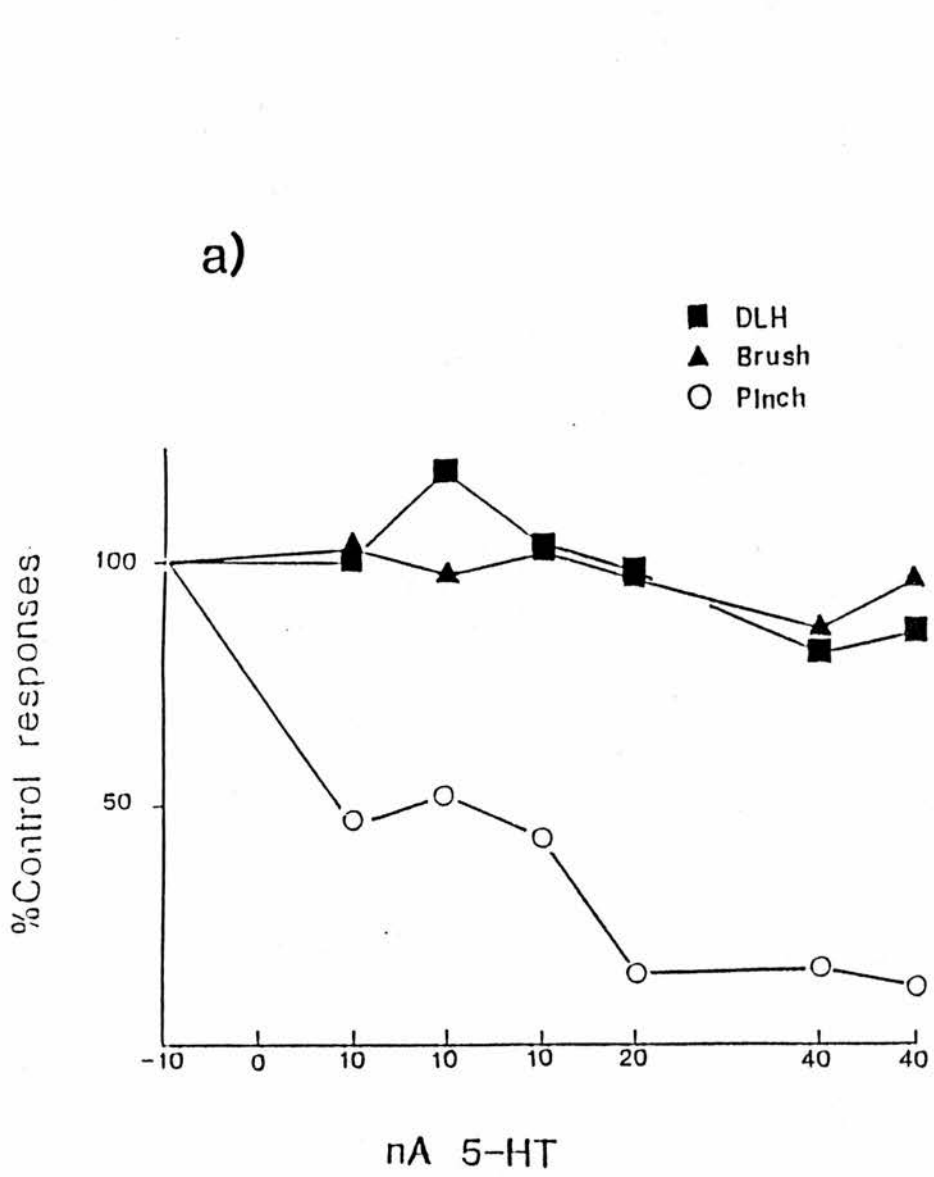

I
II
III
IV
V
VI
VII
500 µm

Figure 4.

The-effect of ionophoretically applied 5-HT on dorsal horn neurones

a) An example of the predominant selective antinociceptive effect of 5-HT. The number of stimulus-evoked action potentials was integrated in selected epochs corresponding to the duration of the response. Integrated test responses were normalised and expressed as percentages of control values. The graph shows percentage of control values for each of the different evoked responses plotted against 5-HT ejection currents (nA) in consecutive test cycles. Pinch-evoked activity was inhibited markedly at very low currents of 5-HT, while corresponding brush- and DLH-evoked activities were unaffected.

b) An example of the non-selective inhibitory effect of 5-HT. Responses were expressed as percentages of control values and plotted against 5-HT ejection currents in consecutive test cycles. Brush- and DLH- as well as pinch-evoked activities were similarly inhibited by 5-HT.


a)
DLH
Brush
Pinch
%Control responses
100
50
-10
0
10
10
10
20
40
40
nA 5-HT

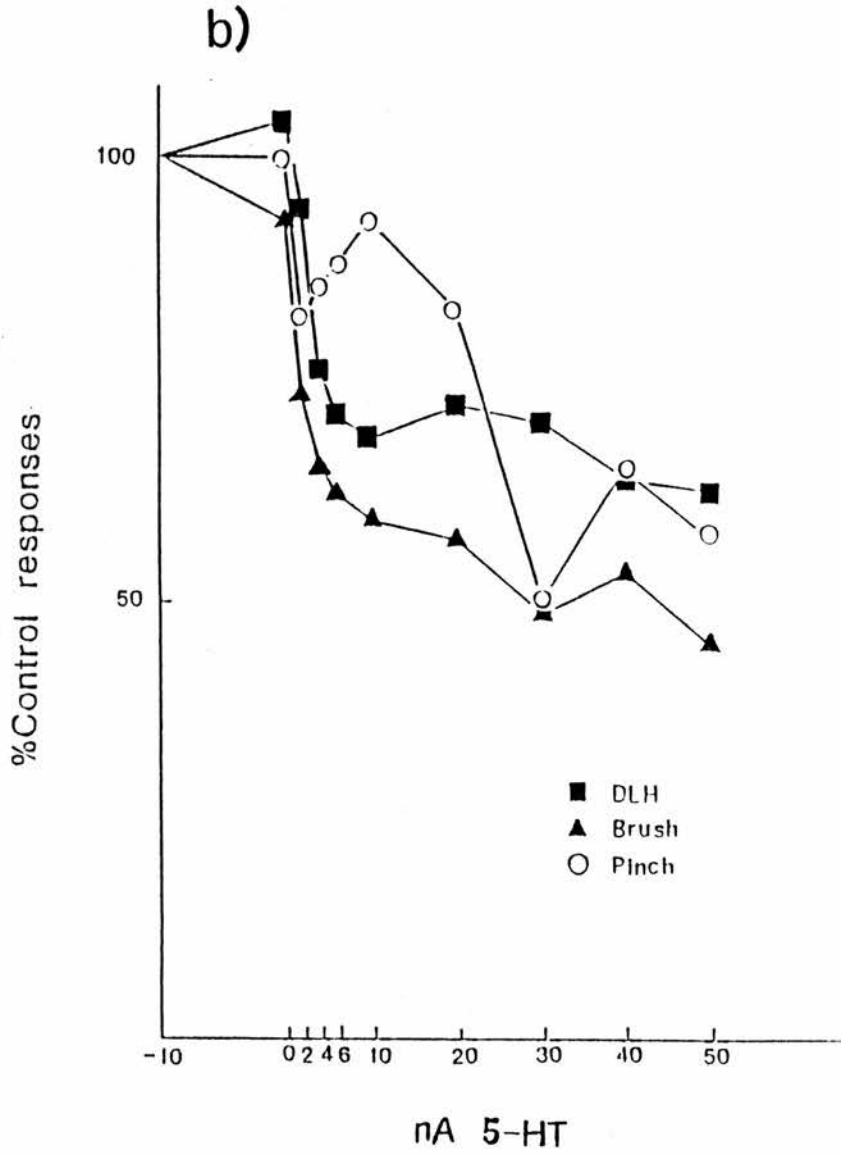

b)
100
50
%Control responses
DLH
Brush
Pinch
-10
0 2 4 6
10
20
30
40
50
nA 5-HT

Figure 5.

The non-selective inhibitory effect of 8-OH-DPAT, a 5-HT_{1A} receptor agonist.

a) Ongoing activity record showing the non-selective inhibitory effect of 8-OH-DPAT on a multireceptive neurone in the deeper dorsal horn. Ongoing firing activity was recorded in 400 ms bins and plotted against time. A. shows the control excitatory responses to DLH, pinch and brush. B. shows the non selective inhibitory effect of 8-OH-DPAT. C. shows the responses 40 minutes after the ionophoretic current of 8-OH-DPAT had been switched off.

b) Ongoing activity record showing the non-selective inhibitory effect of 8-OH-DPAT on a nocispecific lamina I neurone. A. shows control responses to DLH and pinch. B. shows that 8-OH-DPAT inhibited both DLH- and pinch-evoked activities. C. shows recovered responses 17 minutes after 8-OH-DPAT current had been switched off.

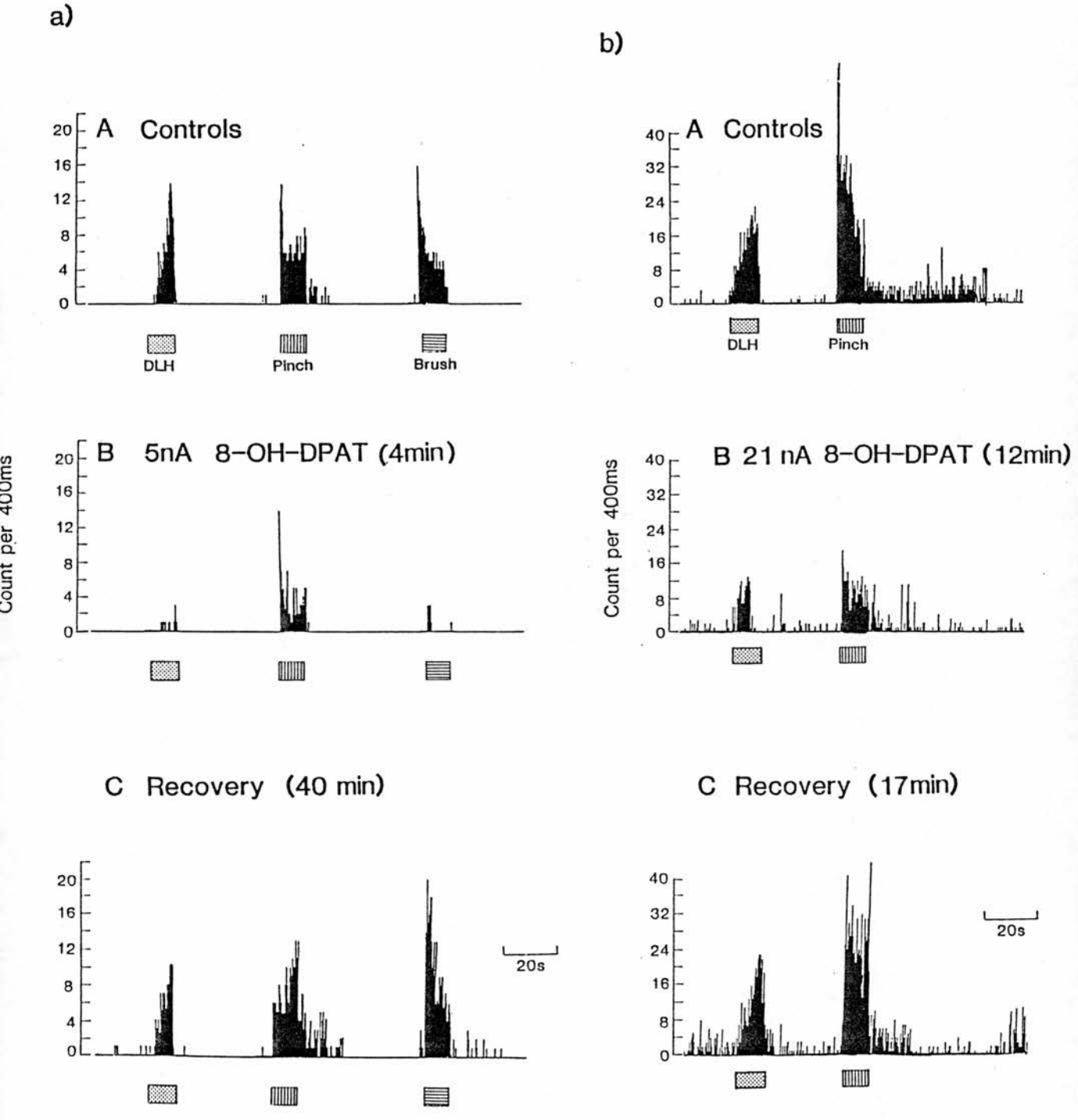

a)
A Controls
B 5nA 8-OH-DPAT (4min)
C Recovery (40 min)
DLH
Pinch
Brush
20s
Count per 400ms
b)
A Controls
B 21 nA 8-OH-DPAT (12min)
C Recovery (17min)
DLH
Pinch
20s
Count per 400ms

Figure 6.

The selective antinociceptive effect of RU 24969, a 5-HT_{1B} receptor agonist

Ongoing activity record of a multireceptive lamina I neurone. A. shows control responses to DLH, brush and pinch. B. shows the selective inhibitory effect of RU 24969, the pinch-evoked activity was markedly reduced, while brush- and DLH-evoked activities were hardly affected. C. shows the recovered pinch-evoked response 5 minutes after the RU 24969 current had been switched off.

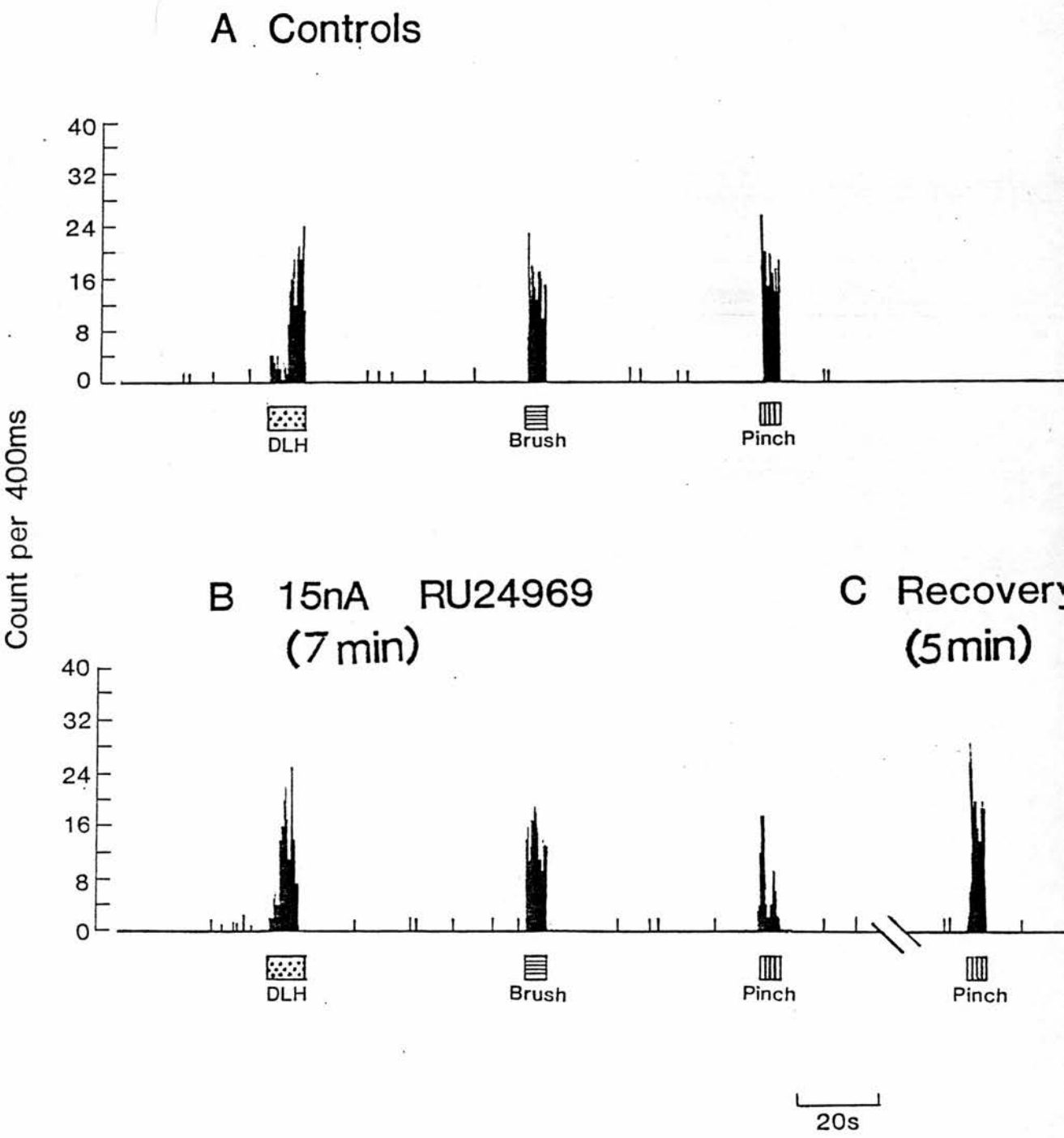
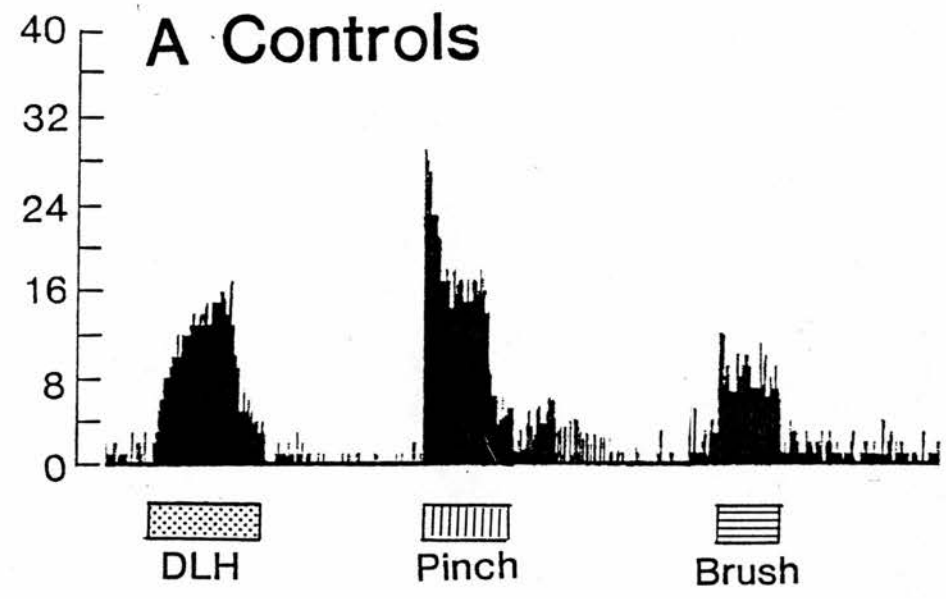
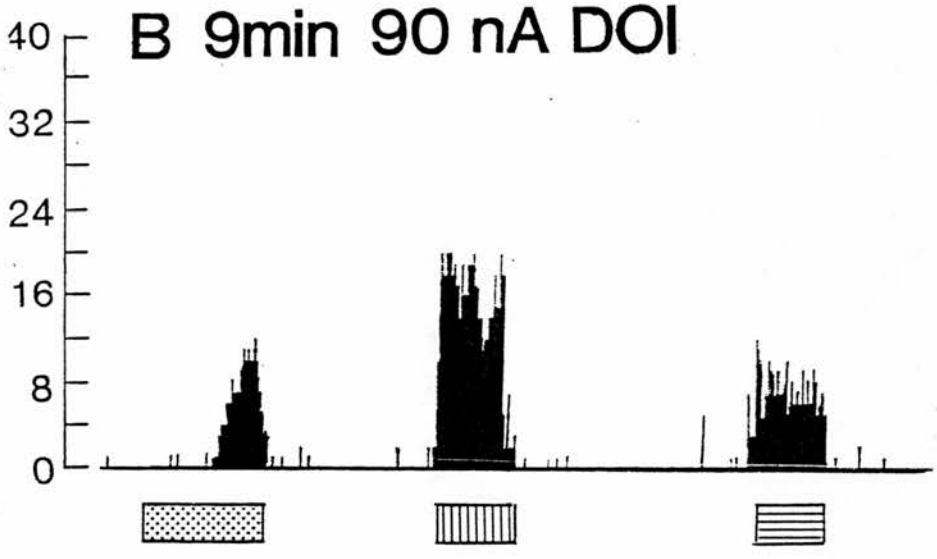

A Controls
40
32
24
16
8
0
DLH
Brush
Pinch
Count per 400ms
B 15nA RU24969
(7 min)
C Recovery
(5 min)
40
32
24
16
8
0
DLH
Brush
Pinch
Pinch
20s

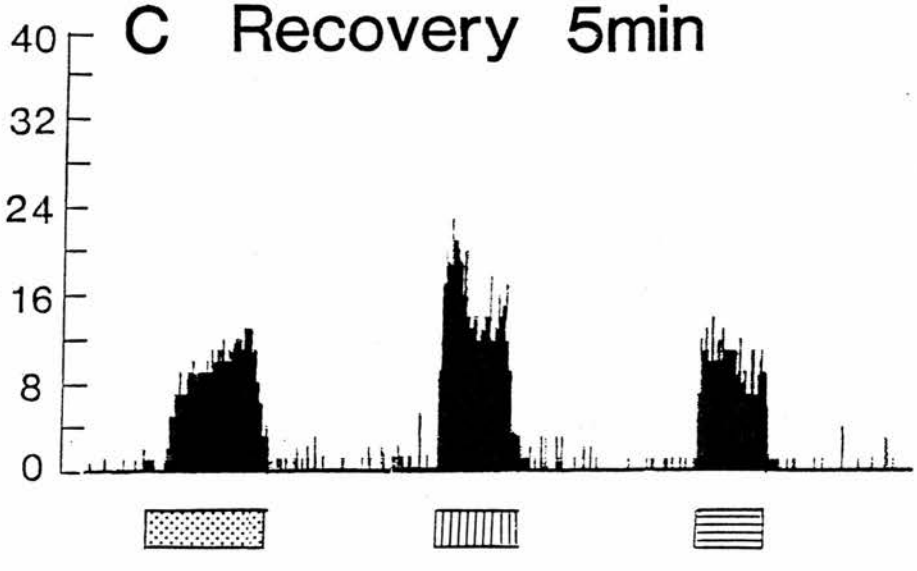
Figure 7.

The effect of DOI, a 5-HT_2 receptor agonist

Ongoing activity record of: A. Control excitatory responses to DLH, pinch and brush. B. High currents of DOI had little effect on nociceptive or non-nociceptive responses, DLH-evoked and spontaneous activities however appeared to be inhibited. C. Recovered responses 5 minutes after DOI current had been switched off.


A Controls
40
32
24
16
8
0
DLH
Pinch
Brush


B 9min 90 nA DOI
40
32
24
16
8
0


C Recovery 5min
40
32
24
16
8
0

20s

Figure 8.

Cyanopindolol, a 5-HT$_{1}$ receptor antagonist, reverses the effect of 8-OH-DPAT

Ongoing activity record of : A. Control responses to Glu, pinch and brush. B. Non-selective inhibitory effect of 8-OH-DPAT on all evoked responses tested. C. Cyanopindolol ejected concurrently with 8-OH-DPAT antagonised the non-selective inhibitory effect of the 5-HT$_{1A}$ receptor agonist.

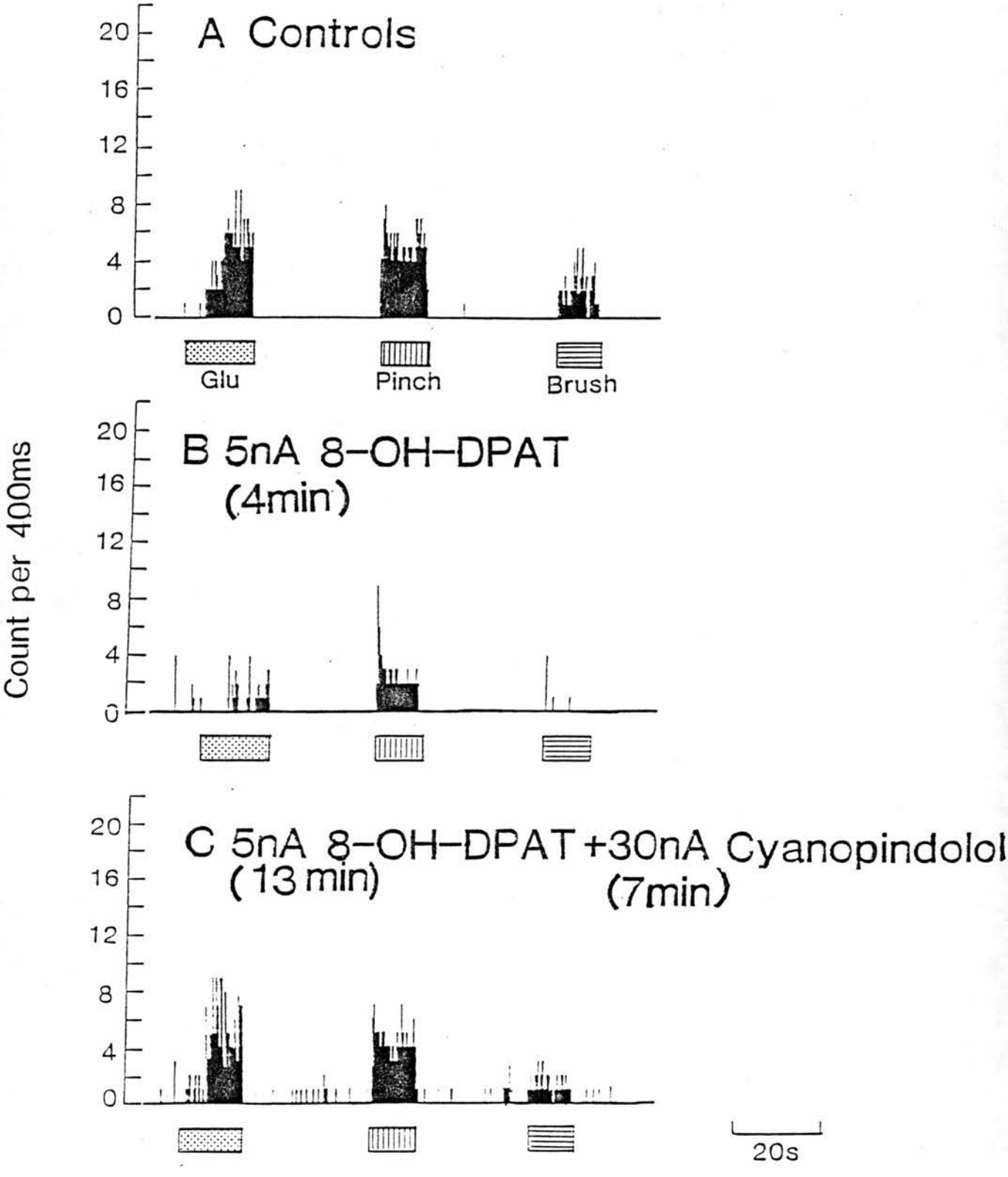
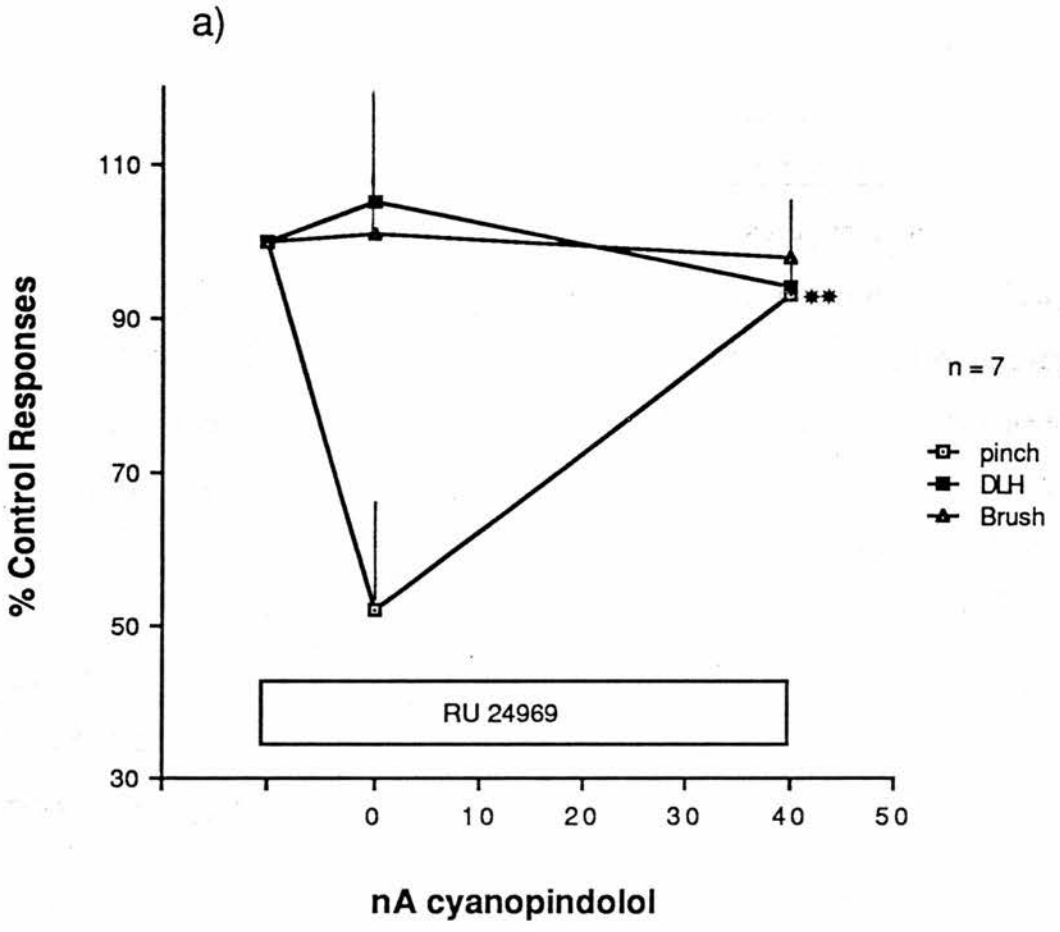

A Controls
20
16
12
8
4
0
Glu
Pinch
Brush
B 5nA 8-OH-DPAT
(4min)
C 5nA 8-OH-DPAT +30nA Cyanopindolol
(13 min)
(7min)
20s
Count per 400ms

Figure 9.

The differential abilities of two 5-HT receptor antagonists to reverse the selective antinociceptive effect of RU 24969

a) The graph depicts percentage control responses (± S.E.M.) plotted against Cyanopindolol ionophoretic current. At 40 (± 8) nA Cyanopindolol on for 10 ± 4 mins, successfully reversed the selective antinociceptive effect of RU 24969, a 5-HT_{1B} receptor agonist. The antagonistic action of Cyanopindolol was found to be statistically significant (**= $0.001 < p < 0.01$, t-test on raw data).

b) Ketanserin, a 5-HT_2 receptor antagonist, at currents reaching 28 (± 2) nA on for 8 ± 2 mins, failed to reverse or even inhibit the selective antinociceptive effect of RU 24969. Responses are shown ± S.E.M.


a)
110
90
70
50
30
% Control Responses
**
n = 7
pinch
DLH
Brush
RU 24969
0
10
20
30
40
50
nA cyanopindolol

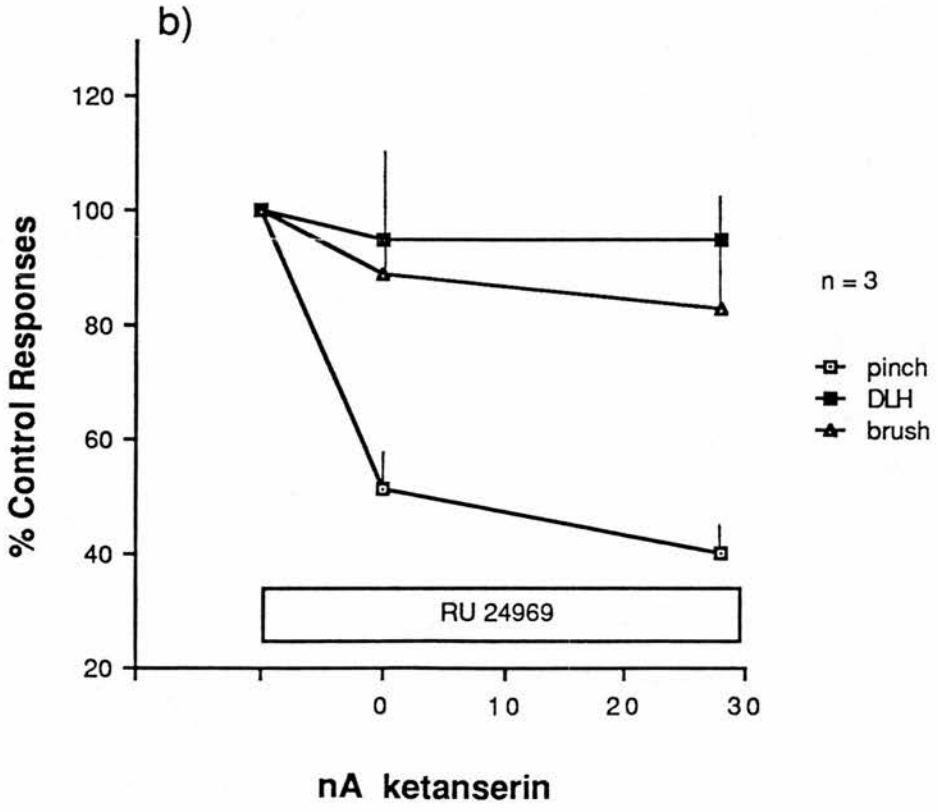

b)
120
100
80
60
40
20
% Control Responses
n = 3
pinch
DLH
brush
RU 24969
0
10
20
30
nA ketanserin

Figure 10.

The differential effect of two agonists selective for 5-HT_1 receptor subtypes on the same multireceptive lamina I cell

Ongoing activity record of : A. Control responses to DLH, pinch and brush. B. the non-selective inhibitory effect of 8-OH-DPAT exerted on all evoked responses. C. Recovered responses 10 minutes after 8-OH-DPAT had been switched off. D. The selective antinociceptive effect of RU 24969; the pinch-evoked responses was markedly inhibited, while DLH- and brush-evoked activities were hardly affected. E. Recovered pinch response 5 minutes after RU 24969 had been switched off.

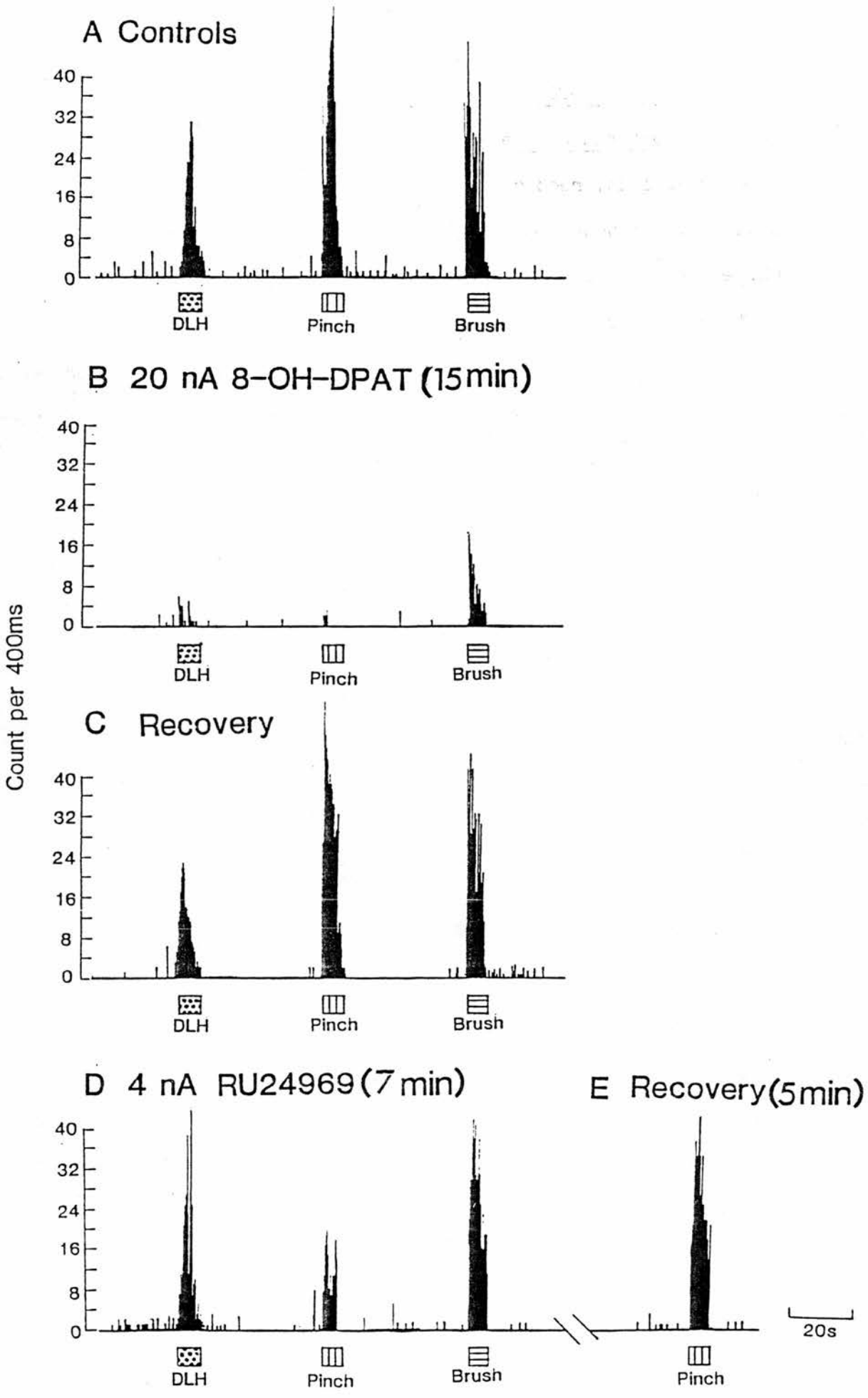

A Controls
B 20 nA 8-OH-DPAT (15 min)
C Recovery
D 4 nA RU24969 (7 min)
E Recovery (5 min)
Count per 400ms
40
32
24
16
8
0
DLH
Pinch
Brush
20s

Figure 11.

The differential abilities of two 5-HT antagonists to reverse the selective inhibitory effect of RU 24969

Ongoing activity record of : A. Control responses to DLH, pinch and brush. B. Responses after acutely applied ketanserin. Ketanserin failed to block the selective inhibitory effect of RU 24969 on the pinch response. C. Acutely applied cyanopindolol successfully reversed the effect of RU 24969.

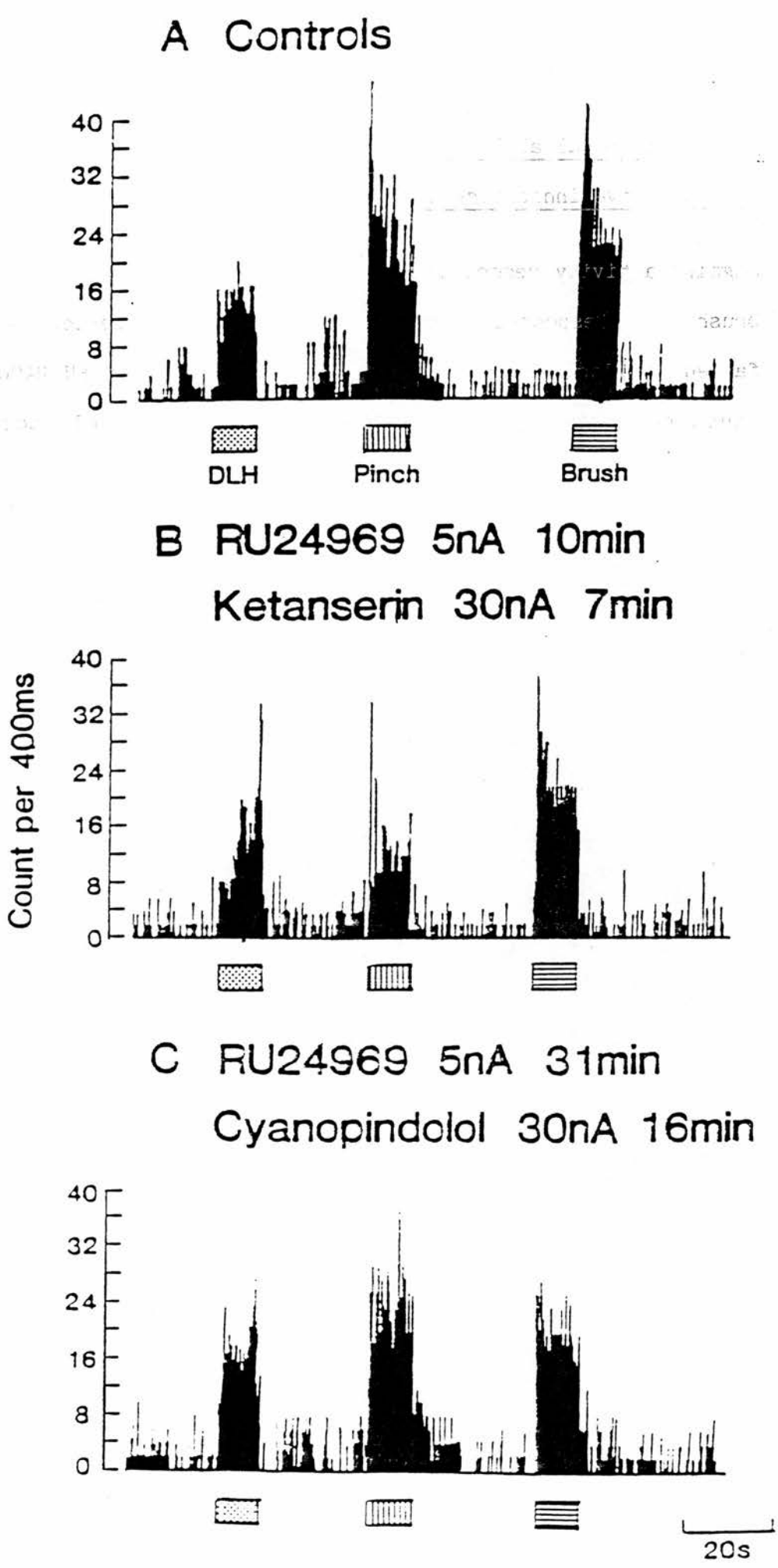

A Controls
40
32
24
16
8
0
DLH
Pinch
Brush
B RU24969 5nA 10min
Ketanserin 30nA 7min
Count per 400ms
40
32
24
16
8
0
C RU24969 5nA 31min
Cyanopindolol 30nA 16min
40
32
24
16
8
0
20s

Figure 12.

Brainstem stimulation sites

A schematic drawing of a cross section of the medulla at different rostro-caudal levels. Stimulating the brainstem at twelve sites in NRM and NRPGL resulted in selectively antinociceptive effect (●). Stimulating the brainstem in six different sites in NRM, NRPGL and NRPG resulted in non-selective inhibitory effect (□). Stimulating the brainstem at high currents at fourteen sites ventral and adjacent to NRM failed to have any effect on neural evoked responses(○).

Abbreviations.

A5 = A5 noradrenaline cells

g7 = genu facial nerve

LsO = lateral superior olive

ML = medial leminiscus

NRGC = nucleus reticularis gigantocellularis

NRM = nucleus raphe magnus

NRPG = nucleus reticularis paragigantocellularis

NRPGL = nucleus reticularis paragigantocellularis lateralis

PnC = nucleus reticularis pontis centralis

PnV = nucleus reticularis pontis ventralis

PT = pyramidal tract

S5 = sensory root of trigeminal nerve

Sp5 = spinal trigeminal tract

SpO = spinal paraolivary nucleus

Tz = trapezoid body

VII = facial nucleus

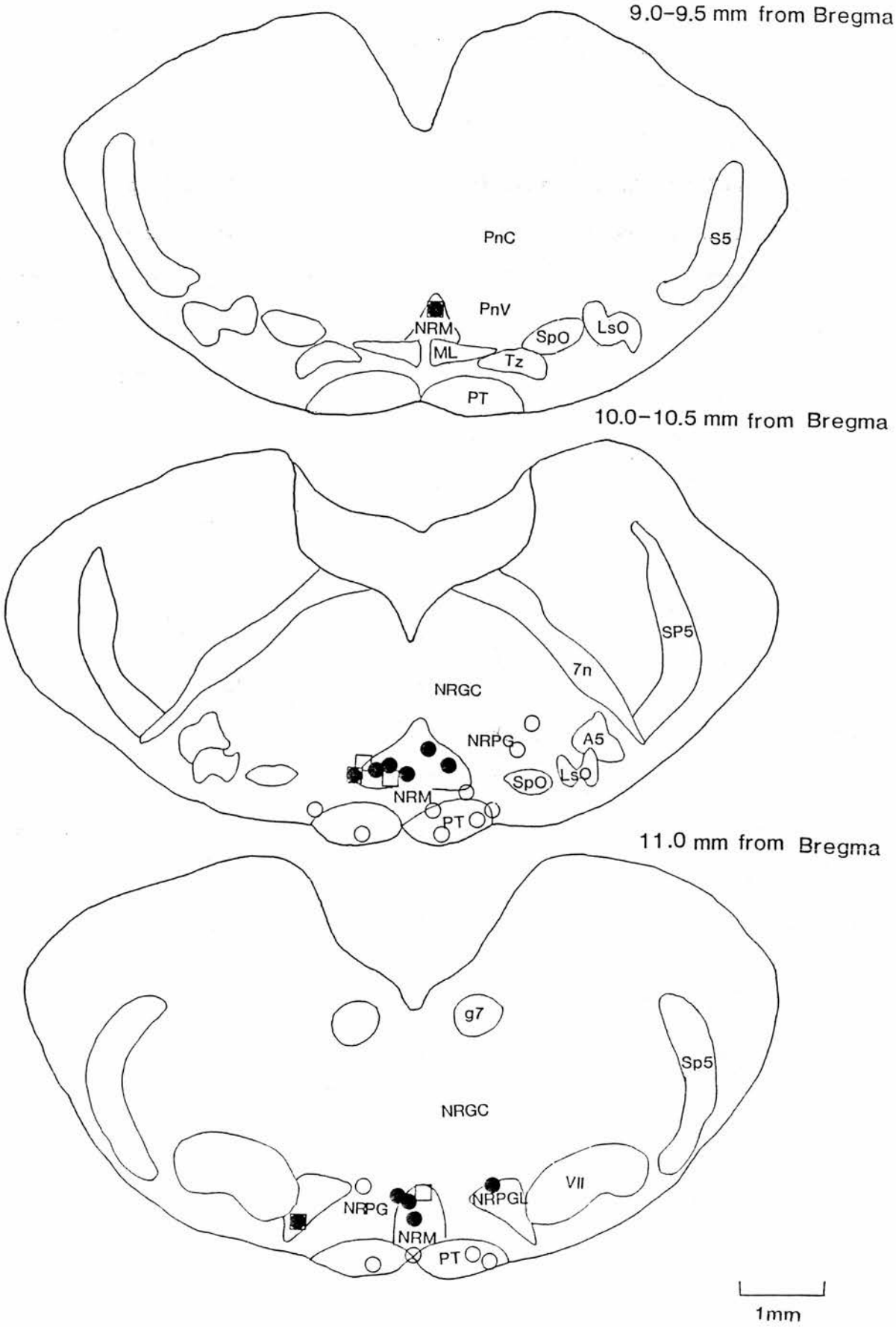

9.0–9.5 mm from Bregma
PnC
S5
PnV
NRM
ML
SpO
LsO
Tz
PT
10.0–10.5 mm from Bregma
SP5
7n
NRGC
NRPG
A5
SpO
LsO
NRM
PT
11.0 mm from Bregma
g7
Sp5
NRGC
VII
NRPG
NRPGL
NRM
PT
1mm

Figure 13.

An example of the ability of cyanopindolol to reverse the effect of NRM stimulation

Ongoing activity record of : A. Control responses to DLH, pinch and brush. B. The selective antinociceptive effect of stimulating NRM (at arrow, 30 μA, at 50 Hz). The pinch-evoked response was markedly inhibited, while DLH- and brush-evoked responses were hardly affected. C. Acutely applied cyanopindolol reversed the effect of NRM stimulation. Inset shows sites in the brainstem where stimulation evoked selective (●) or non-selective (□) inhibitory effects, which were blocked by cyanopindolol, ionophoretically applied near to the dorsal horn neurones which were being recorded.

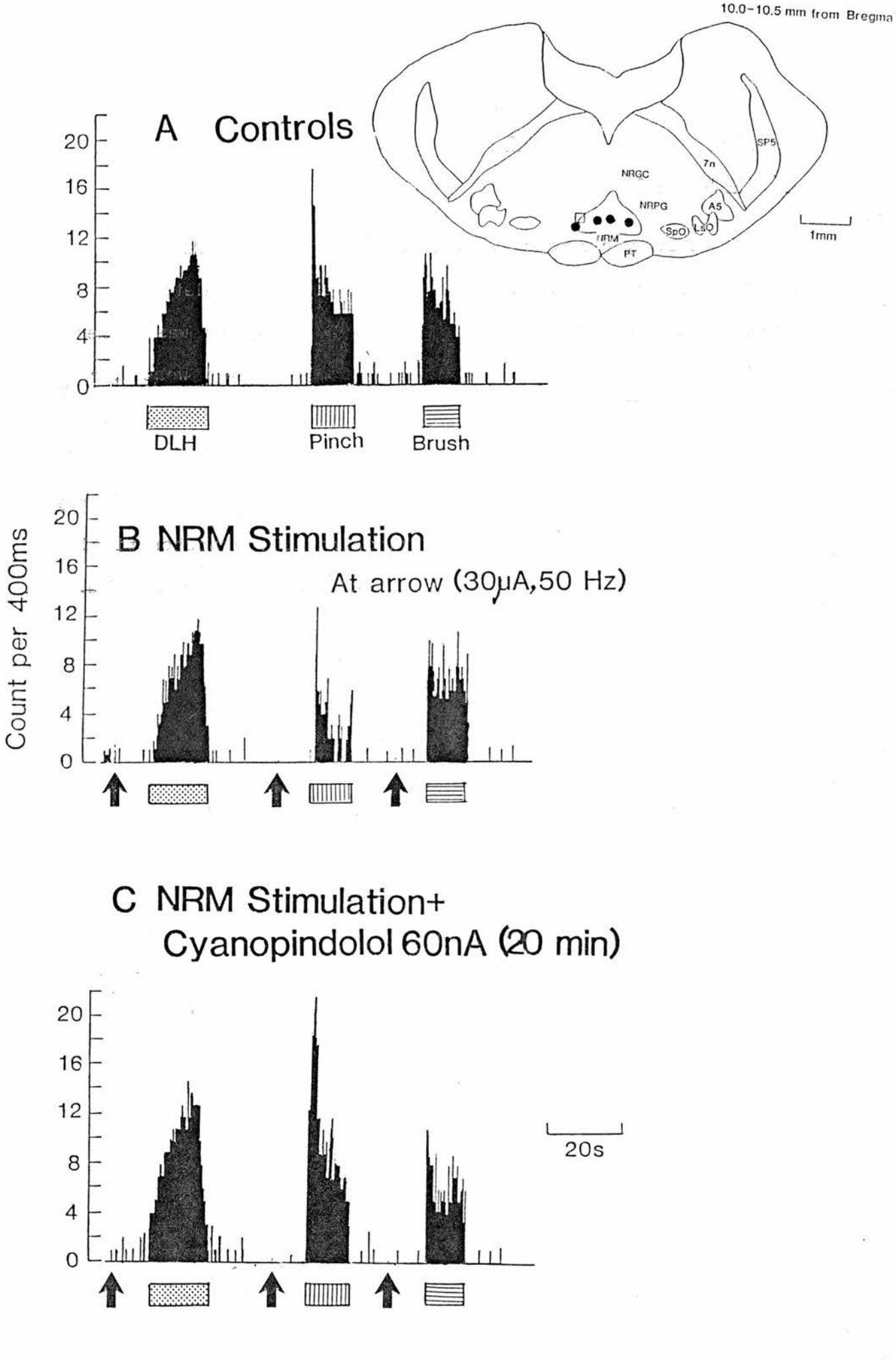

10.0–10.5 mm from Bregma
A Controls
20
16
12
8
4
0
NRGC
7n
SP5
NRPG
A5
SpO
LsO
NRM
PT
1mm
DLH
Pinch
Brush
B NRM Stimulation
At arrow (30μA,50 Hz)
Count per 400ms
C NRM Stimulation+
Cyanopindolol 60nA (20 min)
20s

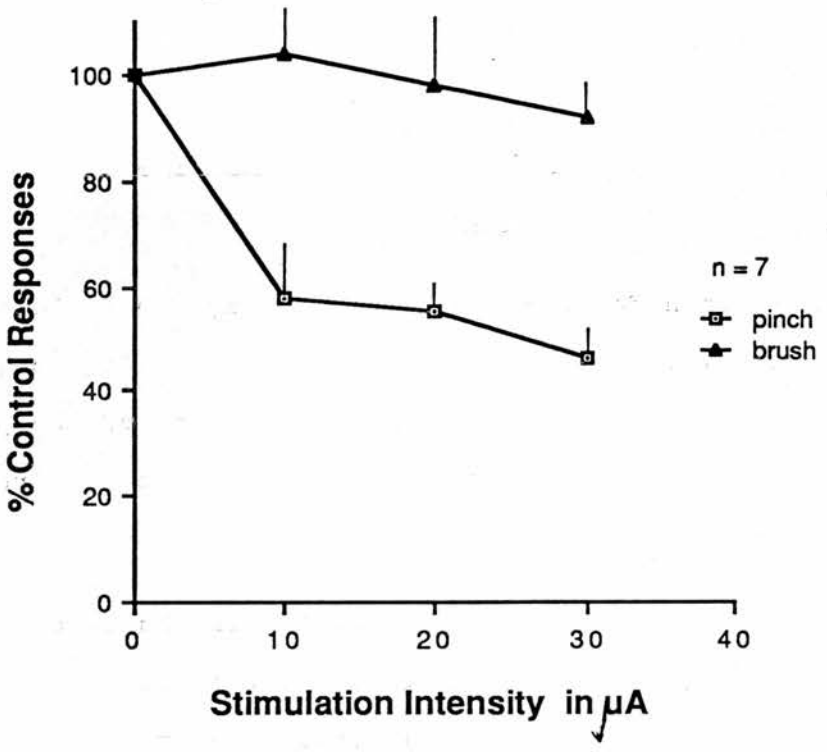
Figure 14.

The relation between stimulation intensity or frequency and the response to brainstem stimulation

a) Cumulative graph for seven cells depicting the relation between stimulus intensity and response. At low current levels shown here, there was little change in percentage inhibition of the pinch-evoked response as stimulation intensity increased. Frequency of stimulation was maintained here at 100 Hz.

b) The relationship between frequency and intensity of stimulation with amplitude of response. Three different frequencies of stimulation were employed ; 33 Hz (n=2), 50 Hz (n=4) and 100 Hz (n=9). The intensity of stimulation required to selectively inhibit pinch-evoked responses to 60% control values are compared here (open squares). Brusn-evoked responses (closed triangles) were not markedly affected. From the graph, it is clear that with lower stimulation frequencies, higher current intensities were required to inhibit the nociceptive response.

a)


% Control Responses
100
80
60
40
20
0
0
10
20
30
40
Stimulation Intensity in µA
n = 7
pinch
brush

b)

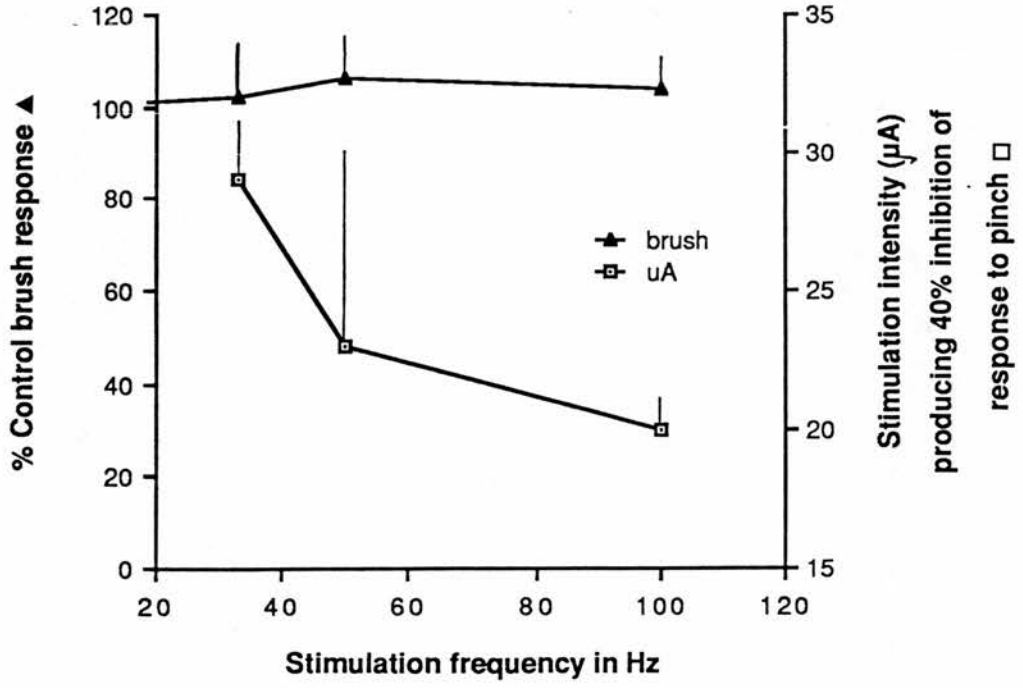
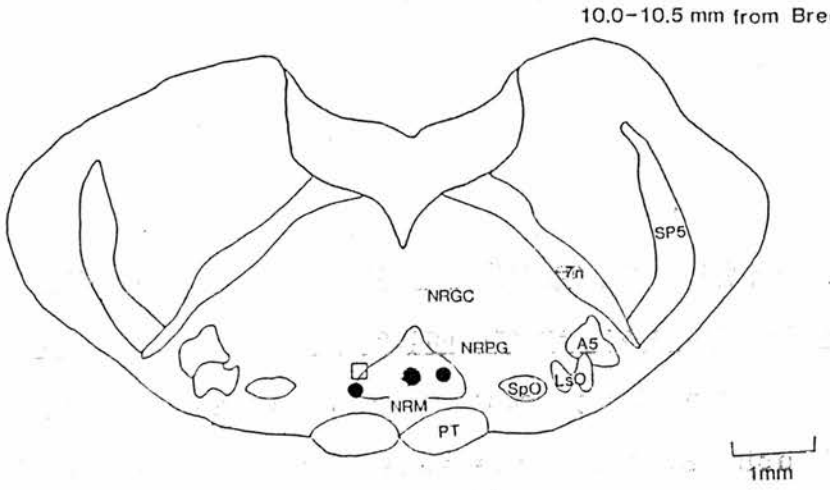

% Control brush response ▲
120
100
80
60
40
20
0
20
40
60
80
100
120
Stimulation frequency in Hz
35
30
25
20
15
Stimulation intensity (µA)
producing 40% inhibition of
response to pinch □
brush
uA

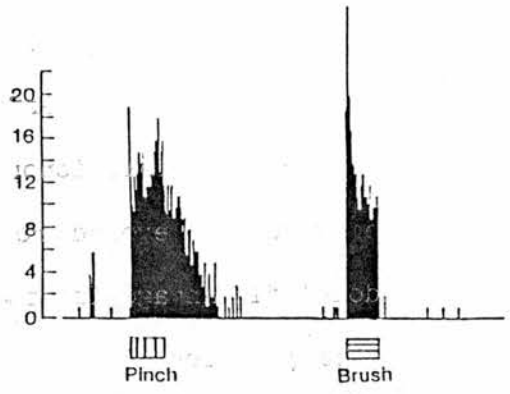
Figure 15.

An example of the differential abilities of two 5-HT receptor antagonists to reverse the effects of NRM stimulation

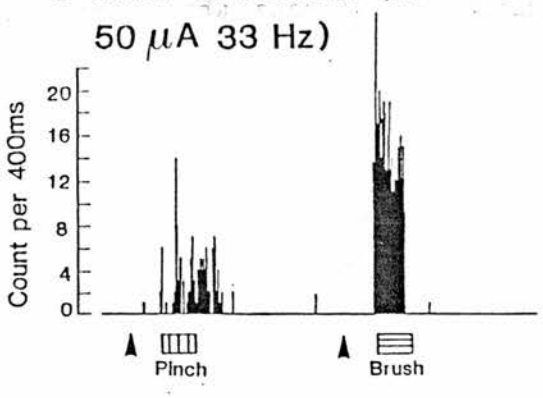
Ongoing activity record of : A. Control responses to DLH, pinch and brush. B. Shows the selective antinociceptive effect of NRM stimulation (recovery not shown). C. Shows the effect of ionophoretically applied ketanserin on its own. As can be seen, ketanserin failed to alter evoked responses markedly. D. Ketanserin also failed to block the selective antinociceptive effect of NRM stimulation (recovery not shown). E. Cyanopindolol, ionophoretically applied on its own, did not markedly affect evoked responses. F. Unlike ketanserin however, Cyanopindolol did antagonise the effect of NRM stimulation. The inset shows the position of four stimulation sites in the brainstem which upon activation produced selective (●) and non selective (□) inhibitory effects which were blocked by cyanopindolol, but not ketanserin.



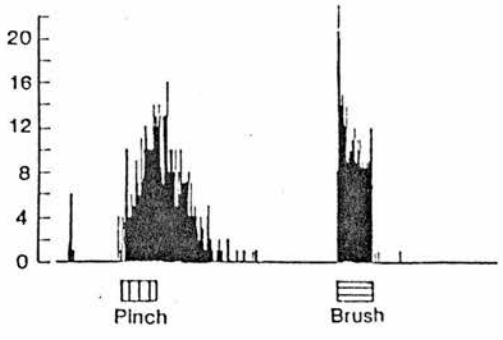
A Controls



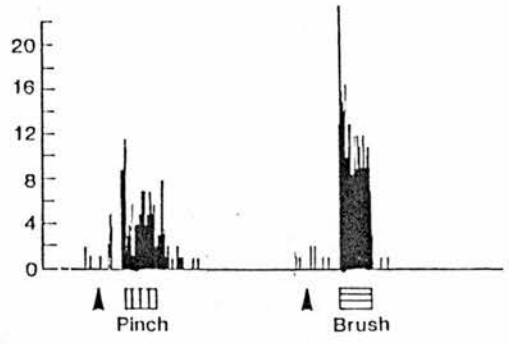
B NRM Stimulation (at arrow 50 μA 33 Hz)



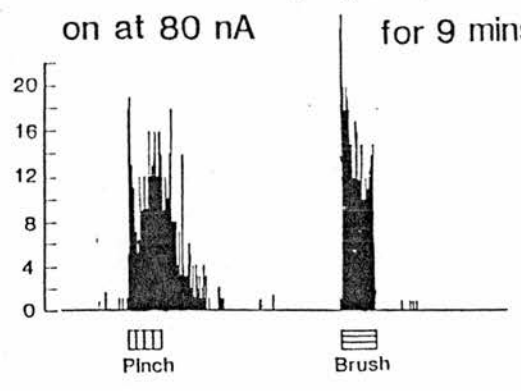
C Post Recovery Ketanserin on at 20 nA for 24 mins



D 20 nA Ketanserin (26 mins) + NRM Stimulation



E Post Recovery Cyanopindolol on at 80 nA for 9 mins



F 80 nA Cyanopindolol (11 mins) + NRM Stimulation

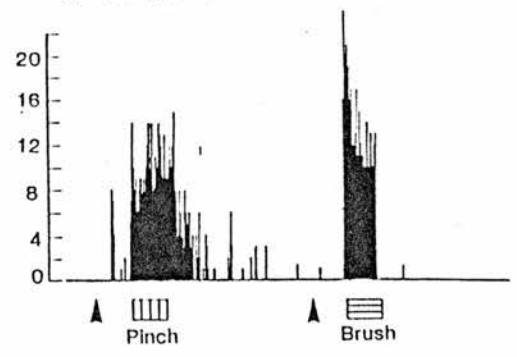


Figure 16.

<u>An example of the ability of Idazoxan (RX) an α_2 receptor antagonist to block the effect of brainstem stimulation</u>

In this graph, % control responses were plotted against time post-application of 30 nA RX. The graph snows that brainstem stimulation (at arrow) at a site on the border between NRM and NRPG (see inset) resulted in selective inhibition of the pinch response (point 0 mins on the graph). This effect was successfully antagonised by RX, applied at quite low currents. RX, however, failed to antagonise the selective (●) and non-selective (□) inhibitory effects of more medial NRM stimulation in two further sites (see inset).

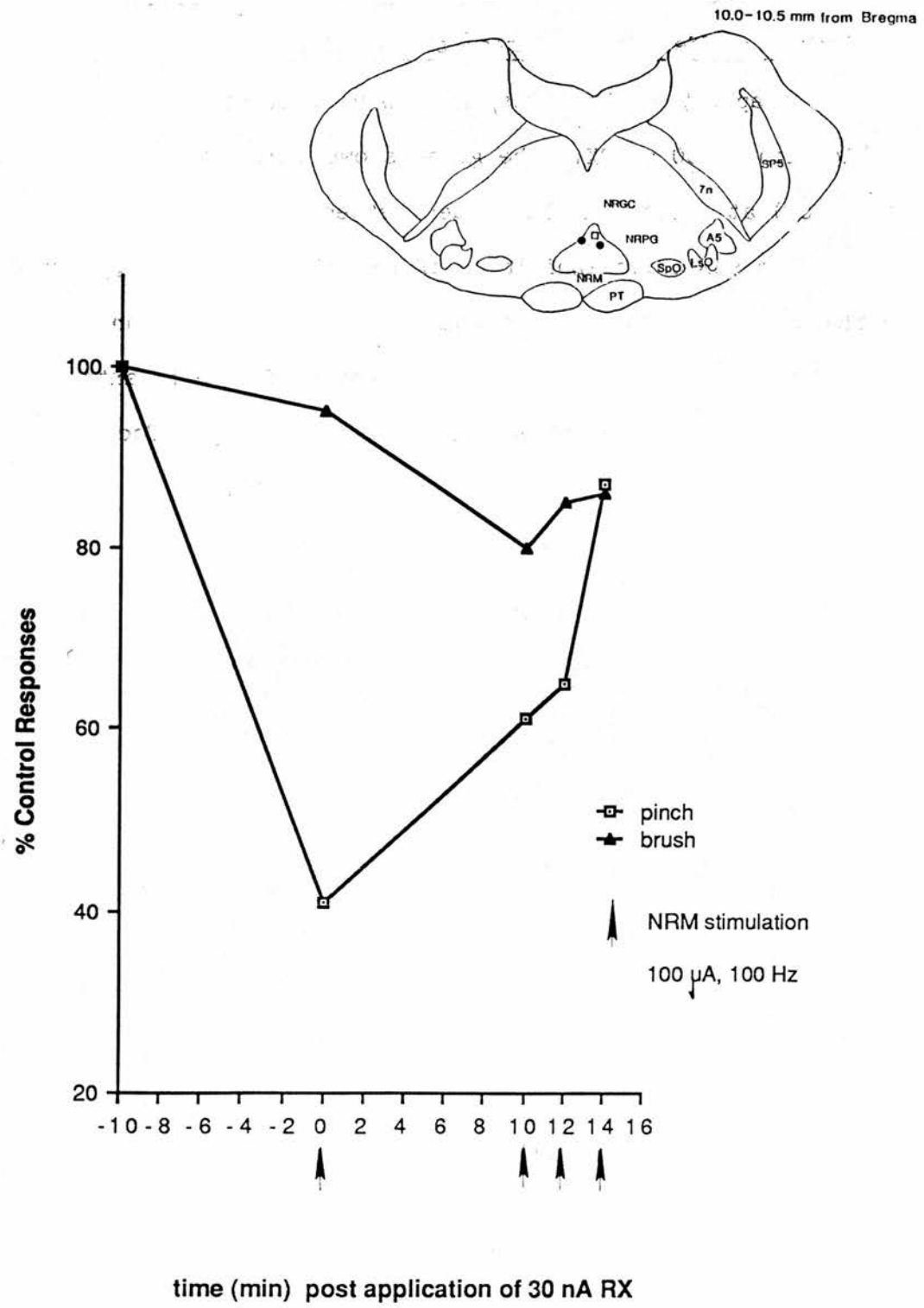

10.0-10.5 mm from Bregma
NRGC
NRPG
NRM
PT
7n
SP5
A5
SpO
LSO
% Control Responses
100
80
60
40
20
-10 -8 -6 -4 -2 0 2 4 6 8 10 12 14 16
pinch
brush
NRM stimulation
100 µA, 100 Hz
time (min) post application of 30 nA RX

Figure 17.

The effect of intrathecally applied RU 24969

a) The effect of RU 24969 on tail flick latency (TFL) over time. Values are shown ± S.E.M. and control values are shown at point 0 of the graph. The effect of 3.7μg RU 24969 was statistically significant and preliminary experiments at 7.4μg (not shown) suggested a similar trend. Moreover, 2.9μg of cyanopindolol, intrathecally applied 10 mins before the application of 3.7μg of RU 24969, successfully reversed the apparently antinociceptive effect of RU 24969 (open squares). Cyanopindolol intrathecally applied on its own (closed triangles) had no apparent effect on TFL. (* = significant, $0.02 < p < 0.05$, ** = highly significant, $0.001 < p < 0.01$, paired t- test).

b) The effect of RU 24969 on hotplate latency (HPL) over time. Values are shown ± S.E.M. and control responses are shown at point 0 on the graph. RU 24969 appeared to cause a transient elevation of HPL, with maximum effects at around 15 mins post-injection. 2.9μg cyanopindolol appeared to antagonise the elevation of HPL by RU 24969 (open squares). Cyanopindolol on its own, had no significant effect on HPL (closed triangles).

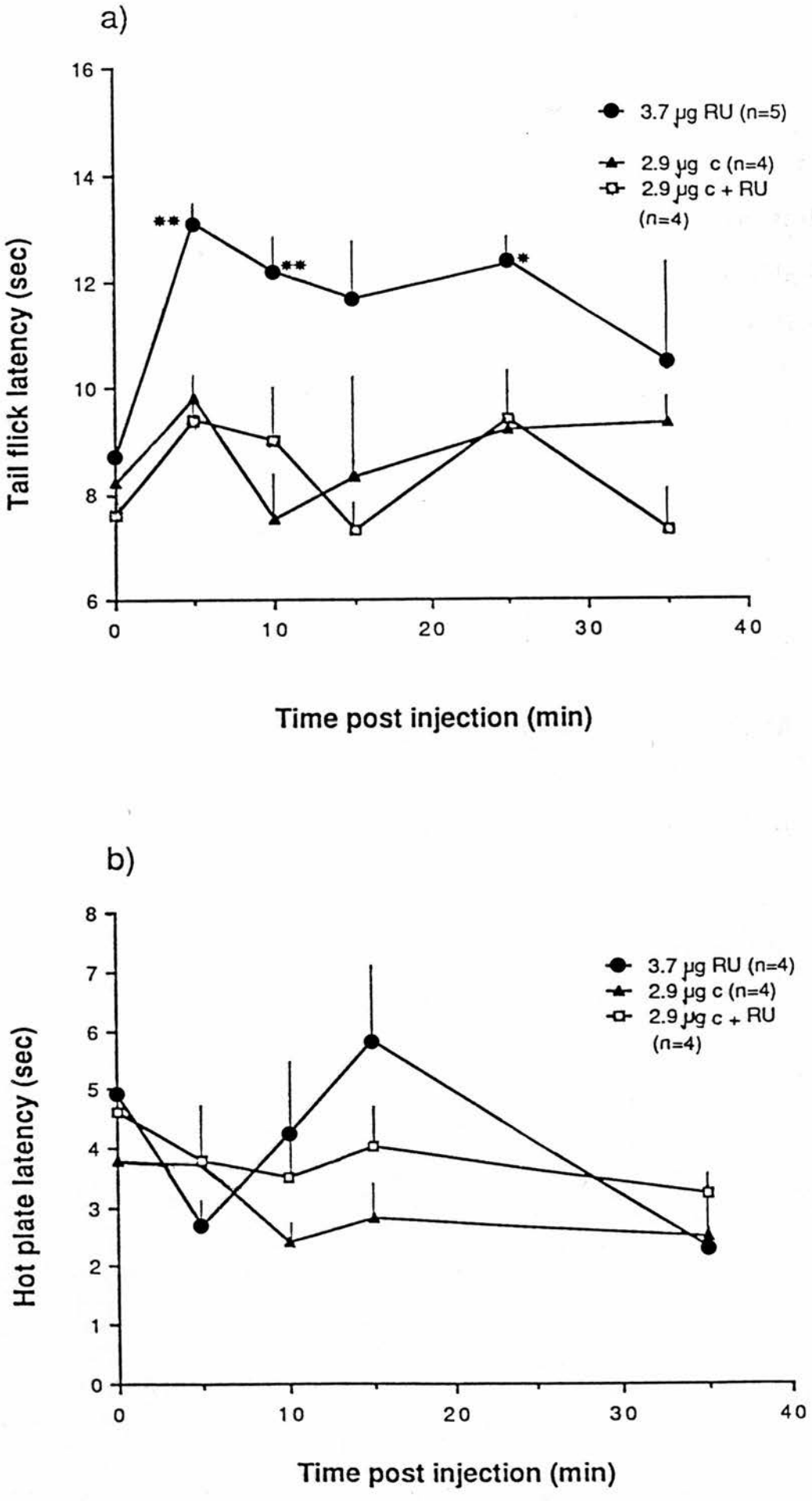

a)
16
14
12
10
8
6
Tail flick latency (sec)
3.7 µg RU (n=5)
2.9 µg c (n=4)
2.9 µg c + RU (n=4)
**
**
*
0
10
20
30
40
Time post injection (min)
b)
8
7
6
5
4
3
2
1
0
Hot plate latency (sec)
3.7 µg RU (n=4)
2.9 µg c (n=4)
2.9 µg c + RU (n=4)
0
10
20
30
40
Time post injection (min)

Figure 18.

Dose-response curve for intrathecally applied RU 24969

a) The graph portrays the effect of different doses of RU 24969 (14 fg-7.4 μg) on TFL assessed 10 mins post-injection. The antinociceptive effect of RU 24969 appears to be dose-dependent, though further testing is required to ascertain this.

b) The graph depicts HPL plotted against log dose of RU 24969. HPL was assessed 15 mins post injection of different doses of RU 24969 and compared with control values. Only at the highest two doses employed did RU 24969 appear to be antinociceptive, but the effect did seem to be dose-dependent. Further testing is required to make definitive statements.

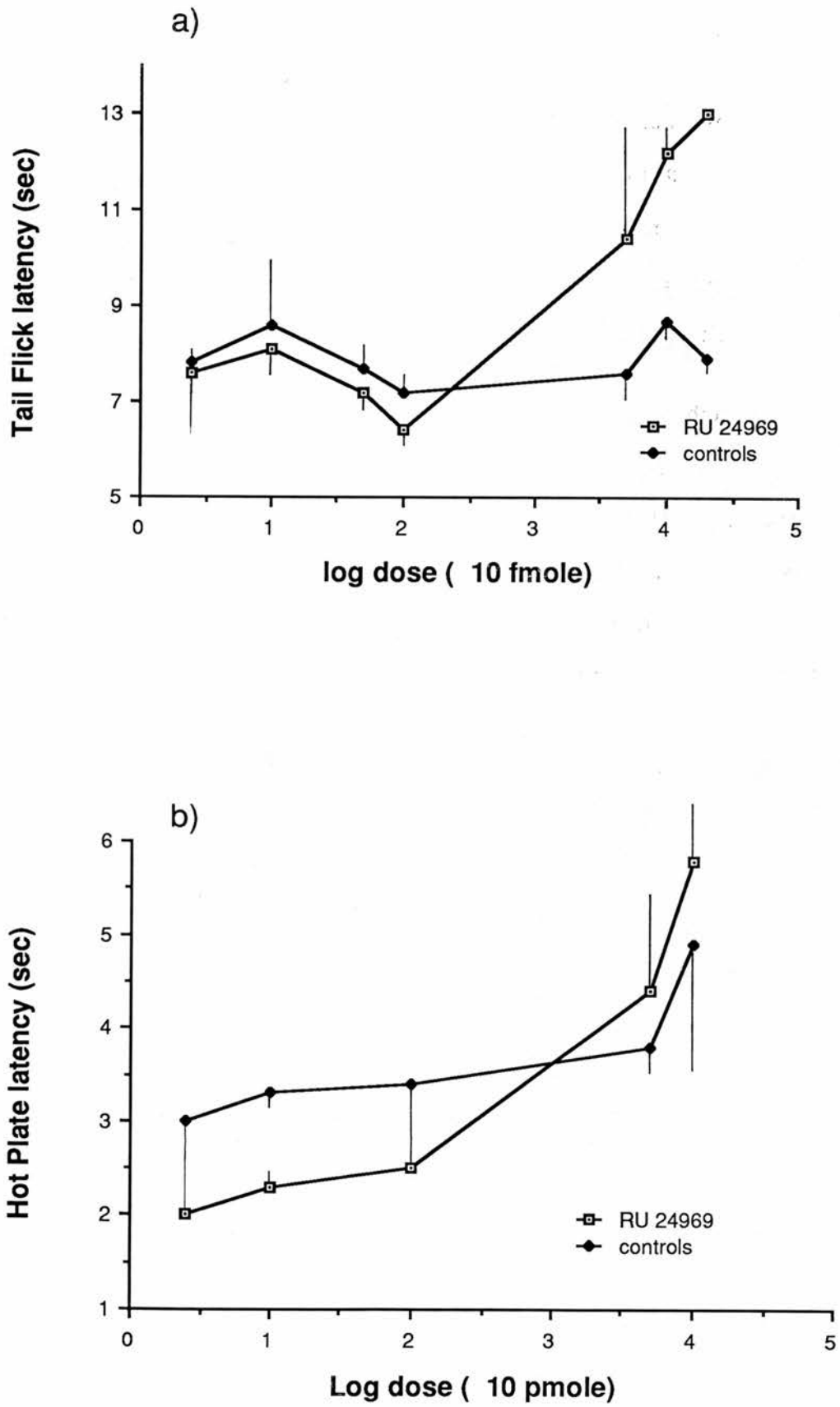

a)
Tail Flick latency (sec)
13
11
9
7
5
0
1
2
3
4
5
log dose (10 fmole)
RU 24969
controls
b)
Hot Plate latency (sec)
6
5
4
3
2
1
0
1
2
3
4
5
Log dose (10 pmole)
RU 24969
controls

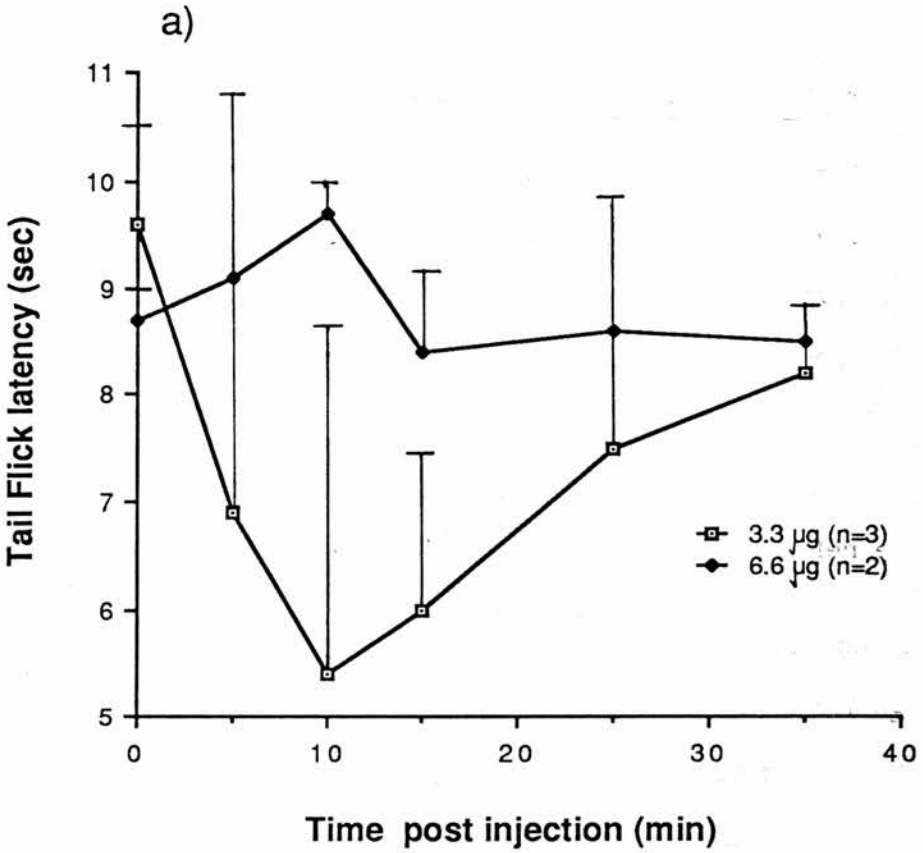
Figure 19.

The effect of intrathecally applied 8-OH-DPAT

Values are shown ± S.E.M. and control responses are depicted at point 0 on the graph.

a) The effect of 8-OH-DPAT on TFL. The lower dose (3.3. µg) appeared to decrease TFL, though this effect was found not to be statistically significant or dose-dependent, since the higher dose (6.6 µg), failed to mimic this apparently facilitatory effect on the nociceptive reflex.

b) The effect of 8-OH-DPAT on HPL. 8-OH-DPAT appeared to increase HPL, though this effect was not found to be significant. It is not clear whether this effect is dose-dependent or not as the sample size was too low for definitive statements to be made here.


a)
Tail Flick latency (sec)
11
10
9
8
7
6
5
0
10
20
30
40
Time post injection (min)
3.3 µg (n=3)
6.6 µg (n=2)

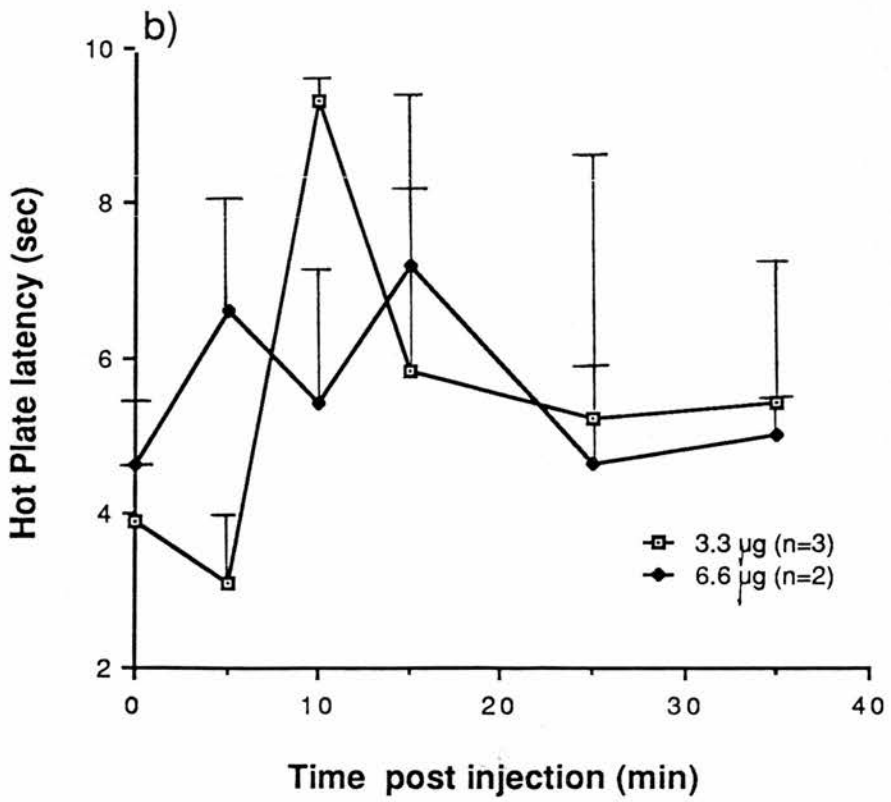

b)
Hot Plate latency (sec)
10
8
6
4
2
0
10
20
30
40
Time post injection (min)
3.3 µg (n=3)
6.6 µg (n=2)

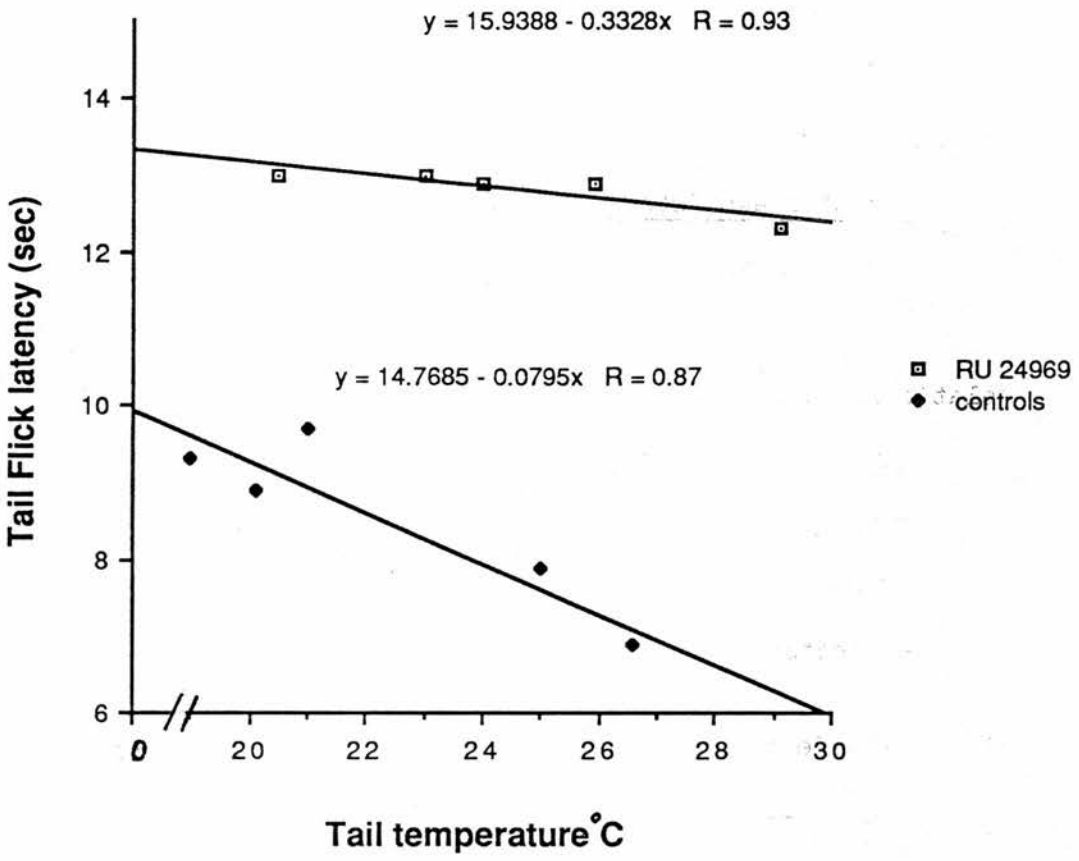
Figure 20.

The relationship between tail temperature and TFL and how different 5-HT_1 receptor agonists influence this relationship

a) The effect of intrathecally applied RU 24969. The relationship between tail temperature and TFL can be expressed as a linear relationship, the colder the skin, the longer the TFL. As can be seen from the graph, RU 24969 (3.7 µg) apparently exerted its effect of elevating TFL regardless of ambient tail temperature. The effect of RU 24969 on TFL therefore appears to be independent of changing skin temperature.

b) The effect of intrathecally applied 8-OH-DPAT. This compound apparently shifts the relationship between tail temperature and TFL observed with control responses slightly to the left. Thus the apparent effect of moderate doses of 8-OH-DPAT on the TFL may be dependent on changes in tail temperature.

a)


y = 15.9388 - 0.3328x R = 0.93
y = 14.7685 - 0.0795x R = 0.87
RU 24969
controls
Tail Flick latency (sec)
Tail temperature °C
14
12
10
8
6
0
20
22
24
26
28
30

b)

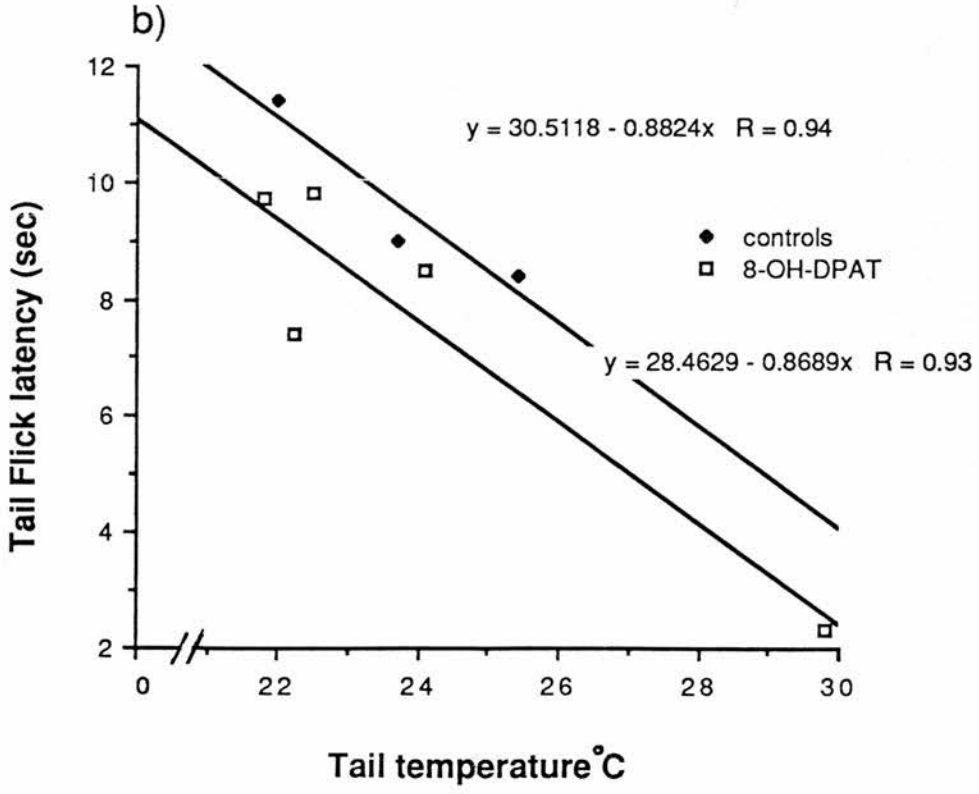

y = 30.5118 - 0.8824x R = 0.94
y = 28.4629 - 0.8689x R = 0.93
controls
8-OH-DPAT
Tail Flick latency (sec)
Tail temperature °C
12
10
8
6
4
2
0
22
24
26
28
30

Table I.

A summary of the effects of 5-HT and two 5-HT_1 receptor agonists on dorsal horn neurones.

a) Effects obsereved in laminae III-VII. Most cells tested were multireceptive, though two appeared to be nocispecific. The selective antinociceptive effect of 5-HT was mimicked by the 5-HT_{1B} receptor agonist, RU 24969. The 5-HT_{1A} receptor agonist, on the other hand, appeared to mimic the non-selective effects of 5-HT.

b) Effects observed on neurones in lamina I. Most cells were classified as multireceptive, though a few, including two SMT neurones appeared to be nocispecific. The effects of 5-HT and its two receptor agonists were similar to those observed in deeper laminae. While RU 24969 mimicked the selective antinociceptive effect of 5-HT, 8-OH-DPAT mimicked the non-selective inhibitory effect of 5-HT. In no instance were excitatory effects observed in lamina I in this study.

a)

Compound	Selective inhibition of the nociceptive response	Non-selective inhibition of all responses tested	Non-selective facilitation of all responses tested	No effect	Total number of cells
5-HT	11	2	1	2	16
8-OH-DPAT	--	17 (inc. 2 nocispecific cells)	3	4	24
RU 24969	27	7	--	8	42

b)

compound	Selective inhibition of the nociceptive response	Non-selective inhibition of of all responses tested	No effect	Total number of cells
5-HT	5 (1 SMT cell)	2 (both nocispecific SMT cells)	2 (1SMT)	9
8-OH-DPAT	--	6 (inc. 1 nocispecific cell)	--	6
RU 24969	5	--	--	5

Table II.

Quantification of the selective antinociceptive effect of 5-HT and RU 24969

The table shows the mean ionophoretic current of 5-HT and RU 24969 required to produce 40 % inhibition of the nociceptive response a) in laminae III-VII and b) in lamina I. Mean brush- and DLH- (where tested) evoked responses at these current levels are also expressed. As can be seen, the effect of both 5-HT and RU 24969 was selectively exerted against the nociceptive response since responses to DLH and brush were hardly affected.

a)

Compound	nA for 40% inhibition of pinch response ± S.E.M.	mins of iono-phoresis ± S.E.M.	as a percentage of control values: mean DLH / Glu response ± S.E.M.	as a percentage of control values: mean brush response ± S.E.M	total no. of cells
5-HT	12 ± 4	5 ±2	95 ± 11	95 ± 4	11
RU 24969	11 ± 2	6 ± 1	97 ± 5	97 ± 2	27

b)

Compound	nA for 40% inhibition of pinch response ± S.E.M.	mins of iono-phoresis ± S.E.M.	as a percentage of control values: mean DLH / Glu response ± S.E.M.	as a percentage of control values: mean brush response ± S.E.M	total no. of cells
5-HT	5 ± 3	7 ± 2	--	94 ± 3	5
RU 24969	10 ± 4	8 ± 2	96 ± 5	93 ± 5	5

Table III.

Quantification of the non-selective inhibitory effect of 5-HT and 8-OH-DPAT

The table shows the mean ionophoretic current of 5-HT and 8-OH-DPAT required to produce 40 % inhibition of the nociceptive response a) in laminae III-VII and b) in lamina I. For comparison, mean brush- and DLH- evoked responses are also expressed. Both 5-HT and 8-OH-DPAT caused the inhibition of all evoked responses tested to a similar degree. In the case of 8-OH-DPAT, this inhibition was found to be significant for all evoked responses (* = $p < 0.05$, paired t-test) both in lamina I and deeper laminae. Unfortunately, the number of cells showing the non-selective inhibitory effect with 5-HT was quite small (n=2, in lmaina I and deeper dorsal horn), making it impossible to conduct meaningful analysis on the data. The effect of 5-HT here was however very similar in extent to that produced by 8-OH-DPAT.

a)

Compound	nA for 40% inhibition of pinch response ± S.E.M.	mins of iono-phoresis ± S.E.M.	as a percentage of control values: mean DLH / Glu response ± S.E.M.	as a percentage of control values: mean brush response ± S.E.M	total no. of cells
5-HT	19 ± 9	16 ± 2	58 ± 1	52 ± 1	2
8-OH-DPAT	21 ± 5	8 ± 1	60 ± 6*	65 ± 5*	17

b)

Compound	nA for 40% inhibition of pinch response ± S.E.M.	mins of iono-phoresis ± S.E.M.	as a percentage of control values: mean DLH / Glu response ± S.E.M.	as a percentage of control values: mean brush response ± S.E.M	total no. of cells
5-HT	5 ± 0	5 ± 2	50 ± 30	--	2
8-OH-DPAT	10 ± 12	8 ± 2	61 ± 8*	43 ± 8*	6

Table IV.

A summary of the effects of 5-HT and two 5-HT_1 receptor agonists on non-nociceptive neurones in the dorsal horn

Serotonin and its two receptor agonists had variable effects on Brush- or DLH- evoked responses of non-nociceptive cells. Inhibitory effects on the brush-evoked response were rare with either 5-HT or RU 24969, though DLH-evoked activity was commonly affected. Facilitatory effects on both brush- and DLH- evoked activity were observed only with 8-OH-DPAT.

Number of cells showing effect / total number of cells

Compound	DLH response facilitated	DLH response inhibited	Brush response facilitated	Brush response inhibited
5-HT	2 / 7	4 / 7	0 / 7	1 / 7
8-OH-DPAT	4 / 10	6 / 10	2 / 10	5 / 10
RU 24969	0 / 6	3 / 6	0 / 6	2 / 6

Table V.

The effect of ionophoretically applied antagonists

a) A summary of the results with antagonists. Whereas cyanopindolol, a 5-HT_1 receptor antagonist readily reversed the effects of both 5-HT_1 receptor agonist employed here, ketanserin, a 5-HT_2 receptor antagonist and RX, an α_2 noradrenergic antagonist, usually failed to block the effects of either RU 24969 or 8-OH-DPAT.

b) The effects of different antagonists, ionophoretically applied on their own, on neuronal responses. Mean maximum ionophoretic current and ejection time employed is expressed for each antagonist and mean % control responses are compared at these levels. As can be seen, neither cyanopindolol, nor ketanserin significantly affected any of the evoked responses. RX, on the other hand, had significantly elevated both DLH- and brush- evoked activity, indicating a possible involvement of NA in tonic inhibition. (* = significant, $p < 0.05$, t-test)

a)

Antagonist	Agonist	Selective inhibition of nociceptive responses	Non-selective inhibition of all responses	Non-selective facilitation of all responses
		Number of cases where block was observed / total number of cells tested		
cyanopindolol	5-HT	1 / 2	--	--
	8-OH-DPAT	--	4 / 5	1 / 1
	RU 24969	7 / 7	1 / 1	--
ketanserin	8-OH-DPAT	--	0 / 1	--
	RU 24969	1 / 5	0 / 2	--
RX	8-OH-DPAT	--	0 / 1	--
	RU 24969	0 / 2	0 / 1	--

b)

			Mean responses as percentages of control values ± S.E.M.			
Antagonist	Mean maximum current ± S.E.M.	Mean iontophoresis time (min) ± S.E.M.	Pinch response	DLH response	Brush response	Number of cells
cyanopindolol	50 ± 13	19 ± 3	100 ± 7	99 ± 22	106 ± 8	4
ketanserin	33 ± 7	12 ± 3	101 ± 6	90 ± 10	94 ± 6	8
RX	33 ± 4	18 ± 4	106 ± 16	140 ± 40*	132 ± 15*	3

Table VI.

The effects of brainstem stimulation and the ability of different antagonists to reverse these effects

a) A summary of the effects of brainstem stimulation. Two effects of stimulation in NRM or NRPGL were discerned. The table shows the mean stimulation current (frequency = 100 Hz) which produced 40% inhibition of the pinch-evoked response. At these current levels, mean DLH- and brush-evoked responses are expressed as % of control values ± S.E.M. The currents required to produce a clear and selective inhibition of the nociceptive response, hardley affected either DLH- or brush- evoked activity. In contrast, stimulation at six different sites in the brainstem, resulted in a non-selective inhibitory effect which significantly affected all responses tested (* = significant, $p < 0.05$, t-test).

b) The ability of different antagonists to reverse the effects of brainstem stimulation. Only cyanopindolol was consistently successful at reversing the effects of brainstem stimulation. Ketanserin and naloxone did not reverse the effects of brainstem stimulation on any of the cells tested. RX, on the other hand, failed to reverse the selective inhibitory effect of stimulating the NRM in two sites, but was successful in reversing the non-slective inhibitory effect of stimulating the lateral NRM (see fig. 16).

a)

Effect of brainstem stimulation	mean current ± S.E.M producing 40% inhibition of the pinch evoked response	total number of cells	Mean responses as percentages of control values ± S.E.M. DLH response	Brush response
Selective antinociception	54 ± 16	14	96 ± 12	101 ± 4
Non-selective	130 ± 50	6	60 ± 4*	67 ± 1*

b)

Antagonist	Effect of brainstem stimulation: Selective inhibition of the pinch-evoked response	Non-selective inhibition of all responses
Cyanopindolol	4 / 4	2 / 2
Ketanserin	0 / 3	0 / 1
RX	0 / 2	1 / 1
Naloxone	0 / 1	0 / 1

DISCUSSION

The results of the present investigation support the involvement of the descending serotonergic raphe-spinal system in antinociception and further provide the first demonstration of the role of different 5-HT receptors in modulating nociceptive transmission at the single neuronal level in the dorsal horn. Findings from this study are discussed in relation to other studies investigating the antinociceptive role of 5-HT.

Ionophoretic Experiments

I Results with Nociceptive Cells

A. Effects of Ionophoretically Applied 5-HT

The present results initially confirm the heterogeneity of effects of 5-HT ionophoretically applied near dorsal horn neurones which had been observed in previous studies (Belcher et al., 1978; Headley et al., 1978; Jordan et al., 1979; Randic and Yu, 1976). Three separate actions of 5-HT were observed; a selective antinociceptive action, a non-selective inhibitory action and a non-selective excitatory action. A minority of cells were unaffected by 5-HT. This heterogeneity may be related to the specific type of sensory input (see for example Jordan et al., 1979) as well as to cell type (i.e projecting or segmental, etc.). Results with identified cells of ascending tracts presented here (SMT cells) and elsewhere (STT cells) (Jordan et al., 1979; Willcockson et al., 1984) indicate that the effects of 5-HT on a supposedly functionally homogeneous population are also varied. The effects of 5-HT were normally of long latency, in the order of one minute or more,

recovery was also prolonged, which confirms previous observations (e.g. Belcher et al., 1978; Randic and Yu, 1976). This may be a reflection of the mode of action of 5-HT which, like other slow transmitters, appears to function through second messenger systems, which ultimately change the excitability of the cell (Hartzell, 1981; Siegelbaum, Camardo and Kandel, 1982). When the effects of 5-HT on nociceptive lamina I and deeper cells were compared (Table Ia,b), it became clear that the effects on the different subpopulations are similar. This is in agreement with Willcockson et al., (1984) who found no differential effects of 5-HT on STT cells located in various laminae. One notable difference however was that lower currents were usually required to produce an effect in lamina I (Table IIa, b). This may be due to the difference in densities between the marginal zone and deeper layers, such that it was easier for 5-HT to diffuse to its target sites in the marginal zone. Alternatively, it may be that 5-HT receptor sites in lamina I are more numerous. The differences may also be a reflection of the position of the ionophoretic electrode with respect to 5-HT target sites.

The predominant effect of 5-HT on both superficial and deeper dorsal horn neurones, was the selective inhibition of nociceptive responses, leaving the non-nociceptive responses unaffected. In contrast, Headley and co workers, found that ejecting 5-HT near cell bodies in laminae IV and V of cat dorsal horn produced non selective inhibition of cells (Headley, et al., 1978). In their study however, they used much higher currents (mean 98 $\pm$ 13 nA) than those employed in the present investigation on a comparable population in the rat (mean 12 $\pm$ 4 nA). Although direct comparison of ejection currents employed is difficult (since the concentration of the drug at its

receptor sites is unknown), it was noted here in three cases that increasing 5-HT currents beyond levels producing clear selective antinociception abolished this selectivity.

One model which may explain the phenomenon of selectivity is to postulate that 5-HT acts upon receptors presynaptic to the recorded cell, that is, situated either directly on nociceptive primary afferent terminals, or on intervening interneurones. Acting on presynaptic terminals, 5-HT may reduce the amount of transmitter released from nociceptive primary afferents by causing depolarisation of the terminal membrane (Eccles, 1964; Schmidt, 1973) and thus decreasing the nociceptive response of the neurone postsynaptic to the afferent input, whilst leaving innocuous responses unaffected. There is some evidence for a direct presynaptic mode of action for 5-HT on primary afferents. Increases in the excitability of terminal membranes, thought to reflect primary afferent depolarisation (PAD) (Wall, 1958), of tooth pulp afferents (Lovick, 1981) and of cutaneous primary afferent fibres relaying both nociceptive and non-nociceptive information (Martin, Haber and Willis, 1979) have been noted on stimulating NRM. If one were to assume that PAD resulting from NRM stimulation was mediated through the release of 5-HT from raphe spinal terminals (which is not necessarily the case as 5-HT is not the only transmitter present in raphe-spinal neurones- see introduction), then this would provide evidence in favour of a presynaptic action by 5-HT.

Hence, ionophoretically applied 5-HT, in vitro, can depolarise bullfrog A- and C-fibre terminals (Holziv and Anderson, 1984; Holziv, et al., 1985). In vivo, 5-HT has been observed to cause non-selective threshold increases in cutaneous A-and C- fibres (Carstens, Gilly, Schreiber and Zimmermann, 1987; Carstens, Klumpp, Randic and Zimmermann, 1981b). Unfortunately, fibres in most of these studies were not identified as nociceptive or non-nociceptive and 5-HT antagonists were not employed to try and counteract 5-HT action and provide evidence for an action through a 5-HT receptor. This may prove very important since noradrenergic receptor antagonists failed to reverse the threshold increases in primary afferents reported to be caused by noradrenaline (Jeftinija, Semba and Randic, 1981). The fact that the above studies do not show a selective action on nociceptive versus non nociceptive primary afferents, where specified, or on C- versus A- terminals, may reflect a genuine indiscriminate effect of 5-HT. Alternatively, it may be that non-nociceptive afferents are affected, but only in specific circumstances and under a specific mode of activation of the raphe-spinal system.

Anatomical evidence for a presynaptic mechanism of action in the dorsal horn is sparse. Descending terminals containing 5-HT have only been reported to form axo-axonic synapses (which constitute the anatomical prerequisite for a conventional type of direct presynaptic inhibition) in one study (La Motte and De Lanerolle, 1983). Other investigators have denied the presence of axo-axonic synapses, but reported a few 5-HT containing terminals located near axons (Light, Kavookjian and Petrusz, 1983; Maxwell, Leranth and Verhofstad, 1983; Ruda, Coffield and Steinbusch, 1982). It is possible that 5-HT released from such sites diffuses out to act non-synaptically at adjacent afferent terminals. A recent study reported that dorsal rhizotomy decreased 5-HT binding sites in the superficial dorsal horn by just over 20% (Daval, Verge, Basbaum, Bourgoin and Hamon, 1987), suggesting that direct presynaptic 5-HT receptors do exist, but that they do not form the dominant population in the superficial laminae. Our results indicate that, also in deeper laminae, a direct presynaptic mode of action of 5-HT, is not likely to be common, since selective inhibitory effects were observed at quite low currents in laminae V-VII. These laminae receive few nociceptive afferent terminals (Light and Perl, 1979a, b) and few, if any cells in these laminae have dendrites that reach laminae I and II (Brown, House, Rose and Snow, 1976; Ritz and Greenspan, 1985). It is therefore unlikely that the drug at current levels such as those employed here diffused upto laminae I and II where most nociceptive primary afferents are known to terminate.

A presynaptic action by 5-HT however does not have to be direct, as it may occur through intermediary interneurones. The long latency of 5-HT action observed in this and other studies is consistent with

the proposal that intermediary neurones are involved.

Though a presynaptic mechanism may be in operation, it is possible that the selective inhibitory effect of ionophoretically applied 5-HT acts through a postsynaptic site located preferentially on the part of the target neurone's dendritic tree receiving the nociceptive input. Indeed, anatomical evidence is consistent with this type of action, since dorsal horn neurones, including cells of origin of ascending tracts in both superficial and deeper laminae, have been shown to receive 5-HT contacts mainly on their dendritic shafts and occasionally on spines and somata (Hoffert, Miletic and Ruda, 1983; Hylden, et al., 1986c; Maxwell, et al. , 1983; Miletic, et al., 1984; Nishikawa, et al., 1983; Ruda and Gobel; 1980; Ruda, et al., 1982). The fact that the same type of contacts are made in superficial and deeper laminae also supports our finding that the effects of 5-HT ionophoretically applied in superficial and deeper dorsal horn are similar. Miletic and coworkers (1984) observed multiple 5-HT contacts on a given dendrite in the marginal zone, in the direction of its long axis. Some primary afferent endings make the same type of contact (Gobel et al., 1981) which led the authors to postulate that if 5-HT contacts were to interdigitate with those of a primary afferent ending on the same part of a neurones' dendrites, it may be possible to inhibit that cell's response to the afferent input by preventing spatial summation. Electrophysiological findings also provide support for a postsynaptic site for 5-HT action. Postsynaptic potentials, for instance, were recorded intracellularly from STT cells upon stimulation of NRM (Light, Casale and Menetrey, 1986; Giesler, Gerhart, Yezierski, Wilcox and Willis, 1981a; Willis, et al., 1977). To accept this as evidence for a

postsynaptic mode of action of 5-HT one must again assume that stimulating NRM in those experiments caused the release of 5-HT near STT neurones. Such a presumption is supported by the study of Miletic, et al., (1984) who related physiological evidence for NRM action of eliciting selective inhibition of marginal zone neuronal responses to noxious stimulation, with anatomical evidence for postsynaptic 5-HT contacts on the somata and dendrites of these same neurones and found a high correlation between these two variables.

In the present study, ionophoresis of 5-HT near a subpopulation of neurones caused the non-selective inhibition of all neuronal responses tested. Such an indiscriminate effect on transmission was also observed in other studies (Belcher et al., 1978; Headley et al., 1978). The simplest model for non-selective inhibition is to postulate the presence of postsynaptic 5-HT receptors on or near the cell body, the activation of which decreases neuronal responses to all peripheral input by changing the properties of the cell's membrane potential (Eccles, 1964). There is anatomical evidence for the presence of serotonergic axo-somatic contacts, both in the superficial and deeper layers (Miletic et al., 1984; Nishikawa et al., 1983). There is also electrophysiological evidence for an inhibitory action of 5-HT on both A- and C- afferent inputs (Carstens et al., 1981). As reported above, the selective antinociceptive action , observed with low 5-HT currents, was abolished upon increasing current levels in three cases. It should be noted however that in some cases, increasing current levels of 5-HT did not abolish the observed selective effect, suggesting that the generalised inhibitory effect of 5-HT may not be ubiquitous. To explain the phenomenon of abolished selectivity, it is simplest to postulate that

phenomenon of abolished selectivity, it is simplest to postulate that the serotonin-containing input to a single neurone is located on two different sites; one mediating the selective antinociceptive action through one of the mechanisms discussed above, the other would be a postsynaptic receptor, probably on or near the soma. The two different sites may be differentially activated through separate mechanisms. Increasing the ionophoretic current of 5-HT may hypothetically cause a spill over of 5-HT from the sites restricted to the nociceptive pathway, activating the somatic postsynaptic receptor, thus altering the cell's membrane potential and impeding excitation by any input. The observation of the phenomenon of abolished selectivity, though not ubiquitous, indicates that the position of the ionophoretic pipette with respect to the neurone is potentially quite important. Most, if not all recordings were presumably made from the region of the somata or dendrites of neurone, since most of the population presented here were excited by DLH or Glu, these excitatory amino acids depolarise cellular membrane at the soma and dendrites, but not at the axon (Fries and Zieglgansberger,1974; Puil, 1981; Zieglgansberger and Puil, 1973). If there are two different sites for 5-HT action on the same neurone, it may be expected that 5-HT may interact with one receptor and , given relatively higher currents are applied, 5-HT may eventually diffuse out to react with the other site. Alternatively, 5-HT may have different affinities to the two putative sites. The non-selective inhibitory effect was observed both in lamina I and in deeper laminae, though the current required to produce this effect was lower in the marginal zone (see table III a, b). It is also notable that proportionately more cells in lamina I (2/9 cells) were

Both marginal cells displaying this type of effect were nocispecific. It is interesting to speculate whether this is the usual effect of 5-HT on nocispecific cells in the marginal layer. Inhibiting the nociceptive input to the cell directly through a postsynaptic site located on or near the soma would be an economical strategy if the cell did not respond to any input other than a nociceptive one. The small sample size of lamina I cells tested here, however may have introduced an element of bias and more tests are necessary to establish whether the non-selective effect of 5-HT occurs with more frequency in the superficial dorsal horn.

One cell only was non-selectively excited by 5-HT. Ionophoretic application of dopamine, a transmitter thought to be involved in antinociception and analgesia (Fleetwood-Walker et al., 1988; Jensen and Yaksh, 1984b) had a selective antinociceptive effect on this neurone. It is possible that the neurone activated was an inhibitory neurone, but the fact that dopamine had an antinociceptive effect on this neurone argues against that. Another possibility is that the non-selective effect of 5-HT was mediated through a tryptamine-type receptor. Tryptamine has been shown to have separate receptors in the central nervous system (Altar, Wasley and Martin, 1986; Kaulen, Bruning, Rommelspacher and Baumgarten, 1986) and to have opposite action to 5-HT at a spinal level (Larson, 1983).

An excitatory 5-HT receptor may also be responsible for the observed facilitation of responses. The 5-HT_3 receptor has been reported to be responsible for most cases of excitation observed with 5-HT in the periphery (see Bradley et al., 1986; Richardson and Engel, 1986). During the course of the present experiments, 5-HT_3 binding sites have been characterised in the central nervous system (Kilpatrick

et al., 1987) and although the central action of this site has not yet been described, it is possible that the 5-HT$_3$ receptor is responsible for the excitatory effects observed in the dorsal horn in the present experiments.

It was noted throughout this study that the effect of 5-HT and its agonists and antagonists on spontaneous activity of cells, whenever present, was often not related to effects on evoked activity. It is not clear why that should be. A possible complicating factor is the altered level of anaesthesia throughout the experiment. Indeed, it has been shown that anaesthetics have wide ranging effects on spontaneous activity (e.g. Heavner, 1975; Kihata, Ghazi-Saidi, Yamashita, Kosaka, Bonikos and Taub, 1975; McIver and Roth, 1987). Another possibility is that 5-HT compounds diffuse from the electrode tip to affect other cells which impinge on the cell recorded from. Serotonin (and its agonists) also had multiple effects on DLH or Glu-evoked activity, comparable complex effects on this type of activity has been reported elsewhere (Belcher et al., 1978). One must consider however that glutamate is a candidate transmitter in its own right in the central nervous system (Watkins and Evans, 1981) and recently, 5-HT has been suggested to modify Glu release and even to interact directly with Glu receptors (Raiteri, Maura, Bonanna and Pittalugat, 1986; Reynolds, Baskys and Carlen, 1988). This may explain the complicated effects of 5-HT observed on DLH- or Glu- evoked activity. The lack of correlation between effects of 5-HT on spontaneous and Glu- or DLH- evoked activity as compared to responses to peripheral stimulation has been reported to occur in other studies (Belcher et al., 1978). This would indicate that interpretation of literature

effects of 5-HT on spontaneous or Glu- activity should be cautious.

The results presented in this report demonstrate multiple effects of 5-HT on transmission of both nociceptive and non nociceptive information in the dorsal horn. There is evidence for both a presynaptic and a postsynaptic mode of action for 5-HT. Anatomical information on the interrelations between dendrites of identified neurones in the dorsal horn and descending 5-HT terminals with inputs from nociceptive and non nociceptive primary afferents is needed before any firm conclusions on the predominant site of 5-HT action can be made . It is likely that the serotonergic raphe-spinal system acts through both presynaptic and postsynaptic modes, depending perhaps on the function of the neurone. The mechanism operating on interneurones involved in flexion reflex pathways, for example, may be different from that operating on neurones projecting to supraspinal levels. The action of a selective antinociceptive system could clearly be of an advantage to the animal in terms of modifying reflexes and behaviour in circumstances of trauma. The raphe-spinal system is probably one of several parallel antinociceptive systems which may be co-activated in situations such as morphine- or stress-induced analgesia. Alternatively, the different systems could be differentially activated under different circumstances.

The non-selective blanket inhibitory effect observed in some cases with 5-HT is hard to reconcile with the observation of selective antinociceptive effects in both SPA and OA. It is possible that such a non-selective inhibitory system, as the one described here, may be involved in sensory discrimination and signal filtering. Indeed, the involvement of 5-HT in DNIC may be evoked as a possible

physiological role for the non-selective effects of 5-HT.

B. Diffuse Noxious Inhibitory Controls : a Role for the Descending Serotonergic System

DNIC has been shown to be mediated, at least partially, through supraspinal structures, since it was reported to be virtually absent in spinal animals (Le Bars et al., 1979b). There is some evidence to imply the involvement of the serotonergic raphe-spinal system in DNIC. For instance, the morphological characteristics of the raphe-spinal axons (described above) allows for such involvement, since collaterals of the same axon spread to several segments and often to two or more regions of the cord (for example, cervical and lumbar). The activation of a single neurone may therefore hypothetically inhibit neurones with receptive fields spread over a wide surface area of the body. Moreover, lesions of the NRM (Dickenson, Le Bars and Besson, 1980) or microinjecting it with local anaesthetics (Morton et al., 1987), pretreatment of animals with a 5-HT neurotoxin (Dickenson, Rivot , Chitour, Besson and Le Bars, 1981) and ionophoretically- or systemically applied 5-HT antagonists (Chitour, Dickenson and Le Bars, 1982; Dickenson, Chitour and Le Bars, 1983) were all found to reduce DNIC. In contrast, pretreatment of animals with a 5-HT precursor was shown to dramatically potentiate DNIC (Kraus, Besson and Le Bars, 1982). Raphe neurones themselves (Anderson, et al., 1977; Behbehani, 1982; Dickenson and Goldsmith, 1986; Eisenhart, Morrow and Casey, 1983), including raphe-spinal neurones (Chiang and Goa, 1986; Dickenson and Goldsmith, 1986; Liu, Zhu and Zhang, 1986), in both rat and cat, respond to noxious stimulation. In addition, stimulation of the sciatic nerve of cat at

intensities sufficient to recruit C- fibres has been shown to cause the release of 5-HT into the intact, but not the cold-blocked spinal cord (Tyce and Yaksh, 1981). The above evidence has suggested that NRM may be part of a spinal-supraspinal loop involved in triggering DNIC, which in turn has been hypothesised to play an important role in stimulus discrimination (Le Bars et al., 1979b; Le Bars and Chitour, 1983; Le Bars, Dickenson and Besson, 1983). According to Le Bars and his colleagues, two pools of multireceptive neurones can signal nociception and pain. It is suggested that when a powerful noxious stimulus is applied to the periphery, the segmental pool of multireceptive neurones are excited by their afferent inputs, whilst this segmental pool, would be surrounded by a larger pool of the remaining multireceptive neurones whose peripheral receptive fields lie outside the area stimulated. Thus the activity of the neurones of this pool would be inhibited simultaneously by the activation of DNIC. Under normal conditions (i.e. in the absence of noxious stimuli), according to this theory, multireceptive neurones would continuously transmit information on non-noxious environmental stimuli, providing supraspinal centres with a constant "noise-signal". Intense noxious stimuli however, is thought to activate DNIC which would then inhibit all multireceptive cells not stimulated by the noxious stimulus, thus cutting down considerably on the "noise-signal", while nociceptive messages remain strong. Le Bars and his colleagues believe that such a contrast between the positive nociceptive messages and the inhibited background "noise" may be a mechanism by which a non-specific system (multireceptive cells) may provide specific information.

The multiple effects of 5-HT on dorsal horn neurones suggested to us that different effects may be mediated through different types of 5-HT receptors and led us to investigate the effects of different 5-HT receptor agonists upon transmission in the dorsal horn.

C. Effects of 5-HT Receptor Agonists

The effects of 5-HT_1 receptor agonists on neurones in lamina I were very similar to those observed in deeper laminae (table I a,b) and therefore the results will not be discussed separately. Quite low currents of 5-HT_1 receptor agonists were sufficient to produce significant responses (see tables II and III). It is clear from the results that the predominant effect (23/30 cases) of the 5-HT_{1A} receptor agonist was a non-selective inhibitory influence on all inputs tested. This indicates that the non selective inhibitory effect of 5-HT is likely to be mediated through a 5-HT_{1A} type receptor. This receptor may function in vivo in a physiological control mechanism such as DNIC, as discussed above, but it seems that this is not the only effect at this site, since non-selective excitations were also produced by 8-OH-DPAT. Only a few cells (3/30) showed non-selective excitation and all were located in laminae III-VII. Whether the excitatory effects observed are specific to a certain type of neurone or whether these excitatory effects occur in lamina I can not be ascertained until a large population of neurones is tested. It can not be determined whether the excitatory effect of 8-OH-DPAT was specific, since the 5-HT_{1A} site is reported to have a predominantly inhibitory function in the central nervous system (see Bradley et al., 1986; Peroutka 1988). It is possible that the excitatory effect of 8-OH-DPAT was mediated through a 5-HT_3-like

site, though this was not tested here, since excitatory effects of 5-HT in the dorsal horn were rarely observed.

In contrast to the effect of 8-OH-DPAT, the predominant response to the 5-HT_{1B} receptor agonist (32/47 cells), was a selective antinociception which was as effective against noxious thermal as against mechanical responses. This would indicate that selective antinociceptive effect of 5-HT is mediated through the 5-HT_{1B} site which is likely to be restricted to the nociceptive pathway. In no case was excitation observed with RU 24969. On a minor population however (7/47 cells), this compound produced a non-selective inhibitory effect. The currents required to produce this effect were relatively low (18 $\pm$ 6 nA, on for 8 $\pm$ 2 mins). It is possible that in these cases, the ionophoretic pipette was positioned close to the putative 5-HT_{1A} sites. Unlike 8-OH-DPAT which is a highly selective agonist at the 5-HT_{1A} site, RU 24969 has significant affinity for both the 1A and 1B sites (Hamon et al., 1986; Middlemiss, 1985; Peroutka, 1986; Sills et al., 1984), it is feasible therefore that the non-selective effects of RU 24969 were mediated through the 5-HT_{1A} sites. In three cases, increasing the current of RU 24969 abolished selectivity of action on the nociceptive input. This may be due to the position of the electrode and may occur by spill over of the drug onto nearby 5-HT_{1A} sites.

Results with sequential agonist testing confirm the two separate actions of the 1A and 1B receptor agonists ; whereas RU 24969 mimicked the selective antinociceptive effects of 5-HT, 8-OH-DPAT mimicked the non-selective inhibitory effects of 5-HT on the same cell. Sequential agonist testing also showed that in 10/11 cases,

the 1A and 1B receptor agonists effects on the same cell were qualitatively different thus demonstrating that both selective and non-selective influences are usually exerted on the same cell. One cell was unaffected by the 5-HT$_{1A}$ agonist, while the nociceptive response was selectively inhibited by the 5-HT$_{1B}$ agonist on the same cell. Another cell was non-selectively inhibited by both agonists. These observations may indicate the importance of the position of the ionophoretic electrode with respect to target sites. 8-OH-DPAT, is highly selective for the 1A site and is therefore unlikely to act on the 1B site except at extremely high currents. At relatively low currents, like the ones used in this study, this compound is likely to reach its site of action without interaction at the 1B site. Thus in no case did we observe selective antinociceptive effects with 8-OH-DPAT. RU 24969, although one of the best available agonists for the 1B site, can interact with the 5-HT$_{1A}$ sites, though its preference is for its own 1B sites. If ejected far away from its target sites and closer to the 5-HT$_{1A}$ sites, it may be likely to interact with these sites and cause the non-selective inhibition sometimes observed with this compound. If RU 24969 were ejected somewhere between the two locations for the two different sites, this compound may first act on its own sites and then spill over to interact with the 1A sites. Such a hypothetical model may explain the phenomenon of abolished selectivity. Anatomical data on the position of different 5-HT receptors on dorsal horn neurones will undoubtedly shed some light on their mode of action. Similarly, labelling studies where the path of ionophoretically applied molecules are traced inside tissue may be helpful in interpreting

In contrast to the ability of the 5-HT_1 agonists to mimic the effects of 5-HT, the 5-HT_2 agonist, DOI, failed to affect either the nociceptive or the non-nociceptive responses of cells tested. The 5-HT_2 agonist however significantly decreased the DLH- evoked response where tested. The reason for this is unclear, although one possibility is that 5-HT and its analogues
may interact with excitatory amino acid receptors (see above).

D. The Effect of Antagonists

Results with antagonists confirm the involvement of 5-HT_1 and not 5-HT_2 sites in mediating both the selective and non selective effects of ionophoretically applied 5-HT on dorsal horn neurones. Cyanopindolol, a 5-HT_1 antagonist successfully reversed the effects of both 5-HT_{1A} and 5-HT_{1B} agonists. Ketanserin, a 5-HT_2 antagonist, on the other hand, failed to attenuate the effects of either agonist. Results where both antagonists were tested on the same cell confirm this finding. Neither cyanopindolol, nor ketanserin had any effect on evoked responses in the majority of cases. Cyanopindolol did however, non-selectively enhance the evoked activity of one cell, while ketanserin depressed spontaneous discharge in three cases. It may be that the 5-HT system is involved in tonic inhibition, although results presented here indicate that such an effect is exerted only on a minor population (see below).

II Results with Non-nociceptive Cells

The effects of 5-HT and its agonists on this type of cell were complex and inconsistent. The non-nociceptive response of only 1/7 cells was inhibited by 5-HT while that of only 2/6 cells was inhibited by RU 24969. In the remaining cells, the brush-evoked

response was unaltered. This is in contrast to the effects of the 5-HT$_{1A}$ agonist which were both excitatory (2/10) and inhibitory (5/10). The differences observed may be a reflection of the heterogeneity of the population examined and further investigation is necessary to elucidate clearer patterns of effect. Two main findings however may be pointed out. Firstly that 5-HT can have an effect on non-nociceptive transmission, though this effect does not seem to be common to all non-nociceptive cells. Secondly, the DLH-evoked activity was consistently affected, but not always in the same direction, since both excitations and inhibitions were observed. It is not clear how 5-HT and its agonists affect this type of activity and whether this effect is direct on the cell recorded or at a site further away.

In summary, the results presented in this section suggest that 5-HT$_1$ and not 5-HT$_2$ sites are involved in mediating 5-HT action in the dorsal horn. Two 5-HT$_1$ receptor subtypes were found to mediate qualitatively different effects. While the 5-HT$_1$ site seems to mediate the non-selective effects of 5-HT, the 5-HT$_{1A}$ site seems to be responsible for the selective antinociceptive effect of 5-HT$_{1B}$. To produce non-selective inhibition, 5-HT presumably acts postsynaptically through a 5-HT$_{1A}$ type site likely to be located on or near the soma, The antinociceptive effect of 5-HT, on the other hand, is likely to be mediated through 5-HT$_{1B}$ sites, probably located at some more distant point, related to the nociceptive input pathway. Serotonin may interact differentially with the 5-HT$_{1A}$ and 5-HT$_{1B}$ sites. Although binding studies indicate that 5-HT has similar affinities to these two sites in brain tissue, in the spinal cord 5-HT

may have better affinity for the 1B site. Such a hypothesis needs to be investigated. Differential affinities of 5-HT for the two different sites is not the only possible explanation for the observed phenomenon of abolished selectivity, if 5-HT$_{1A}$ sites were to be present as a relatively minor population, or if 5-HT action on these sites were to have a slower time course, the phenomenon of abolished selectivity on increasing 5-HT ejection currents would be explained by 5-HT's later action on the 5-HT$_{1A}$ sites. The existence of a sub population of cells which is unaffected by 5-HT is a possibility, but our results suggest that this would be a minor population. Such cells may be under the modulation of different neurotransmitters. Alternatively, they could be part of a specialised system, perhaps a local circuit which may be unaffected by descending control.

Focal Electrical Stimulation Experiments

The results of the brainstem stimulation experiments were entirely consistent with those obtained from ionophoretic experiments. The predominantly selective antinociceptive effect of NRM stimulation observed here confirms previous reports (Duggan and Griersmith, 1979; Fields et al., 1977a; Gerhart et al., 1981; Guilbaud et al., 1977; Miletic et al., 1984). The sites which were effective in producing a selective antinociceptive response were concentrated in the NRM and the lateral NRPG, sites of origin of the major part of the serotonin-containing input to the dorsal horn. The inhibitory effect of brainstem stimulation had a relatively short onset (within seconds) when compared to the effects of ionophoretically applied 5-HT (at least one minute). The difference in latency of response may just reflect methodological differences. Thus, stimulating NRM is likely to release 5-HT directly onto or near

its receptor sites, whereas ionophoretic application does not usually target the receptor sites directly.

Stimulation in and around NRM caused non-selective innibitory effects, such as those observed in other studies (Belcher, et al., 1978; Chapman et al., 1985; Mc Creery et al., 1979; Miletic et al., 1984; Willis et al., 1977). The mean current required to elicit this effect was higher than that noted with selective antinociceptive effects. Non selective effects however are not likely to be due to current spread since these effects were pharmacologically reversible and were observed at low currents in some cases. Moreover, stimulation at high currents, less than 1 mm away from NRM and adjacent NRPG failed to affect either nociceptive or non nociceptive responses. Furthermore, tne bipolar configuration of electrodes used for stimulation in this study allows for relatively good current localisation at the locus of stimulation (Bagshaw and Evans, 1976).

Both selective and non-selective actions of stimulation in the region of the NRM appear to be mediated specifically through a 5-HT$_1$-like receptor, since a 5-HT$_1$ and not a 5-HT$_2$ receptor antagonist could effectively reverse these actions at the level of the dorsal horn. In contrast, a 5-HT$_2$, an α-adrenergic and an opiate receptor antagonist all failed to reverse the effects of NRM stimulation in the majority of cases.

Interaction between 5-HT and Other Transmitters at the level of the Dorsal Horn

Serotonin has been shown to interact with other transmitters at

the spinal level. Taking into account such interaction is important in assessing the role which each transmitter plays.

A. Noradrenaline (NA)

Some reports have suggested the existence of an interaction between the noradrenaline- and serotonin-containing descending systems at the level of the spinal cord. For instance, analgesic effects of NRM stimulation (Barbaro, et al., 1985; Hammond and Yaksh, 1984) or morphine microinjection (Jensen and Yaksh, 1986b) can be attenuated by intrathecally administered α receptor as well as 5-HT receptor antagonists. Neither NRM nor NRPG have noradrenaline-containing cell bodies (Poitras and Parent, 1978; Westlund et al., 1983). This indicates either that the descending noradrenaline-containing system is coactivated with manipulations of NRM, perhaps through an efferent connection. Alternatively, that the 5-HT-containing terminals in the spinal cord impinge on or act through a noradrenergic site. Our results would indicate that the former possibility is more likely. From the ionophoretic experiments, RX, a noradrenergic α_2 blocker, (at currents which had been shown to block the antinociceptive effect of NA), failed to reverse the effects of ionophoretically applied 5-HT receptor agonists. Moreover, intrathecally applied phentolamine, an α_2 - receptor antagonist, in another study, failed to reverse the antinociceptive action of intrathecally applied 5-HT (Schmauss, et al., 1983). In contrast, simulating the lateral NRM was found here to produce selective inhibition at the level of the dorsal horn which was readily reversible by RX. Our results are therefore consistent with the idea that activation of NRM (perhaps from a restricted location) coactivates the descending

serotonin-containing and noradrenaline systems.
The route and mechanism by which such coactivation may occur are yet to be elucidated.

The descending noradrenaline-containing system may however be acting in a tonic manner. Ionophoretically applied RX was found here to increase neuronal evoked and spontaneous responses in 3/4 cells. Similarly, intrathecally applied NA receptor antagonists have been found to decrease nociceptive thresholds in behavioural tests (Janss and Gebhart, 1987). Moreover, spinal NA depletion has been reported to cause hyperalgesia (Fasmer, Berge and Hole, 1984) and to antagonise the antinociceptive effects of intrathecally applied 5-HT (Archer, Jonsson, Minor and Post, 1986; Minor et al., 1985). There seems to be therefore a case for the involvement of NA in tonic innibition, though the extent of this involvement and its effect on reported interactions between NA and 5-HT at the spinal level is yet to be determined.

B. Opioids

There is some anatomical evidence that the descending serotonin-containing system interacts with an opioid receptor at the level of the dorsal horn. Terminals of descending serotonin-containing axons have been demonstrated to be presynaptic to some neurones containing enkephalin immunoreactivity (Glazer and Basbaum, 1984). Furthermore, intrathecally applied naloxone was found to block the antinociceptive effect of NRM stimulation (Jensen and Yaksh, 1984a; Zorman, et al., 1982). In contrast, naloxone was found here and in another study to have no effect on dorsal horn neuronal inhibition by NRM stimulation (Duggan and Griersmith, 1979). Furthermore, the analgesic action of morphine microinjected into PAG was antagonised by both methysergide

and phentolamine, but not naloxone (Jensen and Yaksh, 1986b; Yaksh, 1979) indicating that neither 5-HT nor noradrenaline interact with opioid-containing cells at the spinal level. Importantly, depletion of spinal cord 5-HT content did not attenuate analgesia produced by intrathecally administered morphine (Vasko et al., 1984). Anatomical evidence is also opposed to the mediation of 5-HT action through an opioid link, since Miletic et al. (1984) have provided evidence for direct action of 5-HT on lamina I neurones by finding a very strong correlation between the effects of NRM stimulation with the number of postsynaptic 5-HT contacts on the same cell. Our results are consistent with the above reports which indicate that the descending 5-HT does not prominently act through an opioid link in the dorsal horn, since a 5-HT_1 receptor antagonist but not naloxone reversed the effects of NRM stimulation. The possibility that some 5-HT terminals exert an effect on enkephalinergic neurones can not be ruled out, as there is anatomical evidence to suggest such interaction but the adequate assessment of the role of such a serotonergic-opioid link is still lacking.

Cardiovascular Effects of Brainstem Stimulation

It was noted in the present study that stimulating NRM or adjacent reticular formation often, but not invariably, caused an increase in blood pressure, which accompanied the inhibition exerted on dorsal horn neurones. It is unlikely that the cardiovascular reactions caused by brainstem stimulation are responsible for the antinociceptive action observed. Edeson and Ryall (1983) administered noradrenaline chloride intravenously in their study, a procedure which resulted in a nearly 50% increase in blood pressure,

this did not have any effect on dorsal horn neuronal responses to noxious heat stimulation.

Behavioural Studies

The results of this pilot study on behavioural analgesia may only be taken as indicative evidence for the mediation of the analgesic effect of 5-HT through a 5-HT_{1B} -type receptor. RU 24969 produced an antinociceptive effect of relatively rapid onset (5 minutes) in the tail flick test which was significant at the low dose of 3.7 µg. Two other doses (1.8 and 7.5 µg) caused a similar though statistically insignificant effect in an apparently dose-dependent fashion. The results of the hot plate test were similar to those of the tail flick test, RU 24969 appeared to increase hot plate latency at two doses (1.8 and 3.7 µg), although it did not reach statistical significance. Demonstrating the effects of a drug in the hot plate test as well as the tail flick test is important, since this tries to deal with an inherent problem of behavioural testing of nociception, namely the dependence of the result on the motor component. The contribution of the motor component to a purely spinal reflex (in this case, the tail flick) can not be judged directly. The hot plate test, on the other hand, involves supraspinal circuitry and co-ordinated motor responses . Failure to observe the normal components of such behavioural responses indicates to the experimenter that motor activity is disrupted. In the present experiments, the motor co-ordination of rats was additionally assessed on the rotarod and RU 24969 did not appear to impair rats performance in any way. Moreover, RU 24969 was verified to be genuinely antinociceptive, since animals tested at random retained their responses to light touches but appeared to be analgesic to a noxious mechanical stimulus

(pinch). Moreover, the effect of RU 24969 has been shown here to be mediated through an action at the 5-HT_1 site, since cyanopindolol appeared to reverse the effects of this compound.

In contrast, intrathecally applied 8-OH-DPAT appeared to have no effect on the nociceptive tail flick reflex, but to increase HPL. Although none of the effects of this compound were significant at either dose tested (3.3 and 6.6 µg), these results are in agreement with a study on mice (Fasmer, Berge, Post and Hole, 1986). Some motor impairment was noted with this compound at doses tested which indicates that some of its effects may have been exerted on the motor system, though the extent of diffusion to and action of 8-OH-DPAT upon the ventral horn are unknown.

It is possible that the action of the two different drugs was not restricted to the spinal cord and that they may have diffused rostrally in the CSF, or transported via the circulation to act supraspinally, but this is unlikely. RU 24969 and 8-OH-DPAT, intrathecally applied at the highest doses employed to the cervical level of the cord failed to have any effect on either TFL or HPL. Serotonin is known to have cardiovascular effects (e.g. Garattini and Valzelli, 1965) and it is conceivable that the application of 5-HT agonists crudely to the surface of the cord causes effects which are secondary to a vasoconstrictive action on spinal blood flow. This again seems unlikely, since intrathecally applied papaverine, a non-specific vasodilator, failed to attenuate the antinociceptive action of intrathecally applied 5-HT in a previous study (Yaksh and Wilson, 1979). Moreover, Solomon and Gebhart (1988) found that only intrathecal doses of 10 µg for 8-OH-DPAT and 100 µg of RU 24969

produced significant stimulation in blood pressure. These doses were higher than those employed in the present study. A further possibility is that the action of the agonists employed here was an artefact due to an alteration of the local skin blood flow, effectively decreasing the rate of skin temperature rise in response to a heat stimulus (see Duggan, Griersmith, Headley and Maher, 1978). Serotonin appears to play an important role in body temperature regulation (Cox, Lee and Martin, 1981; Gudelsky, Koenig and Meltzer, 1986) and microinjections of 5-HT into the hypothalamus induces hypothermia in rats (Lin, Wu and Tsay, 1983). A recent study has also shown that interrupting the descending serotonergic system by intrathecal application of serotonergic neurotoxins causes a rise in tail skin temperature and subsequently decreases latencies in the rat tail flick test (Tjolsen, Berge, Eide, Broch and Hole, 1988). It has been suggested that the apparently hyperalgesic action of descending serotonergic pathway lesion was secondary to an increase in skin temperature (presumably through an action on tail skin circulation) which probably reduced the duration of noxious stimulation required before thermal nociceptors reach their threshold level (Tjolsen et al., 1988).

In agreement with Tjolsen et al. (1988), it was noted that a linear relationship exists between tail temperature and tail flick latency. The results presented here indicate that the antinociceptive action of intrathecally applied RU 24969 was not secondary to an action on skin circulation since this compound was found to cause an increase in tail temperature. If the action of RU 24969 was mediated through an action on blood flow, one would expect RU 24969 to cause a decrease in tail flick latency, instead, RU 24969

caused a clearly antinociceptive effect throughout a wide range of resting tail temperatures. This indicates that RU 24969 has a genuinely antinociceptive effect regardless of changes in skin blood flow.

In contrast, 8-OH-DPAT may have caused a small parallel shift of the tail temperature-response line to the left, without affecting the relationship observed in control animals. This would indicate that 8-OH-DPAT may have a somewhat secondary effect on the tail flick response.

Both 5-HT_1 agonists appeared to have an antinociceptive action in the hot plate test. The way in which this test is applied may pose a problem of interpretation. When the animal is placed on the hot plate, two or more paws are normally stimulated at the same time. The relation of this type of general stimulation and the activation of DNIC is not known and consequently the result of any possible interaction (the effects on the measured response) may be difficult to interpret. The use of different behavioural tests involving a single site of stimulation would probably help in eliminating this problem.

The present study has served to highlight several methodological factors which have to be taken into consideration when investigating the effects of intrathecally applied compounds on nociception :

1) The need to assess the motor performance of animals in conjunction with nociceptive testing since an increase in latency or abolition of a nociceptive response may be a reflection of the drug's effect on the motor component of the behavioural response.

2) The need to assess the effects of compounds tested on blood pressure and skin blood flow and temperature when using thermal stimuli in nociceptive testing, since observed responses may be secondary to the cardiovascular or temperature regulatory effects of the drug in question.

3) The need for careful interpretation of results when 5-HT systems (and other systems) are generally manipulated, since such crude manipulations may produce a range of interactions between different systems (for example 5-HT is thought to be involved in temperature control, DNIC, antinociception, cardiovascular and motor control) some of which may be entirely artificial and would not occur under natural circumstances.

In conclusion therefore, the results of this pilot study are indicative of a selectively antinociceptive role for the 5-HT_{1B} sites though any role for the 5-HT_{1A} site remains to be clarified. An extended study is required to confirm and clarify the present results.

Tonic Versus Phasic Inhibition by the Raphe-Spinal System

There seems to be no general consensus in the literature as to whether the inhibition exerted by the descending serotonergic system at the spinal level is phasic or tonic. The raphe-spinal system in cat does not seem to be involved in tonic inhibition, since lesions in NRM (Hall, Duggan, Johnson and Morton, 1981), or microinjecting it with anaesthetic (Gebhart, et al., 1983a) did not affect tonic descending inhibition (investigated by reversible cold block), on any of the spontaneous discharge, C-fibre- or noxious heat-evoked

responses of dorsal horn neurones. Similarly, NRM lesions did not change sensitivity to noxious stimulation in behavioural tests (Abbot and Melzack, 1982; Chance, Krynock and Rosecrans, 1978). In contrast, other groups working on rat found that inactivating NRM either by lesioning (Proudfit and Anderson, 1975; Proudfit, 1980) or by local anaesthetic microinjection (Sandkuhler and Gebhart, 1984b) caused hyperalgesia in some studies but not others (Prieto, et al., 1983). The obvious discrepancies may reflect the use of different species and different preparations

The serotonergic component of the raphe-spinal system is not likely to be prominently involved in tonic inhibition. Thus neurotoxins which destroy 5-HT neurones, when applied to NRM (Bragin and Durinyan, 1983) or intrathecally (Deakin and Dostrovsky, 1978; Johannessen, Watkins, Carlton and Mayer, 1982; Sagen, Winker and Proudfit, 1983; Vasko et al., 1984) had no long lasting effect on nociceptive thresholds. Similarly, intrathecally applied 5-HT antagonists have been reported to have no or only transient effects on nociceptive thresholds (Hammond and Yaksh, 1984; Jensen and Yaksh, 1984a; Jensen and Yaksh, 1986; Schmauss et al., 1983; Yaksh, 1979). In addition, ionophoretically applied 5-HT receptor antagonists have been found to have minimal effects on neuronal responses to A- and C-evoked responses (Dickenson et al., 1983). Our results confirm these findings since cyanopindolol applied on its own ionophoretically or intrathecally, failed to affect evoked responses of the majority of dorsal horn neurones and failed to affect nociceptive threshold in the tail flick test. Cyanopindolol, did however cause excitation of all evoked responses of one neurone and caused a decrease in HPL,

this may indicate that 5-HT has some tonic action over a subpopulation of spinal neurones. Some studies support this contention (Berge, 1982; Berge, Fasmer and Hole, 1983; Fasmer, Berge and Hole, 1985; Proudfit and Hammond, 1981). On the other hand a recent study has pointed out an important point for consideration. Tjolsen and coworkers have found that intrathecally applied 5-HT neurotoxins actually increase skin temperature (Tjolsen et al., 1988). They have therefore suggested that the observed decrease in nociceptive threshold observed in behavioural tests employing thermal stimuli may be secondary to alteration of skin temperature. Such uninvestigated interactions between effects of 5-HT on different systems may have resulted in the often considerable discrepancies in this area of the literature.

Conclusions

The ionophoretic studies presented here have strongly implicated the involvement of 5-HT_1 and not 5-HT_2 sites in modulating nociceptive transmission at the level of the dorsal horn. The involvement of the 5-HT_3 site has not been investigated in this study, but it may be responsible for the few cases of excitation observed. The inhibitory effect of electrically stimulating NRM and lateral NRPG has also been demonstrated here to be mediated through a 5-HT_1 receptor site, since a 5-HT_1 receptor antagonist readily reversed the effects of brainstem stimulation. 5-HT_2, α-adrenergic or opiate receptor antagonists on the other hand were unsuccessful in reversing these effects. It is likely that manipulations of a certain part of the NRM activate separate serotonergic and noradrenergic descending systems. Thus this study, as well as

others, have shown that stimulation in or near NRM produces inhibitory effects which may be attenuated by α- adrenergic blockers.

Ionophoretic studies have discriminated two separate effects of two different 5-HT_1 receptor sites. Paradoxically, the results suggest that 5-HT_1 receptor sub types may mediate functionally opposite effects. The 5-HT_{1B} receptor subtype was found to mediate the selective antinociceptive and analgesic effects of 5-HT. In contrast, the 5-HT_{1A} receptor subtype was found to mimic the non-selective inhibitory effects of 5-HT. Since there is some evidence to implicate the involvement of 5-HT in DNIC, the 5-HT_{1A} site is a likely candidate site for mediating DNIC action. If this proves true, the 5-HT_{1A} site may be involved in discriminating nociceptive transmission, according to the theory of Le Bars et al., an action opposite to 5-HT antinociceptive effect. Results obtained from behavioural studies, though not definitive alone, provide support for the involvement of the 5-HT_{1B} site in antinociception, thus local administration 5-HT_{1B} receptor agonist to the spinal cord has been found to exert a selective antinociceptive and apparently analgesic effect in two behavioural tests. In contrast, the action of the 5-HT_{1A} receptor agonist was difficult to evaluate due to its putative effect on skin temperature and its possible involvement in DNIC. Further studies would greatly aid better understanding of the role of the 5-HT_{1A} site in modulating nociception.

REFERENCES

Abbot, F.V. and Melzack, R. (1982). Brainstem lesions dissociate neural mechanisms of morphine analgesis in different kinds of pain. Brain Res., 251, 149-155.

Abbot, F.V. and Melzack, R. (1983). Dissociation of the mechanisms of stimualtion-produced analgesia in tests of tonic and phasic pain. Adv. Pain Res. Ther., vol.5, J.J. Bonica, U. Lindblum and A.Iggo (Eds.), Raven Press, New York, pp.401-409.

Abols, I.A. and Basbaum, A.I. (1981). Afferent connections of the rostral medulla of the cat : a neuronal substrate for midbrain-medullary interactions in the modulation of pain. J. Comp. Neurol., 201, 285-297.

Adams, J.E. (1976). Naloxone reversal of analgesia produced by brain stimulation in the human, Pain, 2, 161-166.

Adrian, E.D. (1931). The messages in sensory nerve fibres and their interpretation. Proc. R. Soc. Lond, 109, 1-18.

Akaike, A., Shibata, T., Satoh, M. and Takagi, H. (1978). Analgesia induced by microinjection of morphine into, and electrical stimulation of, nucleus reticularis pararagigantocellularis of rat medulla oblangata. Neuropharmacol., 17, 775-778.

Akil, H. and Liebeskind, J.C. (1975). Monoaminergic mechanisms of stimulation-produced analgesia. Brain Res., 94, 279-296.

Akil, H. and Mayer, D.J. (1972). Antagonism of stimulation-produced analgesia by p-CPA, a serotonin synthesis inhibitor. Brain Res., 44, 692-697.

Akil, H., Mayer, D.J. and Liebeskind, J.C. (1976). Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science, 191, 961-962.

Altar, C.A., Wasley, A.M. and Martin, L.L. (1986). Autoradiographic localization and pharmacology of unique [^{3}H] tryptamine binding sites in rat brain. Neurosci., 17, 263-273.

Anderson, S.D., Basbaum, A.I. and Fields, H.L. (1977). response of medullary raphe neurons to peripheral stimulation and to systemic opiates. Brain Res., 123, 363-368.

Angaut-Petit, D. (1975a). The dorsal column system : II. Functional properties and bulbar relay of the postsynaptic fibres of the cat's fasciculus gracilis. Exp. Brain Res., 22, 471-493.

Angaut-Petit, D. (1975b). The dorsal column system : I. Existence of long ascending postsynaptic fibres in the cat's funiculus gracilis. Exp. Brain Res., 22, 457-470.

Appel, N.M., Wessendorf, M.W. and Elde, R.P. (1987). Thyrotropin-releasing hormone in spinal cord : coexistence with serotonin and with substance P in fibers and terminals apposing identified preganglionic sympathetic neuron. Brain Res., 415, 137-143.

Appelbaum, A.E., Leonard, R.B., Kenshalo, D.R., Martin, R.F. and Willis, W.D. (1979). Nuclei in which functionally identified spinothalamic tract neurons terminate. J. Comp. Neurol., 188, 575-586.

Archer, T., Jonsson, G., Minor, B.G. and Post, C. (1986). Noradrenergic - serotonergic interactions and nociception in the rat. Europ. J. Pharmacol., 120, 295-307.

Azami, J., Llewelyn, M.B. and Roberts, M.H.T. (1982). The contribution of nucleus reticularis paragigantocellularis and nucleus raphe magnus to the analgesia produced by systemically administered morphine, investigated with the micro-injection technique. Pain, 12, 229-246.

Bagshaw, E.V. and Evans, M.H. (1976). Measurement of current spread from microelectrodes when stimulating within the nervous system. Exp. Brain Res., 25, 391-400.

Barbaro, N.M., Hammond, D.L. and Fields, H.L. (1985). Effects of intrathecally administered methysergide and yohimbine on microstimulation-produced antinociception in the rat. Brain Res., 343, 223-229.

Barton, C., Basbaum, A.I. and Fields, H.L. (1980). Dissociation of supraspinal and spinal actions of morphine : a quantitative evaluation. Brain Res., 188, 487-498.

Basbaum, A.I. (1973). Conduction of the effects of noxious stimulation by short-fiber multisynaptic systems of the spinal cord in the rat. Exp. Neurol., 40, 699-716.

Basbaum, A.I., Clanton, C.H. and Fields, H.L. (1978). Three bulbospinal pathways from the rostral medulla of the cat. J. Comp. Neurol., 178, 209-224.

Basbaum, A.I. and Fields, H.L. (1978). Endogenous pain control mechanisms : review and hypothesis. Ann. Neurol., 4, 451-462.

Basbaum, A.I. and Fields, H.L. (1979). The origin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat : further studies on the anatomy of pain modulation. J. Comp. Neurol., 187, 513-532.

Basbaum, A.I., Marley, N.J.E., O'Keefe, J. and Clanton, C.H. (1977). Reversal of morphine and stimulus-produced analgesia by subtotal spinal cord lesions. Pain, 3, 43-56.

Basbaum, A.I., Moss, M.S. and Glazer, E.J. (1983). Opiate and stimulation-produced analgesia : the contribution of the monoamines. Adv. Pain Res. Ther., vol.5, J.J. Bonica, U. Lindblum and A.Iggo (Eds.) Raven Press, New York, pp 323-339.

Basbaum, A.I., Ralston, D.D. and Ralston, H.J. (1986). bulbospinal projections in the primate : a light and electron microscopic study of a pain modulating system. J. Comp. Neurol., 250, 311-323.

Basbaum, A.I., Zahs, K., Lord, B. and Lakos, S. (1988). The fiber caliber of 5-HT immunoreactive axons in the dorsolateral funiculus of the spinal cord of the rat and cat. Somatosensory Res., 5, 177-185.

Beall, J.E., Martin, R.F., Appelbaum, A.E. and Willis, W.D. (1976). Inhibition of primate spinothalamic tract neurons by stimulation in the region of the nucleus raphe magnus. Brain Res., 114, 328-333.

Beck, P.W., Handwerker, H.O. and Zimmermann, M. (1974). Nervous outflow from the cat's foot during noxious radiant heat stimulation. Brain Res., 67, 373-386.

Behbehani, M.M. (1982). The role of acetylcholine in the function of the nucleus raphe magnus and in the interaction of this nucleus with the periaqueductal gray. Brain Res., 252, 299-307.

Behbehani, M.M. and Fields, H.L. (1979). Evidence that an excitatory connection between the periaqueductal gray and the nucleus raphe magnus mediates stimulation produced analgesia. Brain Res., 170, 85-93.

Beitel, R.E. and Dubner, R. (1976). Fatigue and adaptation in unmyelinated (C) polymodal nociceptors to mechanical and thermal stimuli applied to the monkey's face. Brain Res., 112, 402-206.

Beitel, R.E. and Dubner, R. (1976). Response of unmyelinated (C) polymodal nociceptors to thermal stimuli applied to monkeys face. J. Neurophysiol., 39, 1160-1175.

Beitz, A.J. (1982a). The sites of origin of brain stem neurotensin and serotonin projections to the rodent nucleus raphe magnus. J. Neurosci., 2, 829-842.

Beitz, A.J. (1982b). The nuclei of origin of brain stem enkephalin and substance P projections to the rodent nucleus raphe magnus. Neurosci, 7, 2753-2768.

Belcher, G., Ryall, R.W. and Schaffner, R. (1978). The differential effects of 5-hydroxytryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dorsal horn interneurones in the cat. Brain Res., 151, 307-321.

Bennett, G.J., Abdelmoumene, M., Hayashi, H. and Dubner, R. (1980). Physiology and morphology of substantia gelatinosa neurons intracellularly stained with horseradish peroxidase. J. Comp. Neurol., 194, 809-827.

Bennett, G.J., Seltzer, Z., Lu, G.W., Nishikawa, N. and Dubner, R. (1983). The cells of origin of the dorsal postsynaptic projection in the lumbrosacral enlargments of cats and monkeys. Somatosenory Res., 1, 131-149.

Berge, O.G. (1982). Effects of 5-HT receptor agonists and antagonists on a reflex response to radiant heat in normal and spinally transected rats. Pain, 13, 253-266.

Berge, O.G., Fasmer, O.B. and Hole, K. (1983). Serotonin receptor antagonists induce hyperalgesia without preventing morphine antinociception.Pharmacol. Biochem. Beh., 19, 873-878.

Berkley, K.J. (1980). Spatial relationships between the terminations of somatic sensory and motor pathways in the rostral brainstem of cats and monkeys. I. Ascending somatic sensory inputs to lateral diencephalon. J. Comp. Neurol., 193, 283-317.

Besson, J.M., Guilbaud, G. and Le Bars, D. (1975). Descending inhibitory influences exerted by the brain stem upon the activities of dorsal horn lamina V cells induced by intra-arterial injection of bradykinin into the limbs. J.Physiol., 248, 725-739.

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

Bjorklund, A., Falck, B. and Stenevi, U. (1970). On the possible existence of a new intraneuronal monoamine in the spinal cord of the rat. J. Pharmacol. Exp. Ther., 175, 525-532.

Blomqvist, A., Flink, R., Bowsher, D., Griph, S. and Westman, J. (1978). Tectal and thalamic projections of dorsal column and lateral cervical nuclei : a quantitative study in the cat. Brain Res., 141, 335-341.

Bobillier, P., Seguin, S., Petitjean, F., Salvert, D., Touret, M. and Jouvet, M. (1976). The raphe nuclei of the cat brain stem : a topographical atlas of their efferent projections as revealed by autoradiography. Brain Res., 113, 449-486.

Boivie, J. (1980). Thalamic projections from lateral cervical nucleus in monkey. A degeneration study. Brain Res., 198, 13-26.

Bourgoin, S., Oliveras, J.L., Bruxelle, J., Hamon, M. and Besson, J.M (1980). Electrical stimulation of the nucleus raphe magnus in the rat. Effects on 5-HT metabolism in the spinal cord.

Brain Res., 194, 377-389.

Bowker, R.M. (1986). The relationship between descending serotonin projections and ascending projections in the nucleus raphe magnus : a double labeling study. Neurosci. Lett., 70, 348-353.

Bowker, R.M., Reddy, V.K., Fung, S.J., Chan, J.Y.H. and Barnes, C.D. (1987). Serotonergic and non-serotonergic raphe neurons projecting to the feline lumbar and cervical spinal cord : a quantitative horseradish peroxidase-immunocytochemical study. Neurosci. Lett., 75, 31-37.

Bowker, R.M., Westlund, K.N. and Coulter, J.D. (1981a). Origins of serotonergic projections to the spinal cord in rat : an immunocytochemical-retrograde transport study. Brain Res., 226, 187-199.

Bowker, R.M., Westlund, K.N. and Coulter, J.D. (1981b). Serotonergic projections to the spinal cord from the midbrain in the rat : an immunocytochemical and retrograde transport study. Neurosci. Lett., 24, 221-226.

Bowker, R.M., Westlund, K.N., Sullivan, and Coulter, J.D. (1982). Organization of descending serotonergic projections to the spinal cord. Prog. Brain Res., 57, 239-265.

Bowker, R.M., Westlund, K.N., Sullivan M.C., Wilber, J.F. and Coulter, J.D. (1983). Descending serotonergic, peptidergic and cholinergic pathways from the raphe nuclei : a multiple transmitter complex. Brain Res., 288, 33-48.

Bowsher, D. (1975). Diencephalic projections from the midbrain reticular formation. Brain Res., 95, 211-220.

Bradley, P.B. (1984). The characterization of 5-HT receptors. Neuropharmacol., 23, 1465-1466.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacol., 25, 563-576.

Bragin, E.O. and Durinyan, R.A. (1983). A study of the catecholaminergic systems of the lateral reticular nuclei and the serotonergic systems of the raphe magnus in various types of analgesia. Pain, 17, 225-234.

Brodal, A., Taber, E. and Walberg, F. (1960a). The raphe nuclei of the brain stem in the cat. II. Efferent connections. J. Comp. Neurol., 114, 239-259.

Brodal, A., Walberg, F. and Taber, E. (1960b). The raphe nuclei of the brain stem in the cat. III. Afferent connections. J. Comp. Neurol., 114, 261-281.

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

Brown, A.G. (1981). Organisation in the Spinal Cord. Springer-Verlag, Berlin.

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

Brown, A.G., and Fyffe, R.E.W. (1981). Form and function of dorsal horn neurones with axons ascending the dorsal columns in cat. J. Physiol., 321, 31-47.

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

Brown, A.G., House, C.R., Rose, P.K. and Snow, P.J. (1976). The morphology of the spinocervical tract neurons in the cat. J. Physiol., 260, 719-738.

Brown, A.G., Noble, R. and Riddell, J.S. (1986). Relations between spinocervical and post-synaptic dorsal column neurones in the cat. J. Physiol., 381, 333-349.

Brown, A.G., Rose, P.K. and Snow, P.J. (1977). The morphology of hair follicle afferent fibre collaterals in the spinal cord of the cat. J. Physiol., 272, 779-797.

Brown, A.G., Rose, P.K. and Snow, P.J. (1978). Morphology and organization of axon collaterals from afferent fibres of slowly adapting Type I units in cat spinal cord. J. Physiol., 277, 15-27.

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

Burgess, P.R. and Perl, E.R. (1973). Cutaneous mechanoreceptors and nociceptors. In : Handbook of Sensory Physiology, vol. II, A. Iggo (ed.). Springer-verlag, Berlin, pp. 29-78.

Cajal, S. Ramon Y, (1909). Histologie du systeme nerveux de l'homme et des vertebres. Vol.I, Maloine, Paris.

Cannon, J.T., Prieto, G.J., Lee, A. and Liebeskind, J.C. (1982). Evidence for opioid and non-opioid forms of stimulation-produced analgesia in the rat. Brain Res., 243, 315-321.

Carlsson, A., Falck, B., Fuxe, K. and Hillarp, N.A. (1964). Cellular localization of monoamines in the spinal cord. Acta. Physiol. Scand., 60, 112-119.

Carstens, E., Fraunhoffer, M. and Zimmermann, M. (1981a). Serotonergic mediation of descending inhibition from midbrain periaqueductal gray, but not reticular formation, of spinal nociceptive transmission in the cat. Pain, 10, 149-167.

Carstens, E., Gilly, H., Schreiber, H. and Zimmermann, M. (1987). Effects of midbrain stimulation and ionophoretic application of serotonin, noradrenaline, morphine and GABA on electrical thresholds of afferent C- and A-fibre terminals in cat spinal cord. Neurosci., 21, 395-406.

Carstens, E., Klumpp, D., Randic, M. and Zimmermann, M. (1981b). Effect of ionophoretically applied 5-hydroxytryptamine on the excitability of single primary afferent C- and A-fibers in the cat spinal cord. Brain Res., 220, 151-158.

Carstens, E., Klumpp, D. and Zimmermann, M. (1980). Differential inhibitory effects of medial and lateral midbrain stimulation on spinal neuronal discharges to noxious skin heating in the cat. J. Neurophysiol., 43, 332-342.

Carstens, E. and Trevino, D.L. (1978). Laminar origins of spinothalamic projections in the cat as determined by the retrograde transport of horseradish peroxidase. J. Comp. Neurol., 182, 151-166.

Carstens, E., Yokota, T. and Zimmermann, M. (1979). Inhibition of spinal neuronal responses to noxious skin heating by stimulation of mesencephalic periaqueductal gray in the cat. J. Neurophysiol., 42, 558-568.

Casey, K.L. (1971). Somatosensory responses of bulboreticular units in awake cat : relation to escape-producing stimuli. Science, 173, 77-80.

Cavada, C., Huisman, A.M. and Kuypers, H.G.J.M. (1984). Retrograde double labeling of neurons : the combined use of horseradish peroxidase and diamidino yellow dihydrochloride (DY.2HCl) compared with True Blue and DY.2HCl in rat descending brainstem pathways. Brain Res., 308, 123-136.

Cechetto, D.F., Standaert, D.G. and Saper, C.B. (1985). Spinal and trigeminal dorsal horn projections to the parabrachial nucleus in the rat. J. Comp. Neurol., 240, 153-160.

Cervero, F. and Iggo (1980). The substantia gelatinosa of the spinal cord : A critical review. Brain, 103, 717-722.

Cervero, F., Iggo, A. and Molony, V. (1977). resposes of spinocervical tract neurones to noxious stimulation of the

skin. J. Physiol., 267, 537-558.

Cervero, F., Iggo, A. and Ogawa, H. (1976). Nociceptor-driven dorsal horn neurones in the lumbar spinal cord of the cat. Pain, 2, 5-24.

Cervero, F. and Tattersall, J.E.H. (1987). Somatic and visceral inputs to the thoracic spinal cord of the cat : marginal zone (Lamina I) of the dorsal horn. J. Physiol., 388, 383-395.

Chance, W.T., Krynock, G.M. and Rosecrans, J.A. (1978). Effects of medial raphe and raphe magnus lesions on the analgesic activity of morphine and methadone. Psychopharmacol., 56, 133-137.

Chan-Palay, V. (1977). Indoleamine neurons and their processes in the normal rat brain and in chronic diet-induced thiamine deficiency demonstrated by uptake of ^{3}H- serotonin. J. Comp. Neurol., 176, 467-494.

Chan-Palay, V., Jonsson, G. and Palay, S.L. (1978). Serotonin and substance P coexist in neurons of the rat's central nervous system. Proc. Nat. Acad. Sci. USA, 75, 1582-1586.

Chapelo, C.B., Doxey, J.C., Myers, P.L. and Roach, A.G. (1981). RX 781094, a new potent, selective antagonist of o_2-adrenoceptors. Br. J. Pharmacol., 74, 842P

Chapman, C.D., Ammons, W.S. and Foreman, R.D. (1985). Raphe magnus Inhibition of feline T_1-T_4 spinoreticular tract cell responses to visceral and somatic inputs. J. Neurophysiol., 53, 773-785.

Cheng, Z.F., Fields, H.L. and Heinricher, M.M. (1986). Morphine microinjected into the periaqueductal gray has differential effects on 3 classes of medullary neurons. Brain Res., 375, 57-65.

Chiang, C.Y. and Gao, B. (1986). The modification by systemic morphine of the responses of serotonergic and non-serotonergic neurons in nucleus raphe magnus to heating the tail. Pain, 26, 245-257.

Chiang, C.Y. and Pan, Z.Z. (1985). Differential responses of serotonergic and non-serotonergic neurons in nucleus raphe magnus to systemic morphine in rats. Brain Res., 337, 146-150.

Christensen, B.N. and Perl, E.R. (1970). Spinal neurons specifically excited by noxious or thermal stimuli : Marginal zone of the dorsal horn. J. Neurophysiol., 33, 293-307.

Chitour, D., Dickenson, A.H. and Le Bars, D. (1982). Pharmacological evidence for the involvement of serotonergic mechanisms in diffuse noxious inhibitory controls (DNIC). Brain Res., 236, 329-337.

Collins, J.G. and Ren, K. (1987). WDR response profiles of spinal dorsal horn neurons may be unmasked by barbiturate anesthesia. Pain, 28, 369-378.

Cox, B., Lee, T.F. and Martin, D. (1981). Different hypothalamic receptors mediate 5-hydroxytryptamine- and tryptamine-induced core temperature changes in the rat. Br. J. Pharmacol., 72, 477-482.

Craig, A.D. (1976). Spinocervical tract cells in cat and dog labeled by the retrograde transport of horseradish peroxidase. Neurosci. Lett., 3, 173-177.

Craig, A.D. (1978). Spinal and medullary input to the lateral cervical nucleus. J. Comp. Neurol., 181, 729-744.

Craig, A.D. and Burton, H. (1979). The lateral cervical nucleus in the cat : anatomic organization of cervicothalamic neurones. J. Comp. Neurol., 185, 329-346.

Craig, A.D. and Kniffki, K.D. (1985). Spinothalamic lumbrosacral lamina I cells responsive to skin and muscle stimulation in the cat. J. Physiol., 365, 197-221.

Crutcher, K.A. and Humbertson, A.O. (1978). The organization of monoamine neurons within the brainstem of the North American opossum (Didelphis virginiana). J. Comp. Neurol., 179, 195-222.

Cruz, F., Lima, D. and Coimbra, A. (1987). Several morpholgical types of terminal arborizations of primary afferents in laminae I-II of the rat spinal cord, as shown after HRP labeling and Golgi impregnation. J. Comp. Neurol., 261, 221-236.

Dahlstrom, A. and Fuxe, F. (1964). Evidence for the existence of monoaminergic neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta. Physiol. Scand., 62 supp 232.

Dahlstrom, A. and Fuxe, F. (1965). Evidence for the existence of monoaminergic neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. Acta. Physio. Scand., supp 247, 7-36.

D' Amour, F.E. and Smith, D.L. (1941). A method for determining loss of pain sensation. J. Pharmacol. Exp. Therp., 72, 74-79.

Daval, G., Verge, D., Basbaum, A.I., Bourgoin, S. and Hamon, M. (1987). Autoradiographic evidence of serotonin $_1$ binding sites on primary afferent fibres in the dorsal horn of the rat spinal cord. Neurosci. Lett., 83, 71-76.

Deakin, J.F.W. and Dostrovsky, J.O. (1978). Involvement of the periaqueductal grey matter and spinal 5-hydroxytryptaminergic pathways in morphine analgesia : effects of lesions and 5-hydroxytryptamine depletion. Br. J. Pharmacol., 63, 159-165.

Dickenson, A.H., Chitour, D. and Le Bars (1983). Influence of serotoninergic receptor blockade on diffuse noxious inhibitory controls in the rat. Advances in Pain Research and Therapy, vol.5, J.J. Bonica, U. Lindblum and A.Iggo (Eds.) Raven Press, New York, pp 155-160.

Dickenson, A.H. and Goldsmith, G. (1986). Evidence for a role of 5-hydroxytryptamine in the response of rat raphe magnus neurones to peripheral noxious stimuli. Neuropharmacol., 25, 863-868.

Dickenson, A.H., Le Bars, D. and Besson, J.M. (1980). An involvement of nucleus raphe magnus in diffuse noxious inhibitory controls (DNIC) in the rat. Neurosci. Lett., supp.5, S375.

Dickenson, A.H., Oliveras, J.L. and Besson, J.M. (1979). Role of the nucleus raphe magnus in opiate analgesia as studied by the microinjection technique in the rat. Brain Res., 170, 95-111.

Dickenson, A.H., Rivot, J.P., Chaouch, A., Besson, J.M. and Le Bars, D. (1981). Diffuse noxious inhibitory controls (DNIC) in the rat with or without pCPA pretreatment. Brain Res., 216, 313-321.

Doods,H.N., Kalkman, H.O., DeJonge, A., Thoolen,. M.J.M.C., Wilffert, B., Timmermans, P.B.M.W.M. and Van Zweiten, P.A. (1985). Differential selectivity of RU 24969 and 8-OH-DPAT for the reported 5-HT_{1A} and 5-HT_{1B} binding sites. Correlation between 5-HT_{1A} affinity and hypotensive activity. Europ. J. Pharmacol., 112, 363-370.

Dostrovsky, J.O., Hu, J.W., Sessle, B.J. and Sumino, R. (1982). Stimulation sites in periaqueductal gray, nucleus raphe magnus and adjacent regions effective in suppressing oral-facial reflexes. Brain Res., 252, 287-297.

Dubner, R. (1985). specialization in nociceptive pathways : sensory discrimination, sensory modulation, and neuronal connectivity. In Adv. Pain Res. Ther., vol. 9, H.L. Fields, R. Dubner and F.Cervero (eds.). Raven Press, New York. pp.111-137.

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

Dubuisson, D. and Wall, P.D. (1980). Medullary raphe influences on units in laminae 1 and 2 of cat spinal cord. J.Physiol., 300, 33P.

Duggan, A.W. and Griersmith B.T. (1979). Inhibition of the spinal transmission of nociceptive information by supraspinal

stimulation in the cat. Pain, 6, 149-161.

Duggan, A.W., Griersmith, B.T., Headley, P.M. and Maher, J.B. (1978). The need to control skin temperature when using radiant heat in tests of analgesia. Exp. Neurol., 61, 471-478.

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

Eccles, J.C. (1964). The physiology of synapses. Springer, Berlin.

Edeson, R.O. and Ryall, R.W. (1983). Systematic mapping of descending inhibitory control by the medulla of nociceptive spinal neurones in cats. Brain Res., 271, 251-262.

Edwards, E. and Whitaker-Azmitia (1987). Selective B-antagonists are equally and highly potent at 5-HT sites in the rat hippocampus. Neuropharmacol., 26, 93-96.

Eisenhart, S.F., Morrow, T.J. and Casey, K.L. (1983). sensory and motor properties of bulboreticular and raphe neurons in awake and anesthetized cats. Adv. Pain Res. Ther., vol.5, J.J. Bonica, U. Lindblum and A.Iggo (Eds.) Raven Press, New York, pp 161-168.

Fang, F.G., Moreau, J.L. and Fields, H.L. (1987). Dose-dependent antinociceptive action of neurotensin microinjected into the rostroventromedial medulla of the rat. Brain Res., 420, 171-174.

Fardin, V., Oliveras, J.L. and Besson, J.M. (1984). projections from the periaqueductal gray matter to the B3 cellular area (Nucleus raphe magnus and nucleus reticularis paragigantocellularis) as revealed by the retrograde transport of horseradish peroxidase in the rat. J. Comp. Neurol., 223, 483-500.

Fasmer, O.B., Berge, O.G. and Hole, K. (1984). Tonic regulation of nociception by descending monoaminergic pathways. Pain, supp. 2, S326.

Fasmer, O.B., Berge, O.G. and Hole, K. (1985). Changes in nociception after lesions of descending serotonergic pathways induced with 5, 6-dihydroxytryptamine. Different effects in the formalin and tail-flick tests. Neuropharmacol., 24, 729-734.

Fasmer, O.B., Berge, O.G., Post, C. and Hole, K. (1986). Effects of the putative 5-HT_{1A} receptor agonist 8-OH-2-(di-n-propylamino) tetralin on nociceptive sensitivity in mice. Pharmacol. Biochem. Behav., 25, 883-888.

Felten, D.L. and Cummings, J.P. (1979). The raphe nuclei of the rabbit brain stem. J. Comp. Neurol., 187, 199-244.

Ferrington, D.G., Sorkin, L.S. and Willis, W.D. (1987). Responses of spinothalamic tract cells in the superficial dorsal horn of the primate lumbar spinal cord. J. Physiol., 388, 681-703.

Fields, H.L. and Anderson, S.D. (1978). Evidence that raphe-spinal neurons mediate opiate and midbrain stimulation-produced analgesias. Pain, 5, 333-349.

Fields, H.L. and Basbaum, A.I. (1978). Brainstem control of spinal pain-transmision neurons. Ann. Rev. Physiol., 40, 217-248.

Fields, H.L., Basbaum, A.I., Clanton, C.H. and Anderson, S.D. (1977a). Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons. Brain Res., 126, 441-453.

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

Fields, H.L., Wagner, G.M. and Anderson, S.D. (1975). Some properties of spinal neurons projecting to the medial brainstem reticular formation. Exp. Neurol., 47, 118-134.

Fitzgerald, M. and Wall, P.D. (1980). The laminar organization of dorsal horn cells responding to peripheral C fibre stimulation. Exp. Brain Res., 41, 36-44.

Fleetwood-Walker, S.M., Hope, P.J. and Mitchell, R. (1988). Antinociceptive actions of descending dopaminergic tracts on cat and rat dorsal horn somatosensory neurones. J. Physiol., 399, 335-348.

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., Molony, V. and Iggo, A. (1984). An o_2 receptor mediates the selective inhibition by noradrenaline of nociceptive responses of identified dorsal horn neurones. Brain Res., 334, 243-254.

Foong, F.W., Terman, G. and Duggan, A.W. (1985). Methysergide and spinal inhibition from electrical stimulation in the periaqueductal grey. Europ. J. Pharmacol., 116, 239-248.

Foreman, R.D., Schmidt, R.F. and Willis, W.D. (1977). Convergence of muscle and cutaneous input onto primate spinothalamic tract neurons. Brain Res., 124, 555-560.

Fries, W. and Ziegelgansberger, W.A. (1974). A method to discriminate axonal from cell body activity and to analyse "silent cells". Exp, Brain Res., 21, 441-445.

Gaddum, J.H. and Picarelli, Z.P. (1957). Two kinds of tryptamine receptor. Br. J. Pharmacol. Chemother., 12,323-328.

Gallager, D.W. and Pert, A. (1978). Afferents to brain stem nuclei (brain stem raphe, nucleus reticularis pontis

caudalis and nucleus gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. Brain Res., 144, 257-275.

Garattini, S. and Valzelli, L. (1965). Serotonin. Elsevier, Amsterdam.

Gebhart, G.F. (1982). Opiate and non opioid peptide effects on brain stem neurons : relevace to nociception and antinociceptive mechanisms. Pain, 12, 93-140.

Gebhart, G.F., Sandkuhler, J., Thalhammer, J.G. and Zimmermann, M. (1983a). Inhibition of spinal nociceptive information by stimulation in midbrain of the cat is blocked by lidocaine microinjected in nucleus raphe magnus and medullary reticular formation. J. Neurophysiol., 50, 1446-1459.

Gebhart, G.F., Sandkuhler, J., Thalhammer, J.G. and Zimmermann, M. (1983b). Quantitative comparison of inhibition in spinal cord of nociceptive information by stimulation in periaqueductal gray or nucleus raphe magnus of the cat. J. Neurophysiol., 50, 1433-1445.

Gerhart, K.D., Wilcox, T.K., Chung, J.M. and Willis, W.D. (1981). Inhibition of nociceptive and non nociceptive responses of primate spinothalamic cells by stimulation in medial brain stem. J. Neurophysiol., 45, 121-136.

Gerhart, K.D., Yezierski, R.P., Wilcox, T.K. and Willis, W.D. (1984). Inhibition of primate spinothalamic tract neurons by stimulation in periaqueductal gray or adjacent midbrain reticular formation. J. Neurophysiol., 51, 450-466.

Giesler, G.J., Cannon, J.T., Urca, G. and Liebeskind, J.C. (1978). Long ascending projections from substantia gelatinosa Rolandi and the subjacent dorsal horn in the rat. Science, 202, 984-986.

Giesler, G.J., Gerhart, K.D., Yezierski, R.P., Wilcox, T.K. and Willis, W.D. (1981a). Postsynaptic inhibition of primate spinothalamic neurons by stimulation in nucleus raphe magnus. Brain Res., 204, 184-188.

Giesler, G.J. and Liebeskind, J.C. (1976). Inhibition of visceral pain by electrical stimulation of the periaqueductal gray matter, Pain, 2, 43-48.

Giesler, G.J., Menetrey, D. and Basbaum, A.I. (1979). differential origins of spinothalamic tract projections to medial and lateral thalamus in the rat. J. Comp. Neurol., 184, 107-126.

Giesler, G.J., Yezierski, R.P., Gerhart, K.D. and Willis, W.D. (1981b). Spinothalamic tract neurons that project to medial and / or lateral thalamic nuclei : evidence for a

physiologically novel population of spinal cord neurons. J. Neurophysiol., 46, 1285-1308.

Glazer, E.J. and Basbaum, A.I. (1984). Axons which take up [$_3$H] serotonin are presynaptic to enkephalin immunoreactive neurons in cat dorsal horn. Brain Res., 298, 386-391.

Glennon, R.A. (1986). Discriminative stimulus properties of 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Pharmacol. Biochem. Behav., 25, 135-139.

Glennon, R.A., Titeler, M. and Young, R. (1986). Structure-activity relationships and mechanism of action of hallucinogenic agents based on drug discrimination and radioligand binding studies. Psychopharmacol. Bull., 22, 953-958.

Gobel, S. (1975). Golgi studies of the substantia gelatinosa neurons in the spinal trigeminal nucleus. J. Comp. Neurol., 162, 397-416.

Gobel, S., Falls, W.M. and Humphrey, E. (1981). Morphology and synaptic connections of ultrafine primary axons in lamina I of the spinal dorsal horn : candidates for the terminal axon arbors of primary neurons with unmyelinated (C) axons. J. Neurosci.,1, 1163-1179.

Goldscheider, A. (1884). The specific energy of the sensory nerves of the skin. In : Classical German Contributions to Pain Research, H.O. Handwerker and K. Brune (eds.), 1987. Tagblatt-Druckerei KG, pp. 49-67.

Goode, G.E., Humbertson, A.O. and Martin, G.F. (1980). projections from the brain stem reticular formation to lamina I and II of the spinal cord. Studies using light and electron microscopic techniques in the North American opossum. Brain Res., 189, 327-342.

Gozlan,H., El Mestikawy, S., Pichat, L., Glowinski, J. and Hamon, M. (1983). Identification of presynaptic serotonin autoreceptors using a new ligand ^{3}H-PAT. Nature, 305, 140-142.

Gray, B.G. and Dostrovsky, J.O. (1983). Descending inhibitory influences from periaqueductal gray, nucleus raphe magnus and adjacent reticular formation. I. Effects on lumbar spinal cord nociceptive and non-nociceptive neurons. J.Neurophysiol., 49, 932-947.

Gray, B.G. and Dostrovsky, J.O. (1985). Inhibition of feline spinal cord dorsal horn neurons following electrical stimulation of nucleus paragigantocellularis lateralis. A comparison with nucleus raphe magnus. Brain Res., 348, 261-273.

Gregor, M. and Zimmermann, M. (1973). Dorsal root potentials produced by afferent volleys in cutaneous group III fibres. J. Physiol., 232, 413-425.

Griersmith, B.T. and Duggan, A.W. (1980). Prolonged depression of spinal transmission of nociceptive information by 5-HT administered in the substantia gelatinosa : antagonism by methysergide. Brain Res., 187, 231-236.

Gudelsky, G.A., Koenig, J.I.and Meltzer, H.Y. (1986). Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. Evidence for opposing roles of 5-HT_2 and 5-HT_{1A} receptors. Neuropharmacol., 25, 1307-1313.

Guilbaud, G., Oliveras, J.L, Giesler, G. and Besson, J.M (1977). Effects induced by stimulation of the centralis inferior nucleus of the raphe on dorsal horn interneurons in cat's spinal cord. Brain Res., 126, 355-360.

Haber, L.H., Martin, R.F., Chung, J.M. and Willis, W.D. (1980). Inhibition and excitation of primate spinothalamic tract neurons by stimulation in region of nucleus reticularis gigantocellularis. J. Neurophysiol., 43, 1578-1593.

Haber, L.H., Moore, B.D. and Willis, W.D. (1982). Electrophysiological response properties of spinoreticular neurons in the monkey. J. Comp. Neurol., 207, 75-84.

Hadjiconstantinou, M., Panula, P., Lackovic, Z. and Neff, N.H. (1984) . Spinal cord serotonin : a biochemical and immunohistochemical study following transection. Brain Res., 322, 245-254.

Hall, J.G., Duggan. A.W., Johnson, S.M. and Morton, C.R. (1981). Medullary raphe lesions do not reduce desceding inhibition of dorsal horn neurones of the cat. Neurosci. lett., 25, 25-29.

Hall, J.G., Duggan, A.W., Morton, C.R. and Johnson, S,M (1982). The location of brainstem neurones tonically inhibiting dorsal horn neurones of the cat. Brain Res., 244, 215-222.

Hall, M.D., El Mestikawy, S., Emerit. M.B., Pichat, L., Hamon, M. and Gozlan, H. (1985). [^{3}H]8-hydroxy-2-(di-n-propylamino) tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. J. Neurochem., 44, 1685-1696.

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

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

Hamon, M., Cossery, J.M., Spampinato, U. and Gozlan, H. (1986). Are there selective ligands for 5-HT_{1A} and 5-HT_{1B} receptor binding sites in brain ? Trends. Pharmacol. Sci., 7, 336-338.

Hancock, M.B., Foreman, R.D. and Willis, W.D. (1975). Convergence of visceral and cutaneous input onto spinothalamic tract cells in the thoracic spinal cord of the cat. Exp. Neurol., 47, 240-248.

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

Harkness, D.H. and Brownfield, M.S. (1986). A thyrotropin-releasing hormone-containing system in the rat dorsal horn seperate from serotonin. Brain Res., 384, 323-333.

Hartzell, H.C. (1981). Mechanisms of slow postsynaptic potentials. Nature, 291, 539-544.

Hayes, R.L., Bennett, G.J., Newlon, P.G. and Mayer, D.J. (1978). behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain enviromental stimuli. Brain Res., 155, 69-90.

Hayes, R.L., Newlon, P.G., Rosecrans, J.A. and Mayer, D.J. (1977). Reduction of stimulation-produced analgesia by lysergic acid dimethylamide, a depressor of serotonergic neuronal activity. Brain Res., 122,367-372.

Hayes, R.L., Price, D.D, Ruda, M.A. and Dubner, R. (1979). Suppression of nociceptive responses in the primate by electrical stimulation of the brain or morphine administration : behavioral and electrophysiological comparisons. Brain Res., 167, 417-421.

Headley, P.M., Duggan, A.W. and Griersmith, B.T. (1978). Selective reduction by noradrenaline and 5-hydroxytryptamineof nociceptive responses of cat dorsal horn neurones. Brain Res., 145, 185-189.

Heavner, J. (1975). Jamming spinal sensory input : effects of anesthetic and analgesic drugs in the spinal cord dorsal horn. Pain, 1, 239-255.

Helke, C.J., Neil, J.J., Massari, V.J. and Loewy, A.D. (1982). Substance P neurons project from the ventral medulla to the intermediolateral cell column and ventral horn in the rat. Brain Res., 243, 147-152.

Helke, C,J., Sayson, S.C., Keeler, J.R. and Charlton, C.G. (1986). Thyrotropin-releasing hormone-immunoreactive neurons project from the ventral medulla to the intermediolateral cell column : partial coexistence with serotonin. Brain Res., 381, 1-7.

Hensel, H. (1973). Cutaneous thermoreceptors. In Handbook of Sensory Physiology, Vol.II. A.Iggo (Ed.), Springer,

Berlin, pp. 75-106.

Hensel, H. (1974). Thermoreceptors. Ann. Rev. Physiol., 36, 233-250.

Hensel, H. and Iggo, A. (1971). Analysis of cutaneous warm and cold fibres in primates. Pflugers Arch. ges. Physiol., 329, 1-8.

Heuring, R.E. and Peroutka, S.J. (1987). Characterization of a novel ^{3}H-5-hydroxytryptamine binding site subtype in bovine brain membranes. J. Neurosci., 7, 894-903.

Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D., Wikstrom, H., Arvidsson, L.E., Hacksell, U. and Nilsson, J.L.G. (1982). 8-hydroxy-2-(di-n-propylamino)tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT receptor stimulating activity. J. Neural. Transm., 55, 169-188.

Hoffert, M.J., Miletic, V., Ruda, M.A. and Dubner, R. (1983). Immunocytochemical identification of serotonin axonal contacts on characterized neurons in laminae I and II of the dorsal horn. Brain Res., 267, 361-364.

Hokfelt, T., Fuxe, K., Johansson, O., Jeffcoate, S. and White, N. (1975). Thyrotropin releasing hormone (TRH)-containing nerve terminals in certain brainstem nuclei and in the spinal cord. Neurosci. lett., 1,133-139.

Hokfelt, T., Ljungdahl, A., Steinbusch, H., Verhofstad, A., Nilsson, G., Brodin, E., Pernow, B. and Goldstein, M. (1978). Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine containing neurons in the rat central nervous system. Neurosci., 3, 517-538.

Holets, V.R., Hokfelt, T., Ude, J., Eckert, M., Penzlin, H., Verhofstad, A.A.J. and Visser, T.J. (1987). A comparative study of the immunohistochemical localization of a presumptive proctolin-like peptide, thyrotropin-releasing hormone and 5-hydroxytryptamine in the rat central nervous system. Brain Res., 408, 141-153.

Holstege, G. and Kuypers, H.G.J.M. (1982). The anatomy of brain stem pathways to the spinal cord in cat. A labeled amino acid tracing study. Prog. Brain Res., 57, 145-175.

Holziv, G.G. and Anderson, E.G. (1984). The actions of serotonin on frog primary afferent terminals and cell bodies. Comp. Biochem. Physiol., 77C, 13-21.

Holziv, G.G., Shefner, S.A. and Anderson, E.G. (1985). Serotonin depolarizes type A and C primary afferents : an intracellular study in bullfrog dorsal root ganglion. Brain Res., 327, 71-79.

Hongo, T., Jankowska, E. and Lundberg, A. (1968). Post-synaptic

excitation and inhbition from primary afferents in neurones of the spinocevical tract. J. Physiol., 199, 569-592.

Howe, P.R.C., Moon, E. and Dampney, R.A.L. (1983). Distribution of serotonin nerve cells in the rabbit brainsstem. Neurosci. lett., 38, 125-130.

Hoyer, D., Engel, G. and Kalkman, H.O. (1985a). Characterization of the 5-HT_{1B} recognition site in rat brain : binding studies with (-) [^{125}I] iodocyanopindolol. Europ. J. Pharmacol., 118, 1-12.

Hoyer, D., Engel, G. and Kalkman, H.O. (1985b). Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes : radioligand binding studies with [^{3}H] 5-HT, [^{3}H] 8-OH-DPAT, (-) [^{125}I] iodocyanopindolol, [^{3}H] mesulergine and [^{3}H] ketanserin. Europ. J. Pharmacol., 118, 13-23.

Huang, J.C. and Peroutka, S.J. (1987). Identification of 5-hydroxytryptamine$_1$ binding site subtypes in rat spinal cord. Brain Res., 436, 173-176.

Huisman, A.M., Kuypers, H.G.J.M. and Verburgh, C.A. (1981). Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique. Brain Res., 209, 271-286.

Huisman, A.M., Kuypers, H.G.J.M. and Verburgh, C.A. (1982). Differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in cat and monkey. Prog. Brain Res., 57, 185-217.

Humphrey, P.P.A. (1984). Peripheral 5-hydroxytryptamine receptors and their classification. Neuropharmacol., 23, 12B, 1503-1510.

Hylden, J.L.K., Hayashi, H., Bennett, G.J. and Dubner, R. (1985a). Spinal lamina I neurons projecting to the parabrachial area of the cat midbrain. Brain Res., 336, 195-198.

Hylden, J.L.K., Ruda, M.A., Hayashi, H. and Dubner, R. (1985b). Descending serotonergic fibers in the dorsolateral and ventral funiculi of cat spinal cord. Neurosci. lett., 62, 299-304.

Hylden, J.L.K., Hayashi, H. and Bennett, G.J. (1986a). Lamina I spiomesencephalic neurons in the cat ascend via the dorsolateral funiculi. Somatosens. Res., 4, 31-41.

Hylden, J.L.K., Hayashi, H., Dubner, R. and Bennett, G.J. (1986b). Physiology and morphology of the lamina I spinomesencephalic projection. J. Comp. Neurol., 247, 505-515.

Hylden, J.L.K., Hayashi, H., Ruda, M.A. and Dubner, R. (1986c).

Serotonin innervation of physiologically identified lamina I projection neurons. Brain Res., 370, 401-404.

Iggo, A. (1959). Cutaneous heat and cold receptors with slowly conducting (C) afferent fibres. Q. J. Exp. Physiol., 44, 362-370.

Iggo, A. (1960). Cutaneous mechanoreceptors with afferent C fibres. J. Physiol., 152, 337-353.

Iggo, A. (1966). Cutaneous receptors with a high sensitivity to mechanical displacement. In: Touch, Heat and Pain., de Reuk E.V.S and Knight, J. (Eds.), Churchill, London, pp. 237-256.

Iggo, A. (1974a). Cutaneous receptors. In: The Peripheral Nervous System, Hubbard, J.I. (Ed.), Plenum Press, New York, pp. 347-404.

Iggo, A. (1974b). Activation of cutaneous nociceptors and their actions on dorsal horn neurons. In : Advances in Neurology, vol. 4, J.J. Bonica (ed.). Raven Press, New York. pp. 1-9

Jacobowitz, D.M. and MacLean, P.D. (1978). A brainstem atlas of catecholaminergic neurons and serotonergic perikerya in a pygmy primate (Cebuella pygmaea). J. Comp. Neurol., 177, 397-416.

Janss, A.J. and Gebhart, G.F. (1987). Spinal monoaminergic receptors mediate the antinociception produced by glutamate in the medullary lateral reticular nucleus. J. Neurosci., 7, 2862-2873.

Jeftinija, S., Semba, K. and Randic, M. (1981). Norepinephrine reduces excitability of single cutaneous primary afferent C-fibers in the cat spinal cord. Brain Res., 219, 456-463.

Jensen, T.S. and Yaksh, T.L (1984a). Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. Brain Res., 321, 287-297.

Jensen, T.S. and Yaksh, T.L. (1984b). Effects of an intrathecal dopamine agonist, apomorphine, on thermal and chemical evoked noxious responses in rats. Brain Res., 296, 285-293.

Jeftinija, S., Raspantini, C., Randic, M., Yaksh, T.L., Go, V.L.W. and Larson, A.A. (1986). Altered responsiveness to substance P snd 5-hydroxytryptamine in cat dorsal horn neurons after 5-HT depletion with p-Chlorophenylalanine. Brain Res., 368, 107-115.

Jensen, T.S. and Yaksh, T.L (1986a). I. Comparison of antinociceptive action of morphine in the periaqueductal gray, medial and paramedial medulla in rat. Brain Res., 363, 99-113.

Jensen, T.S. and Yaksh, T.L. (1986b). II. Examination of spinal monoamine receptors through which brainstem opiate-sensitive

systems act in the rat. Brain Res., 363, 114-127.

Johansson, F. and Von Knorring, L. (1979). A double-blind controlled study of a serotonin uptake inhibitor (zimelidine) versus placebo in chronic pain patients. Pain, 7, 69-78.

Johannessen, J.N., Watkins, L.R., Carlton, S.M. and Mayer, D.J. (1982). Failure of spinal cord serotonin depletion to alter analgesia elicited from the periaqueductal gray. Brain Res., 237, 373-386.

Johansson, O., Hokfelt,T., Pernow, B., Jeffcoate, S.L., White, N., Steinbusch, H.W.M., Verhofstad, A.A.J., Emson, P.C. and Spindol, E. (1981). Immunohistochemical support for three putative transmitters in one neuron : coexistence of 5-hydroxytryptamine-, substance P- and thyrotropin-releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neurosci., 6, 1857-1881.

Jordan, L.M., Kenshalo, D.R., Martin, R.F., Haber, L.H. and Willis, W.D. (1978). Depression of primate spinothalamic tract neurons by iontophoretic application of 5-hydroxytryptamine. Pain, 5, 135-142.

Jordan, L.M., Kenshalo, D.R., Martin, R.F., Haber, L.H. and Willis, W.D. (1979). Two populations of spinothalamic tract neurons with opposite responses to 5-hydroxytryptamine. Brain Res., 164, 342-346.

Jordan, L.M. and Mc Crea, D.A. (1976). Analysis of the effects of p-methoxyphenylethylamine on spinal cord neurons. Brit. J. Pharmacol., 57, 191-199.

Kajander, K.C., Ebner, T.J. and Bloedel, J.R. (1984). Effects of periaqueductal gray and raphe magnus stimulation on the responses of spinocervical and other ascending projection neurons to non-noxious inputs. Brain Res., 291, 29-37.

Kaulen, P., Bruning, G., Rommelspacher, H. and Baumgarten, H.G. (1986). Characterization and quantitative autoradiography of [^{3}H] tryptamine binding sites in rat brain. Brain Res., 366, 72-88.

Kerr, F.W.L. (1975). The vental spinothalamic tract and other ascending systems of the ventral funiculus of the spinal cord. J. Comp. Neurol., 159, 335-356.

Kevetter, G.A., Haber, L.H., Yezierski, R.P., Chung, J.M., Martin, R.F. and Willis, W.D. (1982). Cells of origin of the spinoreticular tract in the monkey. J. Comp. Neurol., 207, 61-74.

Kevetter, G.A. and Willis, W.D. (1982). Spinothalamic cells in the rat lumbar cord with collaterals to the medullary reticular formation. Brain Res., 238, 181-185.

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

Kevetter, G.A. and Willis, W.D. (1984). Collateralization in the spinothalamic tract : new methodology to support or deny phylogenetic theories. Brain Res. Rev., 7, 1-14.

Kilpatrick, G.J., Jones, B.J. and Tyres, M.B. (1987). Identification and distribution of 5-HT3 receptros in rat brain using radioligand binding. Nature, 330, 746-748.

Kitahata, L.M., Ghazi-Saidi, K., Yamashita, M., Kosaka, Y., Bonikos, C. and Taub, A. (1975). The depressant effect of halothane and sodium thiopental on the spontaneous and evoked activity of dorsal horn cells : lamina specificity, time course and dose dependence. J. Pharmacol. Exp. Therp., 195, 515-521.

Kojima, M., Takeuchi, Y., Goto, M. and Sano, Y. (1983). Immunohistochemical study on the localization of serotonin fibers and terminls in the spinal cord of the monkey (Macaca fuscata). Cell. Tissue res., 229, 23-36.

Kolmodin, G.M. and Skoglund, C.R. (1960). Analysis of spinal interneurons activated by tactile and nociceptive stimulation. Acta Physiol. Scand., 50, 337-355.

Kraus, E., Besson, J.M. and Le Bars, D. (1982). Behavioral model for diffuse noxious inhibitory controls (DNIC) : potentiation by 5-hydroxytryptamine. Brain Res., 231, 461-465.

Kumazawa, T. and Perl, E.R. (1976). Differential excitation of dorsal horn and substantia gelatinosa marginal neurons by primary afferent units with fine (A and C) fibers. In: Sensory Function of the Skin of Primates, Zotterman,Y.Z.(Ed.), Perganon Press, Oxford, pp 66-89.

Kumazawa, T. and Perl, E.R. (1977). Primate cutaneous sensory units with unmyelinated (C) afferent fibers. J. Neurophysiol., 40, 1325-1338.

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

Kumazawa, T., Perl, E.R., Burgess, P.R. and Whitehorn, D. (1975). Ascending projections from marginal zone (lamina I) neurons of the spinal dorsal horn. J. Comp. Neurol., 162, 1-12.

Kuraishi, Y., Harada, Y., Satoh, M. and Takagi, H. (1979). Antagonism by phenoxybenzamine of the analgesic effect of morphine injected into the nucleus reticularis gigantocellularis of the rat. Neuropharmacol., 18, 107-110.

Kuypers, H.G.J.M. and Maisky, V.A. (1975). Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. Neurosci. lett., 1, 9-14.

La Motte, C. (1977). Distribution of the tract of Lissauer and the dorsal root fibers in the primate spinal cord. J. Comp. Neurol., 172, 529-562.

LaMotte, C.C., Johns, D.R. and deLanerolle,N.C. (1982). Immunohistochemical evidence of indoleamine neurons in monkey spinal cord. J. Comp. Neurol., 206, 359-370.

LaMotte, C.C. and deLanerolle, N. (1983). Substance P, enkephalin, and serotonin : ultrastructural basis of pain transmission in primate spinal cord. In Adv. Pain Res.Ther., Vol.5, J. Bonica, U. Lindblum and A. Iggo (Eds.), Raven Press, New York, pp.247-255.

Larson, A.A. (1983). Hyperalgesia produced by the intrathecal administration of tryptamine to rats. Brain Res., 265, 109-117.

Le Bars, D. (1988). Serotonin and Pain. In Neuronal Serotonin, N.N.Osborne and M.Hamon (Eds.), John Wiley and Sons, Chichester, pp.171-229.

Le Bars, D., Calvino, B., Villanueva, L. and Cadden, S. (1984). Physiological approaches to counter-irritation phenomena. In Stress Induced Analgesia, M.D. Tricklebank and G. Curzon (Eds.), John Wiley and Sons, Chichester, pp 67-101.

Le Bars, D. and Chitour, D. (1983). Do convergent neurones in the spinal dorsal horn discriminate nociceptive from non-nociceptive information? Pain, 17, 1-19.

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

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

Le Bars, D., Dickenson, A.H. and Besson, J.M (1983). Opiate analgesia and descending control systems. Adv. Pain Res. Ther., vol.5, J.J. Bonica, U. Lindblum and A.Iggo (Eds.) Raven Press, New York, pp 341-372.

Leger, L., Charnay, Y., Dubois, P.M. and Jouvet, M. (1986). Distribution of enkephalin-immunoreactive cell bodies in relation to serotonin-containing neurons in the raphe nuclei of the cat : immunocytochemical evidence for the coexistence of enkephalin and serotonin in certain cells. Brain Res., 362, 63-73.

Leichnetz, G.R., Watkins, L., Griffin, G., Murfin, R. and Mayer, D.J. (1978). The projection from nucleus raphe magnus and other brainstem nuclei to the spinal cord in the rat : a study using the HRP blue-reaction. Neurosci. lett., 8, 119-124.

Lewis, J.W., Terman, G.W., Nelson, L.R. and Liebeskind, J.C. (1984). Opioid and non-opioid stress analgesia. In Stress Induced Analgesia, M.D. Tricklebank and G. Curzon (Eds.), John Wiley and Sons, Chichester, pp 103-133.

Lewis, J.W., Terman, G.W., Watkins, L.R., Mayer, D.J. and Liebeskind, J.C. (1983). Opioid and non-opioid mechanisms of footshock-induced analgesia : role of the spinal dorsolateral funiculus. Brain Res., 267, 139-144.

Leysen, J.E., De Chaffoy De Courcelles, D., De Clerck, F., Niemegeers, C.J.E. and Van Nueten, J.M. (1984). Serotonin-S_2 receptor binding sites and functional correlates. Neuropharmacol., 23, 12B, 1493-1501.

Leysen, J.E., Niemegeers, C.J.E., Van Nueten, J.M. and Laduron, P.M. (1982). [^{3}H] ketanserin (R41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Binding properties, brain distribution, and functional role. Mol. Pharmacol., 21, 301-314.

Liebeskind, J.C., Guilbaud, G., Besson, J.M. and Oliveras, J.L. (1973). Analgesia from electrical stimulation of the periaqueductal gray matter in the cat : behavioral observations and inhibitory effects on spinal cord interneurons. Brain Res., 50, 441-446.

Light, A.R. (1985). The spinal terminations of single, physiologically characterized axons originting in the pontomedullary raphe of the cat. J. Comp. Neurol., 234, 536-548.

Light, A.R., Casale, E.J. and Menetrey, D.M. (1986). The effects of focal stimulation in nucleus raphe magnus and periaqueductal gray on intracellularly recorded neurons in spinal laminae I and II. J. Neurophysiol., 56, 555-571.

Light, A.R. and Kavookjian, A.M. (1985). The ultrastructure and synaptic connections of the spinal terminations from single, physiologically characterized axons descending in the dorsolateral funiculus from the midline, pontomedullary region. J. Comp. Neurol., 234, 549-560.

Light, A.R., Kavookjian, A.M. and Petrusz, P. (1983). The ultrastructural and synaptic connections of serotonin immunoreactive terminals in spinal laminae I and II. Somatosens. res., 1, 33-50.

Light A.R. and Perl, E.R. (1977). differential termination of large and small-diameter primary afferent fibers in the spinal dorsal gray matter as indicated by labeling with

horseradish peroxidase. Neurosci. lett., 6, 59-63.

Light, A.R. and Perl, E.R. (1979a). Reexamination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibres. J. Comp. Neurol., 186, 117-132.

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

Lima, D. and Coimbra, A. (1983). The neuronal population of the marginal zone (laminaI) of the rat spinal cord. A study based on reconstruction of serially sectioned cells. Anat. Embryol. Berl., 167, 273-288.

Lin, M.T., Wu, J.J. and Tsay, B.L. (1983). Serotonergic mechanisms in the hypothalamus mediate thermoregulatory responses in rats. Naunyn-Schmiedeberg's Arch. Pharmacol., 322, 271-278.

Lipski, J. (1981). Antidromic activation of neurones as an analytical tool in the study of the central nervous system. J. Neurosci. Meth., 4, 1-32.

Liu, R.P.C. (1986). Spinal neuronal collaterals to the intralaminar thalamic nuclei and periaqueductal gray. Brain Res., 365, 145-150.

Liu, X., Zhu, B. and Zhang, S.X. (1986). relationship between electroacupuncture analgesia and descending pain inhibtory mechanism of nucleus raphe magnus. Pain, 24, 383-396.

Llewelyn, M.B., Azami, J. and Roberts, M.H.T. (1983). Effects of 5-hydroxytryptamine applied into nucleus raphe magnus on nociceptive thresholds and neuronal firing rate. Brain Res., 258, 59-68.

Lovick, T.A. (1981). Primary afferent depolarization of tooth pulp afferents by stimulation in nucleus raphe magnus and the adjacent reticular formation in the cat : effects of bicuculline. Neurosci. lett., 25, 173-178.

Lovick, T.A., West, D.C. and Wolstencroft, J.H. (1978). Responses of raphespinal and other bulbar raphe neurones to stimulation of the periaqueductal gray in the cat. Neurosci. lett., 8, 45-49.

Mac Iver, M.B. and Roth, S.H. (1987). Anestheics produce differntial actions on the discharge activity of a single neuron. Europ. J. Pharmacol., 139, 43-52.

Madden, J., Akil, H., Patrick, R.L. and Barchas, J.D. (1977). Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat. Nature, 265, 358-360.

Mantyh, P.W. and Peschanski, M. (1982). Spinal projections from the periaqueductal grey and dorsal raphe in the rat, cat and monkey. Neurosci., 7, 2769-2776.

Martin, G.F., Cabana, R., Humbertson, A.O., Laxson, L.C. and Panneton, W.M. (1981). Spinal projections from the medullary reticular formation of the North American opossum : evidence for connectional heterogeniety. J. Comp. Neurol., 196, 663-682.

Martin, R.F., Haber, L.H.and Willis, W.D. (1979). Primary afferent depolarization of identified cutaneous fibers following stimulation in medial brianstem. J. Neurophysiol., 42, 779-790.

Maunz, R.A., Pitts, N.G. and Peterson, B.W. (1978). Cat spinoreticular neurons : locations, responses and changes in responses during repetitive stimulation. Brain Res., 148, 365-379.

Maxwell, D.J., Leranth, Cs. and Verhofstad, A.A.J. (1983). Fine structure of serotonin-containing axons in the marginal zone of the rat spinal cord. Brain Res., 266, 253-259.

Mayer, D.J. and Hayes, R.L. (1975). Stimulation-produced analgesia : development of tolerance and cross-tolerance to morphine. Science, 188, 941-943.

Mayer, D.J. and Liebeskind, J.C. (1974). Pain reduction by focal electrical stimulation of the brain : an anatomical and behavioral analysis. Brain Res., 68, 73-93.

Mayer, D.J. and Price, D.D. (1976). Central nervous system mechanisms of analgesia. Pain, 2, 379-404.

Mayer, D.J., Wolfe, T.L., Akil, H., Carder, B. and Liebeskind, J.C. (1971). analgesia from electrical stimulation in the brainstem of the rat. Science, 174, 1351-1354.

Mc Creery, D.B., Bloedel, J.R. and Hames, E.G. (1979). Effects of stimulating in raphe nuclei and in reticular formation on response of spinothalamic neurons to mechanical stimuli. J. Neurophysiol., 42, 166-182.

Mc Mahon, S.B. and Wall, P.D. (1983). A system of rat spinal cord lamina I cells projecting through the contralateral dorsolateral funiculus. J. Comp. Neurol., 214, 217-223.

Mc Mahon, S.B. and Wall, P.D. (1985). Electrophysiological mapping of brainstem projections of spinal cord lamina I cells in the rat. Brain Res., 333, 19-26.

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

Mendell, L.M. (1966). Physiological properties of unmyelinated

fibre projection to the spinal cord. Exp. Neurol., 16, 316-332.

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

Menetrey, D., Chaouch, A., Binder, D. and Besson, J.M. (1982). The origin of the spinomesencephalic tract in the rat : an anatomical study using the retrograde transport of horseradish peroxidase. J. Comp. Neurol., 206, 193-207.

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

Messing, R.B. and Lytle, L.D. (1977). Serotonin-containing neurons : their possible role in pain and analgesia. Pain, 4, 1-21.

Middlemiss, D.N. (1985). The putative 5-HT_1 receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. J. Pharm. Pharmacol., 37, 434-437.

Middlemiss, D.N. and Fozard, J.R. (1983). 8-hydroxy-2-(di-n-propylamino)tetralin discriminates between sub types of 5-HT_1 recognition site. Europ. J. Pharmacol., 90, 151-153.

Miletic, V., Hoffert, M.J., Ruda, M.A., Dubner, R. and Shigenaga, Y. (1984). Serotoninergic axonal contacts on identified cat spinal dorsal horn neurons and their correlation with nucleus raphe magnus stimulation. J. Comp. Neurol., 228, 129-141.

Millhorn, D.E., Hokfelt, T., Seroogy, K., Oertel, W., Verhofstad, A.A.J. and Wu, J.Y. (1987a). Immunohistochemical evidence for colocalization of Y-aminobutyric acid and serotonin in neurons of the ventral medulla oblangata projecting to the spinal cord. Brain Res., 410, 179-185.

Millhorn, D.E., Seroogy, K., Hokfelt, T., Schmued, L.C., Terenius, L., Buchan, A. and Brown, J.C. (1987b). Neurons of the ventral medulla oblangata that contain both somatostatin and enkephalin immunoreactivities project to nucleus tractus solitari and spinal cord. Brain Res., 424, 99-108.

Minor, B.G., Post, C. and Archer, T. (1985). Blockade of intrathecal 5-hydroxytryptamine-induced antinociception in rats by noradrenaline depletion. Neurosci. lett., 54, 39-44.

Mitchell, R. and Riley, S. (1985). Characterization of receptors for 5-hydroxytryptamine in spinal cord. Biochem. Soc. Trans., 13, 995.

Mohrland, J.S., Mc Manus, D.Q. and Gebhart, G.F. (1982). Lesions in nucleus reticularis gigantocellularis : effect on the antinociception produced by microinjection of morphine and focal electrical stimulation in the periaqueductal gray matter. Brain Res., 231, 143-152.

Mokha, S.S., Mc Millan, J.A. and Iggo, A. (1981). Descending influences on spinal nociceptive neurons from locus coeruleus. Pain, supp. 1, S103.

Monroe, P.J. and Smith, D.J. (1983). Characterization of multiple [^{3}H] 5-hydroxytryptamine binding sites in rat spinal cord tissue. J. Neurochem., 41, 349-355.

Morton, C,R., Duggan, A.W. and Zhao, Z.Q. (1984). The effects of lesions of medullary midline and lateral reticular areas on inhibition in the dorsal horn produced by periaqueductal grey stimulation in the cat. Brain Res., 301, 121-130.

Morton, C.R., Maisch, B. and Zimmermann, M. (1987). Diffuse noxious inhibitory controls of lumbar spinal neurons involve a supraspinal loop in the cat. Brain Res., 410, 347-352.

Muller, (1842). Elements of Physiology, Vol. 2, Taylor and Walton, London.

Nathan, P.W. and Wall, P.D. (1974). Treatment of post-herpetic neuralgia by prolonged electric stimulation. Br. Med. J., 3, 645-647.

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

Neale, R.F., Fallon, S.L., Boyar, W.C., Wasley, J.W.F., Martin, L.L., Stone, G.A., Glaeser, B.S., Sinton, C.M. and Williams, M. (1987). Biochemical and pharmacological characterization of CGS 12066B, a selective serotonin-1B agonist. Europ. J. Pharmacol., 136, 1-10.

Newton, B.W., Maley, B.E. and Hamill, R.W. (1986). Immunohistochemical demonstration of serotonin neurons in autonomic regions of the rat spinal cord. Brain Res., 376, 155-165.

Nishikawa, N., Bennett, G.J., Ruda, M.A., Lu, G.W. and Dubner,R. (1983). Immunocytochemical evidence for a serotoninergic innervation of dorsal column postsynaptic neurons in cat and monkey : light- and electron-microscopic observations. Neurosci., 10, 1333-1340.

Noble, R. and Riddell, J.S. (1988). Cutaneous excitatory and inhibitory input to neurones of the postsynaptic dorsal column system in the cat. J. Physiol., 396, 497-513.

Oleson, J.D., Twombly, D.A. and Liebeskind, J.C. (1978). Effects of pain-attenuating brain stimualtion and morphine on electrical activity in the raphe nuclei of the awake rat. Pain, 4, 211-230.

Oliveras, J.L., Besson, J.M., Guilbaud, G. and Liebeskind, J.C. (1974). Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. Exp. Brain Res., 20, 32-44.

Oliveras, J.L., Bourgoin, S., Hery, F., Besson, J.M. and Hamon, M. (1977). the topographical distribution of serotonergic terminals in the spinal cord of the cat : biochemical mapping by the combined use of microdissection and microassay procedures. Brain Res., 138, 393-406.

Oliveras, J.L., Guilbaud, G. and Besson, J.M. (1979). A map of serotoninergic structures involved in stimulation produced analgesia in unrestrained freely moving cats. Brain Res., 164, 317-322.

Oliveras, J.L., Hosobuchi, Y., Guilbaud, G. and Besson, J.M. (1978). Analgesic electrical stimulation of the feline nucleus raphe magnus : development of tolerance and its reversal by 5-HTP. Brain Res., 146, 404-409.

Oliveras, J.L., Redjemi, F., Guilbaud, G. and Besson, J.M. (1975). Analgesia induced by electrical stimulation of the inferior centralis of the raphe in the cat. Pain, 1, 139-145.

Oliveras, J.L., Woda, A., Guilbaud, G. and Besson, J.M. (1974). Inhibition of the jaw opening reflex by electrical stimulation of the periaqueductal gray matter in the awake, unrestrained cat. Brain Res., 72, 328-331.

Parent, A., Poitras, D. and Dube, L. (1984). Comparative anatomy of central monoaminergic systems. In Handbook of Chemical Neuroanatomy, vol.2, A.Bjorklund and T.Hokfelt (Eds.), Elseiver, Amsterdam, pp.409-439.

Paxinos, G. and Watson, C. (1986). The rat brain atlas. Academic Press, New York.

Pazos, A., Cortes, R. and Palacios, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2-receptors. Brain Res., 346, 231-249.

Pazos, A., Hoyer, D. and Palacios, J. (1984). The binding of serotonergic ligands to the porcine choroid plexus : characterization of a new type of serotonin recognition site. Europ. J. Pharmacol., 106, 539-546.

Pazos, A. and Palacios, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1-receptors, Brain Res., 346, 205-230.

Pechura, C.M. and Liu, R.P.C. (1986). Spinal neurons which project to the periaqueductal gray and the medullary reticular formation via axon collaterals : a double-label fluorescence study in the rat. Brain Res., 374, 357-361.

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. (1981). Discrimination of multiple [^{3}H] 5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem., 36, 220-226.

Pelletier, G., Steinbusch, H.W.M. and Verhofstad, A.A.J. (1981). Immunoreactive substancce P and serotonin present in the same dense-core vesicles. Nature, 293, 71-72.

Perl, E.R. (1968). Myelinated afferent fibres innervating the primate skin and their response to noxious stimuli. J. Physiol., 197, 593-615.

Perl, E.R. (1984). Why are selectively responsive and multireceptive neurons both present in somatosensory pathways? In : Somatosensory Mechanisms, , C. Von Euler, O. Franzen, U. Lindblom, and D. Ottoson (Eds.). The MacMillan Press, London. pp. 141-161

Peroutka, S.J. (1986). Pharmacological differentiation and characterization of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites in rat frontal cortex. J. Neurochem., 47, 529-540.

Peroutka, S.J. (1988). 5-hydroxytryptamine receptor subtypes. Ann. Rev. Neurosci., 11, 45-60.

Peroutka, S.J., Lebovitz, R.M. and Snyder, S.H. (1981). Two distinct central serotonin receptors with different physiological functions. Science, 212, 827-829.

Peroutka, S.J. and Snyder, S.H. (1979). Multiple serotonin receptors : differential binding of [^{3}H] 5-hydroxytryptamine, [^{3}H] lysergic acid diethylamide and [^{3}H] spiroperidol. Molec. Pharmacol., 16, 687-699.

Peroutka, S.J. and Snyder, S.H. (1981). Two distinct serotonin receptors : regional variations in receptor binding in mammalian brain. Brain Res., 208, 339-347.

Peschanski, M. and Besson, J.M. (1984). A spino-reticulo-thalmic pathway in the rat : an anatomical study with reference to pain transmission. Neurosci., 12, 165-178.

Poitras, D. and Parent, A. (1978). Atlas of the distribution of monoamine-containing nerve cell bodies in the brain stem of the cat. J. Comp. Neurol., 179, 699-718.

Pomeranz, B., Wall, P.D. and Weber, W.V. (1968). Cord cells responding to fine myelinated afferents from viscera, muscle and skin. J. Physiol., 199, 511-532.

Pomperoy, S.L. and Behbehani, M.M. (1979). Physiologic evidence for a projection from periaqueductal gray to nucleus raphe magnus in the rat. Brain Res., 176, 143-147.

Price, D.D, and Dubner, R. (1977). Neurons that subserve the sensory-discriminative aspects of pain. Pain, 3, 307-338.

Price, D.D. and Mayer, D.J. (1975). Neurophysiological characterization of the anterolateral quadrant neurons subserving pain in M.Mulatta. Pain, 1, 59-72.

Prieto, G.J., Cannon, J.T. and Liebeskind, J.C. (1983). N. raphe magnus lesions disrupt stimulation-produced analgesia from ventral but not dorsal midbrain areas in the rat. Brain Res., 261, 53-57.

Proudfit, H.K. (1980). Reversible inactivation of raphe magnus neurons : effects on nociceptive threshold and morphine-induced analgesia. Brain Res., 201, 459-464.

Proudfit, H.K. and Anderson, E.G. (1974). New long lasting bulbospinal evoked potentials blocked by serotonin antagonists. Brain Res., 65, 542-546.

Proudfit, H.K. and Anderson, E.G. (1975). Morphine analgesia : blockade by raphe magnus lesions. Brain Res., 98, 612-618.

Proudfit, H.K. and Hammond, D.L. (1981). Alterations in nociceptive threshold and morphine-induced analgesia produced by intrathecally administered amine antagonists. Brain Res., 218, 393-399.

Proudfit, H.K., Larson, A.A. and Anderson, E.G. (1980). Thr role of GABA and serotonin in the mediation of raphe-evoked spinal cord dorsal root potentials. Brain Res., 195, 149-165.

Puil, E. (1981). S-glutamate : its interactions with spinal neurons. Brain Res. Rev., 3, 229-322.

Raiteri, M., Maura, G., Bonanno, G. and Pittaluga, A. (1986). Differential pharmacology and function ot two 5-HT_1 receptors modulating transmitter release in cerebellum. J. Pharmacol. Exp. Therp., 237, 644-648.

Ralston, H.J. and Ralston, D.D. (1979). The distribution of dorsal root axons in lamina I, II and III of the Macaque spinal cord : a quantitative electron microscope study. J. Comp. Neurol., 184, 643-644.

Randic, M. and Yu, H.H. (1976). Effects of 5-hydroxytryptamine and bradykinin in cat dorsal horn neurones activated by noxious stimuli. Brain Res., 111, 197-203.

Reynolds, D.V. (1969). Surgery in the rat during electrical

analgesia induced by focal brain stimulation. Science, 164, 444-445.

Reynolds, J.N., Baskys, A. and Carlen, P.L. (1988). The effects of serotonin on N-methyl-D-aspartate and synaptically evoked depolarizations in rat neocortical neurons. Brain Res., 456, 286-292.

Rexed, b. (1952). The cytoarchitectonic organisation of the spinal cord in the cat. J. Comp. Neurol., 96, 415-495.

Rexed, B. (1954). A cytoarchitectonic atlas of the spinal cord in the cat. J. Comp. Neurol., 100, 297-379.

Rexed, B. (1964). Some aspects of the cytoarchitectonic and synaptology of the spinal cord. Prog. Brain Res., vol.11, pp.58-92. J.C. Eccles and J.P Schade (eds.). Elsevier, Amsterdam.

Richardson, D.E. and Akil, H. (1977). Pain reduction by electrical stimulation in man (part2) : chronic self-administeration in the periventricular gray matter. J. Neurosurg., 47, 184-194.

Richardson, B.P. and Engel, G. (1986). The pharmacology and function of 5-HT_3 receptors. Trends Neurosci., 7, 424-428.

Rivot, J.P., Chaouch, A. and Besson, J.M. (1980). Nucleus raphe magnus modulation of response of rat dorsal horn neurons to unmyelinated fiber inputs : partial involvement of serotonergic pathways. J. Neurophysiol., 44, 1039-1057.

Rivot, J.P, Chiang, C.Y. and Besson, J.M. (1982). Increase of serotonin metabolism within the dorsal horn of the spinal cord during nucleus raphe magnus stimulaton, as revealed by in vivo electrochemical detection. Brain Res., 238, 117-126.

Rivot, J.P., Ory-Lavollee, L. and Chiang, C.Y. (1983). Differential pulse voltammetry in the dorsal horn of the spinal cord of the anesthetized rat : are voltammograms related to 5-HT and / or to 5-HIAA ? Brain Res., 275, 311-319.

Ritz, L.A. and Greenspan, J.D. (1985). Morphological features of lamina V neurons receiving nociceptive input in cat sacrocaudal spinal cord. J. Comp. Neurol., 238, 440-452.

Roberts, M.H.T. (1984). 5-hydroxytryptamine and nociception. Neuropharmacol., 23, 12B, 1529-1536.

Roberts, M.H.T. and Rees, H. (1986). The antinociceptive effects of stimulating the pretectal nucleus of the rat. Pain, 25, 83-94.

Rolando, L. (1824). Ricerche anatomiche sulla struttura del midollo spinale. Torino : Dalla Stamperia Reale.

Ruda, M.A., Coffield, J. and Steinbusch, H.W.M. (1982).

Immunocytochemical analysis of serotonergic axons in lamina I and II of the lumbar spinal cord of the cat. J. Neurosci., 2, 1660-1671.

Ruda, M.A. and Gobel, S. (1980). Ultrastructural characterization of axonal endings in the substantia gelatinosa which take up [^{3}H] serotonin. Brain Res., 184, 57-83.

Rustioni, A. and Kaufman, A.B. (1977). Identification of cells of origin of non-primary afferents to the dorsal column nuclei of the cat. Exp. Brain Res., 27, 1-14.

Sagen, J., Winker, M.A. and Proudfit, H.K. (1983). Hypoalgesia induced by the local injection of phentolamine in the nucleus raphe magnus : blockade by depletion of spinal cord monoamines. Pain, 16, 253-263.

Sandkuhler, J. and Gebhart, G.F. (1984a). Characterization of inhibition of a spinal nociceptive reflex by stimulation medially and laterally in the midbrain and medulla in the pentobarbital-anestheized rat. Brain Res., 305, 67-76.

Sandkuhler, J. and Gebhart, G.F. (1984b). Relative contributions of the nucleus raphe magnus and adjacent medullary reticular formation to the inhibition by stimulation in the periaqueductal gray of a spinal nociceptive reflex in the pentobarbital-anesthetized rat. Brain Res., 305, 77-87.

Satoh, M., Akaike, A., Nakazawa, T. Masuda, C. and Takagi, H. (1983a). Different roles of the nucleus reticularis paragigantocellularis and nucleus raphe magnus of the rat in the production of analgesia by microinjection of opioids. Adv. Pain Res. Ther., vol.5, J.J. Bonica, U.Lindblum and A.Iggo (Eds.), Raven Press, New York, pp 381-386.

Satoh, M., Akaike, A., Nakazawa, T. and Takagi, H. (1980). Evidence for involvement of separate mechanisms in the production of analgesia by electrical stimulation of the nucleus reticularis paragigantocellularis and nucleus raphe magnus in the rat. Brain Res., 194, 525-529.

Satoh, M., Oku, R. and Akaike, A. (1983b). Analgesia produced by microinjection of L-glutamate into the rostral ventromedial bulbar nuclei of the rat and its inhibition by intrathecal a-adrenergic blocking agents. Brain Res., 261, 361-364.

Scheibel, M.E. and Scheibel, A.B. (1968). Terminal axonal patterns in cat spinal cord. II. The dorsal horn. Brain Res., 9, 32-58.

Scheibel, M.E. and Scheibel, A.B. (1969). Terminal patterns in cat spinal cord. III. Primary afferent collaterals. Brain Res., 13, 417-443.

Schmauss, C., Hammond, D.L., Ochi, J.W and Yaksh, T.L. (1983). Pharmacological antagonism of the antinociceptive effects of serotonin in the rat spinal cord. Europ. J. Pharmacol., 90, 349-357.

Schmidt, R.F. (1973). Control of the access of afferent activity to somatosensory pathways. In Handbook of Sensory Physiology, Vol.II, A.Iggo (Ed.), Springer-Verlag, Berlin, pp. 151-206.

Segu, L. and Calas, A. (1978). The topographical distribution of serotoninergic terminals in the spinal cord of the cat : quantitative radioautographic studies. Brain Res., 153, 449-464.

Shah, Y. and Dostrovsky, J.O. (1980). Electrophysiological evidence for a projection of the periaqueductal gray matter to nucleus raphe magnus in cat and rat. Brain Res., 193, 534-538.

Shannon, M., Battaglia, G., Glennon, R.A. and Titeler, M. (1984). 5-HT_1 and 5-HT_2 binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). Europ. J. Pharmacol., 102, 23-29.

Sherrington, C.S. (1906). The Integrative Action of the Nervous System. Scribner's, New York.

Sherrington, C.S. and Sowton, S.C.M. (1915). Observations on reflex responses to single break shocks. J. Physiol., 49, 331-343.

Siegelbaum, S.A., Camardo, J.S. and Kandel. E.R. (1982). Serotonin and cyclic AMP close single K^+ channels in aplysia sensory neurones. Nature, 299, 413-417.

Sills, M.A., Wolfe, B.B. and Frazer. A. (1984). Determination of selective and nonselective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. J. Pharmacol. Exp. Therp., 231, 480-487.

Singer, E., Sperk, G., Placheta, P. and Leeman, S.E. (1979). Reduction in substance P levels in the ventral cervical spinal cord of the rat after intracisternal 5,7-dihydroxy-tryptamine injection. Brain Res., 174, 362-365.

Skagerberg, G. and Bjorklund, A. (1985). Topographic principles in the spinal projections of serotonergic and non-serotonergic brainstem neurons in the rat. Neurosci., 15, 445-480.

Sladek, J.R., Garver, D.L. and Cummings, J.P. (1982). Monoamine distribution in primate brain-IV. Indoleamine-containing perikarya in the brain stem of Macaca arctoides. Neurosci., 7, 477- 493.

Solomon, R.E. and Gebhart, G.F. (1988). Mechanisms of effects of intrathecal serotonin on nociception.

J. Pharmacol. Exp. Therp., to be published.

Soper, W.Y. (1976). Effects of analgesic midbrain stimualtion on reflex withdrawal and thermal escape in the rat. J. Comp. Physiol. Psychol., 90, 91-101.

Sorkin, L.S., Steinman, J.L., Hughes, M.G., Willis, W.D. and McAdoo, D.J. (1988). Microdialysis recovery of serotonin released in spinal cord dorsal horn. J. Neurosci. Meth., 23, 131-138.

Steedman, W., Molony, V. and Iggo, A. (1985). Nociceptive neurones in the superficial dorsal horn of cat lumbar spinal cord and their primary afferent inputs. Exp. Brain Res., 58, 171-182.

Steinbusch, H.W.M. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat - cell bodies and terminals. Neurosci., 6, 557-618.

Suzuki, Y. and Taguchi, K. (1986). In vivo voltammetric studies of the effect of morphine on the serotonergic system in the cat spinal cord. Brain Res., 398, 413-418.

Sweet, W.H. (1981). Animal models of chronic pain : their possible validation from human experience with posterior rhizotomy and cogenital analgesia. Pain, 10, 275-295.

Swett, J.E., Mc Mahon, S.B. and Wall P.D. (1985). Long ascending projections to the midbrain from cells of lamina I and nucleus of the dorsolateral funiculus of the rat spinal cord. J. Comp. Neurol., 238, 401-416.

Szentagothi, J. (1964). Propriospinal pathways and their synapses. Prog. Brain Res., vol.11, 115-174.

Takeuchi, Y., Kimura, H. and Sano, Y. (1982). immunohistochemical demonstration of the distribution of serotonin neurons in the brainstem of the rat and cat. Cell Tissue Res., 224, 247-267.

Tashiro, T., Satoda, T., Takahashi, O., Matsushima, R. and Mizumo, N. (1988). Distribution of axons exhibiting both enkephalin- and serotonin-like immunoreactivities in the lumbar cord segments : an immunohistochemical study in the cat. Brain Res., 440 :357-362.

Thalhammer, J.G., Du, H.J., Kitahata, L.M. and Zimmermann, M. (1983). Inhibition of nociceptive responses in cat spinal dorsal horn neurones by morphine microinjection in the nucleus raphe magnus. Naunyn-Schmiedeberg's Arch. Pharmacol., suppl. 322, R83.

Tjolsen, A., Berge, O. G., Eide, P.K., Broch, O.J. and Hole, K. (1988). Apparent hyperalgesia after lesions of the descending serotonergic pathways is due to increased tail skin temperature. Pain, 33, 225-231.

Todd, A.J. and Millar, J. (1983). Receptive fields and responses to ionophoretically applied noreadrenaline and 5-hydroxytryptamine of units recorded in laminae I-III of cat dorsal horn. Brain Res., 288, 159-167.

Torebjork, H.E. and Hallin, R.G. (1973). Perceptual changes accompanying controlled preferential blocking of A and C fibre responses in intact human skin nerves. Exp. Brain Res., 16, 321-332.

Towle, A.C., Breese, G.R., Mueller, R.A., Hunt, R. and Lauder, J.M. (1986). Early postnatal administration of 5,7-dihydroxytryptamine on substance P and thyrotropin releasing hormone neurons and terminals in rat brain. Brain Res., 363, 38-46.

Tyce, G.M. and Yaksh, T.L (1981). Monoamine release from cat spinal cord by somatic stimuli : an intrinsic modulatory system. J. Physiol., 314, 513-529.

Uzan, A., Kabouche, M., Rataud, J. and Le Fur, G. (1980). Pharmacological evidence of a possible tryptaminergic regulation of opiate receptors by using indalpine, a selective 5-HT uptake inhibitor. Neuropharmacol., 19, 1075-1079.

Vasko, M.R., Pang, I.H. and Vogt, M. (1984). Involvement of 5-hydroxytryptamine-containing neurons in antinociception produced by injection of morphine into nucleus raphe magnus or onto spinal cord. Brain Res., 306, 341-348.

Von Frey, M. (1896). The pain sensation. In : Classical German Contributions to Pain Research, H.O. Handwerker and K. Brune (eds.), 1987, Tagblatt-Druckerei KG, pp. 91-131.

Wall, P.D. (1958). Excitability changes in afferent fibre terminations and their relation to slow potentials. J. Physiol., 142, 1-21.

Wall,P.D. (1967). The laminar organization of dorsal horn and the effects of descending impulses. J. Physiol., 188, 403-423.

Wall, P.D. (1980). The substantia gelatinosa. A gate control mechanism set across a sensory pathway. Trends Neurosci., 3, 221-224.

Wall. P.D. and Sweet, W.H. (1967). Temporary abolition of pain in man. Science, 155, 108-109.

Watkins, J.C. and Evans, R.H. (1981). Excitatory amino acid transmitters. Ann. Rev. Pharmacol. Toxicol., 21, 165-204.

Watkins, L.R., Cobelli, D.A. and Mayer, D.J. (1982). Opiate vs non-opiate foot shock induced analgesia (FSIA) : descending and intraspinal components. Brain Res., 245, 97-106.

Watkins, L.R., Griffin, G., Leichnetz, G.R. and Mayer, D.J. (1980). The somatotopic organization of the nucleus raphe magnus and surrounding brain stem structures as revealed by HRP slow gel release. Brain Res., 181, 1-15.

Weight, F.F and Salmoiraghi, G.C. (1966). Responses of spinal cord interneurons to acetylcholine, norepinephrine and serotonin administered by microelectrophoresis. J. Pharmacol. Exp. Therep., 153,420-427.

Weil-Fugazza, J., Godefroy, F., Coudert, D. and Besson, J.M. (1981). Morphine analgesia and newly synthesized 5-hydroxytryptamine in the dorsal and the ventral halves of the spinal cord of the rat. Brain Res., 214, 440-444.

Wessendorf, M.W., Proudfit, H.K. and Anderson, E.G. (1981). The identification of serotonergic neurons in the nucleus raphe magnus by conduction velocity. Brain Res., 214, 168-173.

West, D.C. and Wolstencroft, J.H. (1977). location and conduction velocity of raphe spinal neurones in nucleus raphe magnus and raphe pallidus in the cat. Neurosci. Lett., 5, 147-151.

Westlund, K.N., Bowker, R.M., Ziegler, M.G. and Coulter, J.D. (1983). Noradrenergic projections to the spinal cord of the rat. Brain Res., 263, 15-31.

Wiberg, M. and Blomqvist, A. (1984). The spinomesencephalic tract in the cat : its cells of origin and termination pattern as demonstrated by the intraaxonal transport method. Brain Res., 291, 1-18.

Wiklund, L., Leger, L. and Persson, M. (1981). Monoamine cell distribution in the cat brainstem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. J. Comp. Neurol., 203, 613-647.

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

Willcockson, W.S., Gerhart, K.D., Cargill, C.L. and Willis, W.D. (1983). Effects of biogenic amines on raphe-spinal tract cells. J. Pharmacol. Exp. Therp., 225, 637-645.

Willis, W.D. (1981). Ascending pathways from the dorsal horn. In : Spinal Cord Sensation. A.G. Brown and M. Rethelyi (Eds.), Scottish Academic Press, Edinburgh. pp. 169-178

Willis, W.D. (1982). Mechanisms of medial brain stem stimulation-induced inhibition in spinothalamic neurons. In

Brain Stem Control of Spinal Mechanisms, B.Sjolund and A. Bjorklund (Eds.), Elseiver, Amsterdam, pp.411-438.

Willis, W.D., Haber, L.H. and Martin, R.F. (1977). Inhibition of spinothalamic tract cells and interneurons by brainstem stimulation in the monkey. J. Neurophysiol., 40, 968-981.

Willis, W.D., Kenshalo, D.R. and Leonard, R.B. (1979). The cells of origin of the primate spinothalamic tract. J. Comp. Neurol., 188, 543-574.

Willis, W.D., Leonard, R.B. and Kenshalo, D.R. (1978). Spinothalamic tract neurons in the substantia gelatinosa. Science, 202, 986-988.

Willis, W,D., Trevino, D.L., Coulter, J.D. and Maunz, R.A. (1974). Responses of primate spinothalamic tract neurons to natural stimulation of hind limb. J. Neurophysiol., 37, 358-372.

Woolf, C.J. and Fitzgerald, M. (1983). The properties of neurones recorded in the superficial dorsal horn of the rat spinal cord. J. Comp. Neurol., 221, 313-328.

Woolfe, G. and McDonald, A.D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Therp., 80, 300- .

Yaksh, T.L. (1979). Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. Brain Res., 160, 180-185.

Yaksh, T.L. and Rudy, T.A. (1976). Chronic catheterization of the spinal subarachnoid space. Physiol. Beh., 17, 1031-1036.

Yaksh, T.L. and Tyce, G.M. (1979). Microinjection of morphine into the periaqueductal gray evokes the release of serotonin from spinal cord. Brain Res., 171, 176-181.

Yaksh, T.L. and Wilson, P.R. (1979). Spinal serotonin terminal system mediates antinociception. J. Pharmacol. Exp. Therp., 208, 446-453.

Yaksh, T.L., Yeung, J.C. and Rudy, T.A. (1976). An inability to antagonize with naloxone the elevated nociceptive thresholds resulting from electrical stimulation of the mesencephalic central gray. Life Sci., 18, 1193-1198.

Yeung,J.C. and Rudy T.A. (1980). Sites of antinociceptive action of systemically injected morphine : involvement of supraspinal loci as revealed by intracerebroventricular injection of naloxone. J. Pharmacol. Exp. Ther., 215, 626-632.

Yeung, J.C., Yaksh, T.L. and Rudy, T.A. (1977). Concurrent mapping

of brain sites for sensitivity to the direct application of morphine and focal electrical stimulation in the production of antinociception in the rat. Pain, 4, 23-40.

Yezierski, R.P. and Schwartz, R.H. (1986). Response and receptive-field properties of spinomesencephalic tract cells in the cat. J. Neurophysiol., 55, 76-96.

Yezierski, R.P., Sorkin, L.S. and Willis, W.D. (1987). response properties of spinal neurons projecting to midbrain or midbrain-thalamus in the monkey. Brain Res., 437, 165-170.

Yezierski, R.P., Wilcox, T.K. and Willis, W.D. (1982). The effects of serotonin antagonists on the inhibition of primate spiothalamic tract cells produced by stimulation in nucleus raphe magnus or periaqueductal gray. J. Pharmacol. Exp. Therep., 220, 266-277.

Young, R.F. and Chambi, I. (1987). Pain relief by electrical stimulation of the periaqueductal gray matter. J. Neurosurg., 66, 364-371.

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

Zemlan, F.P., Behbehani, M.M. and Beckstead, R.M. (1984). Ascending and descending projections from nucleus reticularis magnocellularis and nucleus reticularis gigantocellularis : an autoradiograpghic and horseradish peroxidase study in the rat. Brain Res., 292, 207-220.

Zieglgansberger, W. and Puil, E. (1973). Actions of glutamic acid on spinal neurones. Exp. Brain Res., 17, 35-49.

Zivin, J.A., Reid, J.L., Saavedra, J.M. and Kopin, I.J. (1975). Quantitative localization of biogenic amines in the spinal cord. Brain Res., 99, 293-301.

Zorman, G., Belcher, G., Adams, J.E. and Fields, H.L. (1982). Lumbar intrathecal naloxone blocks analgesia produced by microstimulation of the ventromedial medulla in the rat. Brain Res., 236, 77-84.

Zorman, G., Hentall, I.D., Adams, J.E. and Fields, H.L. (1981). Naloxone-reversible analgesia produced by microstimulation in the rat medulla. Brain Res., 219, 137-148.

Zotterman, Y. (1939). Touch, pain and tickling : an electrophysiological investigation on cutaneous sensory nerves. J. Physiol., 95, 1-28.

Kosterlitz, H.W and Patreson, S.J. (1985). Types of opioid receptors : relation to antinociception. Phil. Trans. R. Soc. Lond., B 308, 291-297.

SEROTONERGIC EFFECTS ON SUPERFICIAL AND DEEP DORSAL HORN NEURONES.
N. EL-YASSIR, S.M. FLEETWOOD-WALKER and P.J. HOPE, Department of Preclinical Veterinary Sciences, University of Edinburgh, Edinburgh EH9 1QH, U.K.

The major descending serotonergic input to the spinal cord originates from the nucleus raphe magnus (NRM). 5-HT has been shown to have an antinociceptive action at the spinal level. 5-HT, however, can interact with a number of different receptor types. The present report investigates the receptor type involved. Extracellular recordings were made from rat dorsal horn neurones through a multibarreled electrode, also used for ionophoresis. 5-HT, ionophoretically-applied near lamina I cells, selectively inhibited responses to noxious pinch or heat, leaving responses to innocuous brush unaffected in 5 multireceptive cells. On the other hand, 5-HT inhibited responses to both pinch and DL-homocysteic acid in two nocispecific cells. Two cells were unaffected by 5-HT. The selective inhibitory effect of 5-HT on the nociceptive response was mimicked by RU 24969, a 5-HT_{1B} receptor agonist (5/5 cells). In contrast, 8-OH-DPAT, a 5-HT_{1A} receptor agonist mimicked the non-selective inhibitory effect of 5-HT (6/6 cells). These results are similar to those obtained from cells in the deeper dorsal horn (Brain Res., in press). Electrical stimulation of NRM inhibited both superficial and deeper dorsal horn neuronal responses to noxious stimulation. This effect was reversed by cyanopindolol, a 5-HT_1 receptor antagonist, but not ketanserin, a 5-HT_2 receptor antagonist. These results indicate that the modulatory effect of NRM stimulation on the nociceptive respones of dorsal horn neurones is mediated through a 5-HT_1 site.

SELECTIVE ANTINOCICEPTIVE EFFECTS OF 5-HT IN DORSAL HORN ARE MEDIATED BY 5-HT 1B RECEPTORS. N. El-Yassir, S.M. Fleetwood-Walker, Department of Preclinical Veterinary Sciences, University of Edinburgh, Summerhall, Edinburgh EH9 1 QH.

It has long been suggested that 5-HT plays an important role in modulating nociception. The dorsal horn of rat spinal cord has been shown to receive a serotonergic input from specific supraspinal loci (Dahlstrom and Fuxe (1965). Intrathecal application of 5-HT produces behaviourally defined analgesia (Yaksh and Wilson, 1979). Moreover, ionophoretically applied 5-HT depresses reponses of cat dorsal horn neurones to noxious stimulation (Headley, Duggan and Griersmith, 1978). It is now clear, however, that 5-HT can interact with a number of different receptor subtypes (Peroutka and Snyder, 1979; Pedgio, Yamamura and Nelson, 1981). The present experiments investigate the action of agonists, selective for subtypes of 5-HT, receptors on the responses of multireceptive dorsal horn neurones in the rat.

Rats were anaesthetized with α-chloralose (38mg/Kg) and urethane (700mg/Kg). Extracellular recordings were made in the lumbar spinal cord using multibarrelled glass electrodes containing: 1M NaCl for automatic current balancing; D,L-homocysteic acid (DLH); 5-HT creatinine sulphate; the 5-HT1A receptor agonist (+)-8-hydroxy-2-(Di-N-propylamino) tetralin hydrobromide (8-OH-DPAT) and a 5-HT1B receptor agonist 5-Methoxy-3 (1,2,3,6 tetralydropyridin 4-yl) 1H indole (Ru24969). The responses of neurones to innocuous stimulation (motorized brush), noxious stimulation (graduated pinch) and to ionophoretically applied DLH were investigated.

Compound	Selective depression of the pinch response	Non-selective depression of all responses	Non-selective excitation of all responses	No effect	Total no. of cells
5-HT	7	4	1	-	12
8-OH-DPAT	-	17	3	4	24
Ru24969	20	4	-	6	30

Low ejecting currents (2-30 nA) were usually sufficient to produce clear effects. The above results suggest that the selective depression of the response to the noxious stimulus by 5-HT is mediated through the 5-HT1B receptor subtype. Whilst the non selective action of 5-HT is mediated through the 5-HT1A receptor subtype.

References

1. Dahlstrom, A. and Fuxe, K. (1965). Acta physiol. scand. 64 Supp. 247: 1-36
2. Headley, P.J., Duggan, A.W. and Griersmith, B.T. (1978). Brain Res. 145, 185-189
3. Pedgio, N.W., Yamamura, H.I. and Nelson, D.L. (1981). J. Neurochem. 36, 220-226
4. Peroutka, S.J. and Snyder, S.H. (1979). Mol. Pharmacol. 16, 687-699
5. Yaksh, T.L. and Wilson, P.R. (1979). J. Pharmacol. Exp. Ther. 208, 446-453

Brain Research, 456 (1988) 147–158
Elsevier

BRE 13813

Heterogeneous effects of serotonin in the dorsal horn of rat: the involvement of 5-HT_1 receptor subtypes

Nada El-Yassir, Susan M. Fleetwood-Walker and R. Mitchell*

Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh (U.K.)

(Accepted 23 February 1988)

Key words: Serotonin (5-HT); 5-HT receptor; Ionophoresis; Nociception; Dorsal horn

The effect of ionophoretically applied serotonin (5-HT) was tested on cutaneous sensory responses of multireceptive dorsal horn neurones in the anaesthetized rat. Three types of 5-HT action were discerned: selective inhibition of nociceptive responses (10/18 cells), non-selective inhibition of responses to both noxious and innocuous stimuli as well as to excitatory amino acids (4/18 cells) and non-selective excitation of evoked responses (1/18 cells). A few cells (3/18) were unaffected by 5-HT. The use of agonists, shown to discriminate between subtypes of 5-HT_1 receptor revealed that a 5-HT_{1A} receptor agonist mimicked the non-selective effects of 5-HT, whereas a 5-HT_{1B} receptor agonist mimicked the selective antinociceptive effects of 5-HT. A 5-HT_2 receptor agonist, in contrast, was without effect. Both the selective and the non-selective effects were reversed by a 5-HT_1 receptor antagonist, but not a 5-HT_2 antagonist.

INTRODUCTION

It has long been considered that serotonin (5-HT) may play an important role in modulating nociception. The spinal cord appears to be a major site for serotonin action, since intrathecally applied 5-HT produces dose-dependent behavioural analgesia in rat, cat and rabbit[69], an action which can be reversed by 5-HT antagonists[65,69]. The serotonergic innervation of the spinal cord originates from specific brainstem loci[3,4,9,54]. In the dorsal horn, serotonergic terminals are concentrated in the superficial laminae I and II and the lateral aspects of laminae V and VI in rat[4,9,54], cat[34,54,66] and monkey[39].

Electrical stimulation of nucleus raphe magnus, a major source of descending serotonergic input to the dorsal horn[3,4,54], can inhibit responses of dorsal horn neurones (including cells of ascending tracts) to noxious[12,14,23,63], but also to innocuous stimuli[7,17,21,37,44,46,47,51,68]. Furthermore, the action of ionophoretically applied 5-HT on responses of dorsal horn neurones in cat[2,6,22,26,62], and monkey[36] is predominantly inhibitory, though excitatory effects were also observed. There does not seem to be agreement, however, as to whether the inhibitory action of 5-HT is selectively exerted upon the nociceptive input. In view of the multiplicity of serotonin actions in the dorsal horn, we addressed the possibility that different effects of 5-HT are mediated through different classes of 5-HT receptor sites.

As early as 1957, two distinct sites for 5-HT were described in the guinea pig ileum[16]. In the central nervous system, classification of 5-HT receptors has been based on high-affinity binding studies with agonists and antagonists. Two specific binding sites for 5-HT were described in 1979 (ref. 59); one to which 5-HT and its agonists have high affinity, the other displaying high affinity to 5-HT antagonists[60]. The two sites were named 5-HT_1 and 5-HT_2 sites. Since then, the 5-HT_1 site has been subclassified[28,55,58]. A selective agonist at the 5-HT_{1A} site, (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT), recently described[20,29,48,61], has low affinity for both the 5-HT_2 and 5-HT_{1B} sites[24,25,48,61].

* Present address: MRC Brain Metabolism Unit, 1 George Square, Edinburgh EH9 9JZ, U.K.
Correspondence: N. El-Yassir, Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, U.K.

5-Methoxy-3(1,2,3,6-tetrahydropyridin-4-yl) 1H indole (RU 24969), on the other hand, is a high-affinity agonist at the 5-HT_{1B} site[32,33,61]. Although it has little affinity for the 5-HT_2 site[49], it retains moderate activity at the 5-HT_{1A} site[25,27,33,49,61,67]. For the 5-HT_2 site, a potent and selective agonist, (±)-1-2,5-dimethoxy-4-iodophenyl)-2-amino-propane hydrochloride (DOI) has been described[19]. Selective antagonists are now also available for 5-HT_2 sites, such as ketanserin[42,43] and for 5-HT_1 sites, such as cyanopindolol[25,32,33,53,56]. In the spinal cord of rat, both 5-HT_{1A} and 5-HT_{1B} receptor subtypes are present[52,56]. Although the 5-HT_2 site also exists in the spinal cord, it is present at quite low levels[52,57].

The present experiments investigated the effects of ionophoretically applied 5-HT on dorsal horn neuronal responses to natural cutaneous stimulation as well as to locally applied excitatory amino acids. Employing recently identified receptor-selective compounds, our aim was to assess which type of site(s) mediate(s) the effects of 5-HT on nociceptive processing in the dorsal horn.

MATERIALS AND METHODS

Experiments were carried out on 42 adult Wistar male rats anaesthetized with a mixture of α-chloralose (35 mg/kg) and urethane (700 mg/kg) administered i.v. after initial induction with halothane. Additional doses (0.2 ml of 10 mg/ml α-chloralose and 200 mg/ml urethane) were given when required. The trachea was cannulated and blood pressure was continuously monitored via a transducer connected to a cannula placed in the carotid artery. A thermostatic rectal probe connected to a heating blanket maintained core temperature between 37 and 38 °C. A laminectomy was performed exposing the spinal cord between T_{13} and L_3. To improve extracellular recording stability, 4% agar solution at 38 °C was injected under the spinal bone of T_{12} then poured over the surface of the exposed cord. Once the agar had set, it was removed from the laminectomy area, the dura was retracted and the exposed area covered with mineral oil at 37 °C to prevent dehydration. The rat was allowed to breathe humidified oxygen passed close to a Y-shaped attachment to the tracheal cannula.

Electrophysiological recording and ionophoresis of drugs

Single unit extracellular recordings from dorsal horn neurones were made through the central barrel (4 M NaCl, pH 4.0–4.5) of a 7-barrel electrode made from 1.5 mm o.d. filamented glass tubing. Electrodes were of 3.5–5 μm tip size (DC resistance 4–8 MΩ). One side-barrel of the electrode contained 1 M NaCl at pH 4.0–4.5 for automatic current balancing and independent current controls (Neurophore BH2 system). The remaining side-barrels contained a combination of the following compounds all at pH 4.0–4.5 and dissolved in 0.1 M NaCl unless otherwise stated: 0.1 M D,L-homocysteic acid, DLH (Sigma), in aqueous solution, pH 8.0–8.5; 0.1 M glutamate (Sigma) in aqueous solution, pH 8.0–8.5; 2.5 mM-serotonin creatinine sulphate (Sigma); 1.25 mM RU 24969 (Roussel); 1.25 mM 8-OH-DPAT (Research Biochemicals Inc.), 1 mM DOI (Research Biochemicals Inc.), in aqueous solution; 1 mM ketanserin tartrate (Janssen), in aqueous solution; 1 mM (±)-cyanopindolol, (Sandoz), in aqueous solution. Drugs were applied with cathodal currents (DLH and glutamate with anodal) at 2–100 nA. Retaining currents of 10–15 nA were applied to minimize leakage from the electrode tip between tests. One side barrel was always filled with Pontamine sky blue (2% in 0.5 M NaAc) for marking the position of recording sites in the dorsal horn 4–7 min at 10 nA. Neurones were located by light tactile stimulation of the hind limb whilst searching the ipsilateral dorsal horn of the spinal cord with the multibarrel electrode. The electrode was advanced through the dorsal horn using a microdrive system in steps of 4 μm. Action potentials were regarded as belonging to a single unit only when both the shape and amplitude of the spike were uniform.

Quantification of cutaneous sensory responses

The peripheral receptive field of each cell was determined and its responses were briefly characterized. Cells were classified as multireceptive when they were excited by innocuous tactile stimuli and had a progressively higher discharge rate to increasing levels of pressure applied by pinching with rat-toothed forceps. Some of these cells when tested, also responded to radiant heat. Cells which did not

appear to respond to innocuous stimulation and only responded to high levels of mechanical or thermal stimulation, were classified as nocispecific. Cells which responded maximally to tactile innocuous stimulation were classified as low threshold only cells and they were discarded from our sample. Innocuous stimulation was provided by a motorized brush (10 s duration) whilst the noxious stimulation was usually provided by a quantifiable constant-pressure mechanism (10 s duration). This mechanism consisted of a pair of serrated forceps connected to a graduated scale, modified from a glass syringe barrel and was operated manually to constant displacement. The local pressures (equivalent to a loading of approx. 200–700 g) applied to the skin by this mechanism caused a withdrawal reflex in lightly anaesthetized rats and were confirmed to be distinctly noxious in humans. In some cases, noxious thermal stimuli were provided by a thermistor-controlled radiant heat lamp giving a skin temperature range from 30 to 48 °C (10–15 s duration). Noxious stimulation was applied once every 3–4 min to avoid damage to the skin. Noxious and innocuous stimuli were applied to adjacent areas within the excitatory receptive field on the paw or hind limb, ipsilateral to the recording site. Activity evoked by ionophoretically applied DLH or glutamate was also assessed (4–100 nA, 10–20 s duration). The evoked responses were always submaximal. Regular cycles (3–4 min duration) of control responses to noxious, innocuous and DLH or glutamate stimuli were repeated until two or more sets of these responses corresponded closely, varying by less than 15%. Drugs were then ejected for 1 min before the start of a test cycle and the ejected current was increased between each cycle until a clear response was observed. Care was taken to monitor and discriminate neuronal spikes (Digitimer spike processor, D130) throughout the tests. After testing a neurone with a particular drug, the cell was allowed to recover (up to 100 min) before further study was undertaken. Continuous records of firing (over 400 ms bin width), together with the analogue signals from the stimula-

TABLE I

Summary of results obtained with 5-HT and 5-HT$_1$ receptor agonists

The majority of cells tested with 5-HT displayed selective inhibition of the pinch-evoked response. This effect was reproduced by RU 24969, a 5-HT$_{1B}$ receptor agonist in most cells tested. A subpopulation of cells tested with 5-HT displayed non-selective inhibition of all evoked responses tested to broadly the same extent. This effect was reproduced by 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist. A minor population displayed non-selective excitation of all evoked responses when tested with either 5-HT or 8-OH-DPAT. Some cells were apparently unaffected by 5-HT. (See text for further details.)

Compound	*Effect observed*				
	Selective inhibition of the response to pinch	*Non-selective inhibition of all evoked responses*	*Non-selective excitation of all evoked responses*	*No effect*	*Total number of cells tested*
5-HT	10	4	1	3	18
8-OH-DPAT	–	21	3	4	28
RU 24969	22	8	–	6	36

TABLE II

Summary of the selective inhibitory effects of 5-HT and RU 24969 on the nociceptive response

A comparison of the effects of 5-HT and RU 24969 on different evoked responses. The table was compiled by obtaining values for DLH/glutamate-evoked and brush-evoked activity as percentages of control responses at currents of 5-HT or RU 24969 which produced inhibition of the pinch-evoked response to 50% control response for each cell. Values are the mean ± S.E.M.

Compound	*Current at which the pinch response was inhibited to 50% of control (nA)*	*% Control responses to DLH or glutamate*	*% Control responses to brush*	*Number of cells*
5-HT	12 ± 5	95 ± 3	92 ± 4	10
RU 24969	16 ± 4	96 ± 3	94 ± 2	22

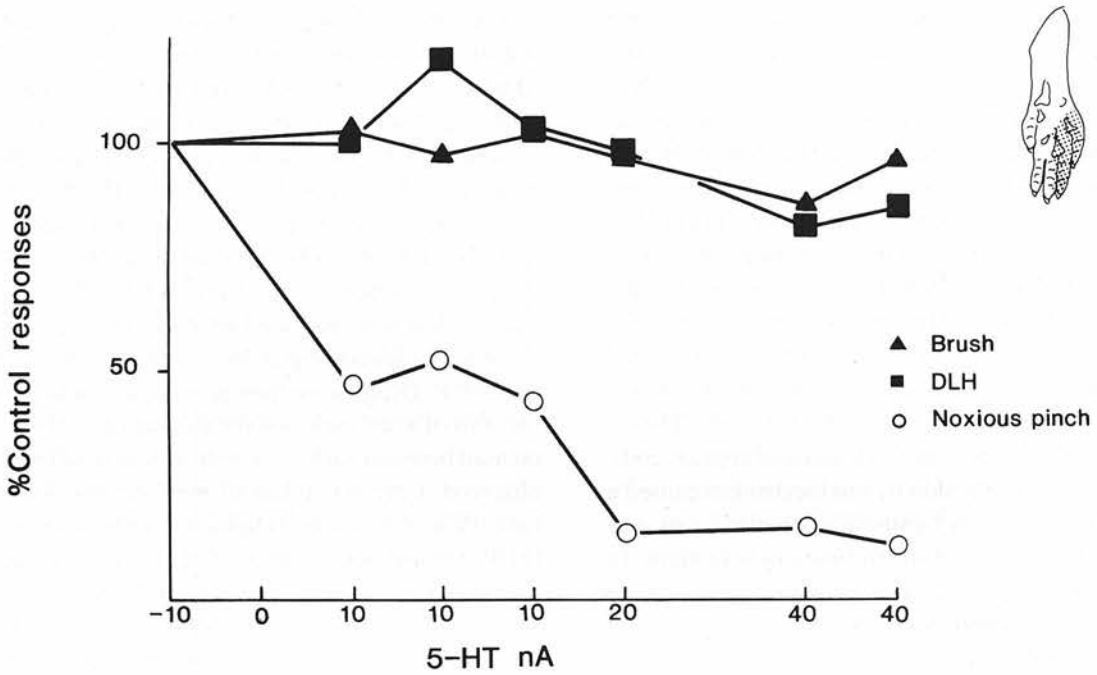


Fig. 1. Example of the selective antinociceptive effect of 5-HT. The number of stimulus-evoked action potentials was integrated in selected epochs corresponding to the duration of the response. Integrated test responses were then expressed as percentages of control values. The graph above shows percentages of control values for each of the different evoked responses plotted against 5-HT ejection currents (nA) in consecutive test cycles. Pinch-evoked activity (open circles) was inhibited markedly at very low currents of 5-HT (10 nA). On the other hand, corresponding brush-evoked activity (triangles) and DLH-evoked activity (squares) were unaffected. The inset shows the extent of the low-threshold excitatory receptive field on the hind-foot ipsilateral to the recording site (shaded area).

tors were plotted and stored by computer (Cromemco system III) using custom-designed software. Data was stored on disc and analyzed 'off-line' by integrating the number of stimulus-evoked action potentials in selected epochs. To permit comparison of the effects of a single drug on the different evoked responses of the same neurone, integrated test responses were expressed as percentages of control values. Graphs were constructed plotting percentage of the control values for each of the evoked responses against ejecting currents.

RESULTS

Seventy cells were tested in these experiments. The sample was deliberately biased towards multireceptive cells, two cells apparently nocispecific, however, were also tested. The position of 35 recording

TABLE III

Summary of the non-selective inhibitory effects of 5-HT and 8-OH-DPAT

The table was compiled by obtaining currents at which maximum inhibition of the pinch-evoked response occurred in a test together with the percentage of pinch-evoked, brush-evoked and DLH/glutamate-evoked responses at that current (expressed as % control responses). Values are the mean ± S.E.M.

Compound	*Current producing maximum inhibition of the pinch response observed in a test (nA)*	*% Control responses to pinch*	*% Control responses to DLH or glutamate*	*% Control responses to brush*	*Number of cells*
5-HT	31 ± 8	33 ± 7	55 ± 10	52 ± 20	7*
8-OH-DPAT	23 ± 5	41 ± 4	33 ± 5	45 ± 6	21

* Including 3 cells which at low currents of 5-HT had initially displayed selective inhibition of response to pinch.

sites was marked by Pontamine sky blue and all the sites occurred in the dorsal horn, mainly in laminae III–V, but also in lamina I.

Effects of ionophoretically applied 5-HT on multireceptive neurones

Eighteen cells were tested with 5-HT (see Table I). In 10/18 cells 5-HT caused a selective inhibition of the pinch response (see Table II). An example of this type of effect is shown in Fig. 1. Serotonin produced non-selective inhibition of all responses tested in 4/18 cells (see Table III). Fig. 2 shows an example of this type of effect. In one cell, 5-HT was observed to have a non-selective excitatory effect, whilst there was no effect on a further 3 cells, even at high currents.

The nociceptive response was judged to be selectively inhibited by a particular compound when this response was significantly reduced whilst responses to brush or to the excitatory amino acids were not significantly affected (statistical analysis on original data using dependent-treatment *t*-test). A compound was judged to have a non-selective effect when the different evoked responses were found to have altered significantly from control responses and to broadly the same extent.

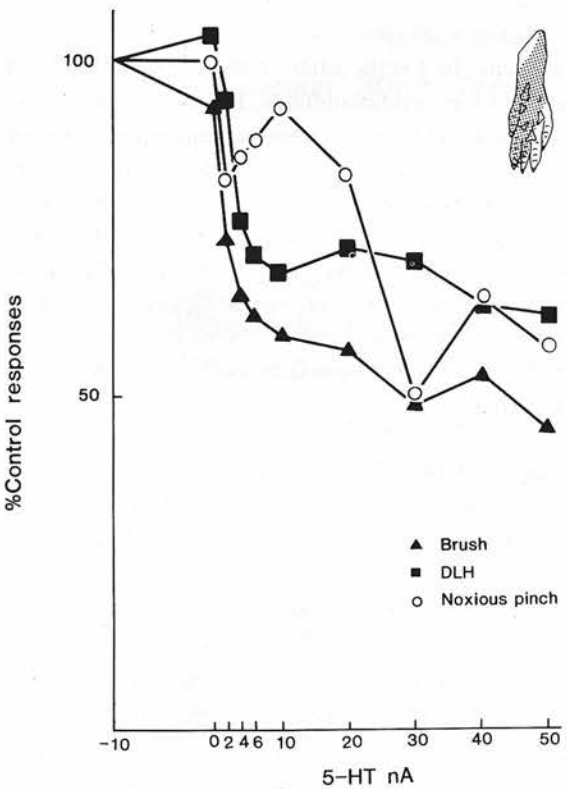


Fig. 2. Example of the non-selective inhibitory effect of 5-HT. Integrated test responses, expressed as a percentage of control responses, are plotted against the ionophoretic ejection current for 5-HT in consecutive test cycles. Brush-evoked activity (triangles), DLH-evoked activity (squares) as well as pinch-evoked activity (open circles) were similarly inhibited by 5-HT. The inset shows the extent of the low-threshold excitatory receptive field on the foot ipsilateral to the recording site (shaded area).

Serotonin had no effect on the spontaneous activity of 10/18 cells, whilst increasing it in 3 and decreasing it in 5 cases. There seemed to be no clear correlation between the effect of 5-HT on the evoked responses and that on background activity of the cell. It was notable that 5-HT ejection currents required to produce a selective inhibition of the noxious input were very low (see Table II), the mean current to produce a 50% inhibition of the pinch response was 12 ± 5 nA (in 8/10 cells, the current required was <10 nA). Upon increasing 5-HT currents (30–40 nA), 3 cells which had displayed selective inhibition at low currents, no longer displayed selectivity and all evoked responses were inhibited. Altering the sequence of the stimuli did not modify neuronal responses to 5-HT. Ionophoresis of 40–80 nA of NaCl (pH 4.0–4.5) had no effect on neuronal responses, thus discounting the possibility that the effects observed were due to current changes and not the compound under testing.

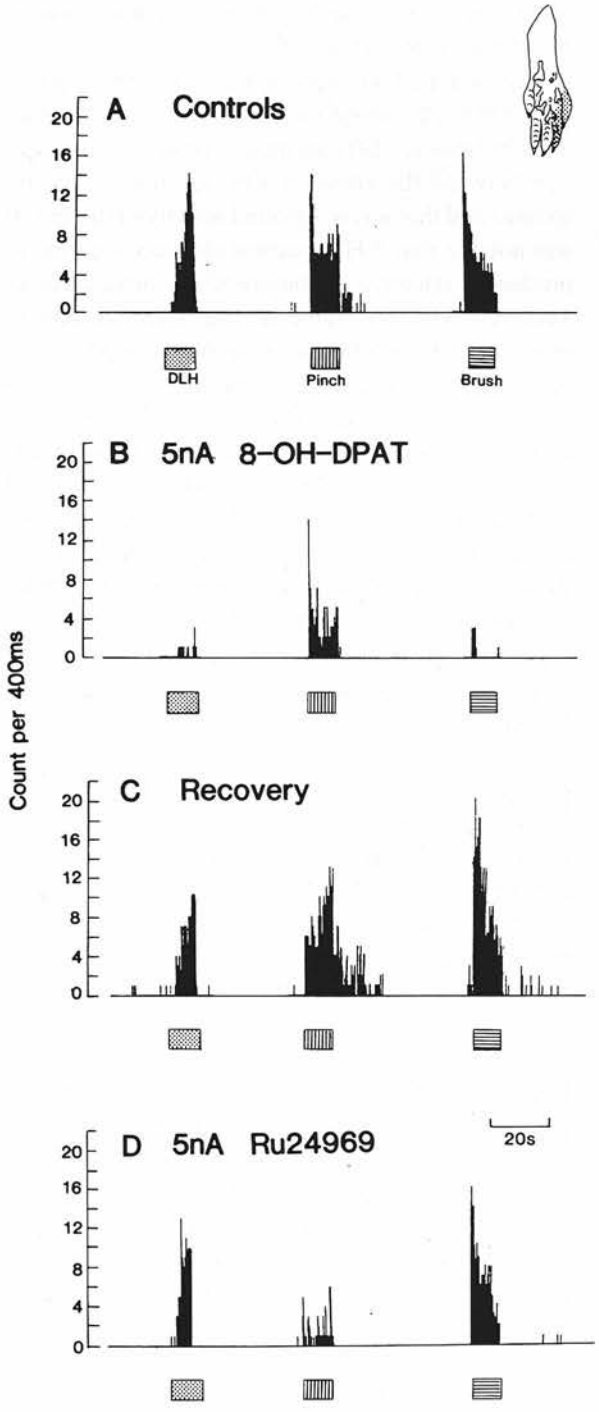
Effects of 5-HT receptor agonists

Two selective 5-HT_1 agonists were used; 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist[1,20,29], and RU 24969, a 5-HT_{1B} receptor subtype agonist[15,32,33,61]. Any involvement of the 5-HT_2 receptor site in mediating the effects of 5-HT on dorsal horn neurones was tested with the selective 5-HT_2 agonist DOI[19].

Effects of 8-OH-DPAT: a 5-HT_{1A} receptor agonist

Twenty eight cells were tested with this compound (Table I). The predominant effect of this compound was a non-selective inhibition of all evoked responses (21/28 cells) (see Table III). An example of this effect is shown in Fig. 3A–C. For the majority of cells tested (18/21), ejection currents of 8-OH-DPAT less than or equal to 30 nA were sufficient to produce clear non-selective inhibition. Non-selective excitation was observed in 3/28 cells with this compound.

Finally, while 4/28 cells were unaffected by 8-OH-DPAT, one cell which had not been affected by this compound at currents up to 55 nA, displayed selective inhibition of the pinch response at a current of 75 nA. It was noted, however, that 8-OH-DPAT reduced spike amplitude when ejected at high currents. 8-OH-DPAT had no effect on the spontaneous activity of 15/28 cells, whilst increases were observed in 6/28 cells and decreases in 5/28 cells. In two cells, 8-OH-DPAT had a biphasic effect on spontaneous activity.



Effects of RU 24969: a 5-HT$_{1B}$ receptor agonist

Thirty-six cells were tested with this compound, Table I summarizes the results obtained. Thirty-two cells were multireceptive while two appeared to be nocispecific. Twenty-two cells (including the two nocispecific cells) displayed clear selective inhibition of pinch while other evoked responses were not significantly altered. (Fig. 3C,D shows an example of this type of effect.) Table II shows the mean current of RU 24969 required to produce 50% inhibition of the pinch response. In 18/22 cells, this current was less than 20 nA. Higher currents abolished selectivity in two cells. In 4 cells, where heat was substituted for pinch as the noxious stimulus, RU 24969 again selectively inhibited the nociceptive responses. A minor population (8/36 cells) tested with low currents of RU 24969 displayed non-selective inhibition, all evoked responses were significantly depressed. In some other cases (6/36 cells), RU 24969 (ejected at up to 80 nA) had no effect on evoked responses. Spontaneous activity was not altered by this compound in 26/36 cells, though it was increased in one cell and decreased in 9.

Effects of DOI: a 5-HT$_2$ receptor agonist

This compound was tested on 4 cells with currents up to 100 nA. No effect was observed on the pinch- or

←

Fig. 3. Ongoing activity record showing the differential effects of two agonists selective for 5-HT$_1$ receptor subtypes on the same cell. Ongoing firing frequency was recorded in 400-ms bins and plotted against time. A shows the control excitatory responses to DLH, noxious pinch and brush. B shows the effect of ionophoresis of 8-OH-DPAT, a selective 5-HT$_{1A}$ receptor agonist (5 nA for 4 min) which caused marked inhibition of all evoked responses tested. C shows recovered responses, 40 min after 8-OH-DPAT was switched off. D shows the effect of ionophoresis of RU 24969, a 5-HT$_{1B}$ receptor agonist (5 nA for 1 min) which caused a selective inhibition of the response to noxious pinch. The inset shows the extent of the low-threshold excitatory receptive field on the foot ipsilateral to the recording site (shaded area).

brush-evoked responses, the response to DLH, however, was markedly decreased in two cells. The spontaneous activity of 3 cells was unaffected by DOI, whilst it was decreased in one cell.

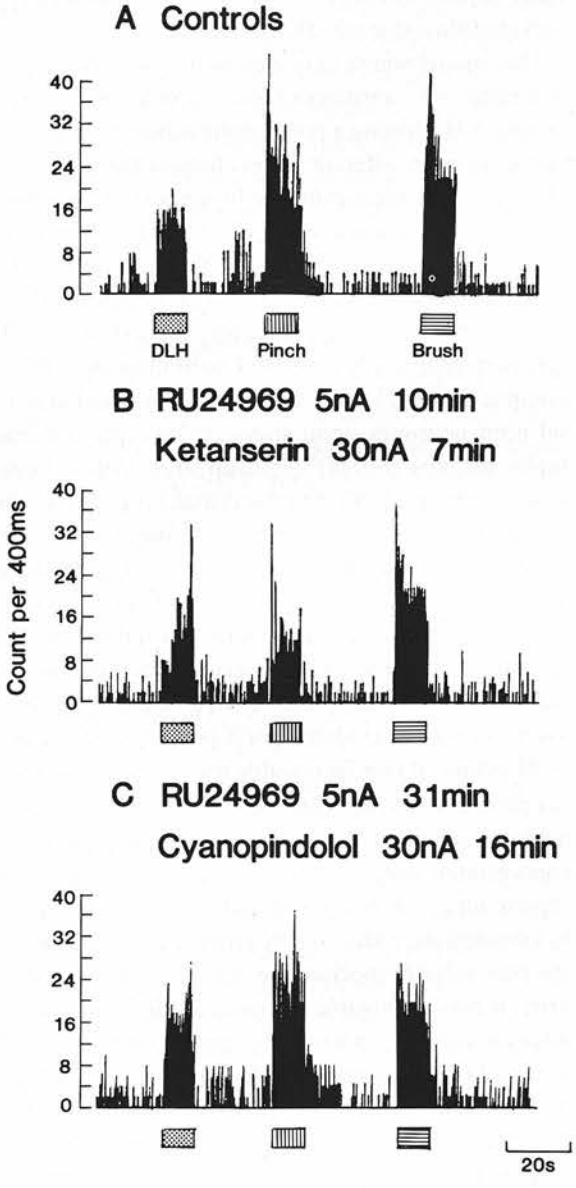


Fig. 4. Ongoing activity record of a cell showing the differential abilities of two 5-HT antagonists to reverse the selective inhibitory effect of RU 24969 on the pinch response. A shows the control excitatory responses to DLH, noxious pinch and brush. B shows that acutely applied ketanserin, a 5-HT_2 antagonist, failed to block the selective inhibitory effect of RU 24969 on the pinch response. C shows that acutely applied cyanopindolol, a 5-HT_1 antagonist, successfully reversed the effect of RU 24969.

Sequential agonist testing on cells

Six cells were tested with 5-HT and additionally with one or both 5-HT_1 receptor subtype agonists sequentially. The effect of 5-HT on 3/6 cells was a selective inhibition of pinch. In one of these cells, however, increasing the 5-HT current caused this selectivity to be abolished. The selective action of 5-HT was mimicked by RU 24969 in 3/3 cells, whilst 8-OH-DPAT caused a non-selective inhibition in all 3 cells. In the remaining 3 cells tested with 5-HT, the compound caused a non-selective decrease in all evoked responses. This effect was mimicked in 2/2 cells tested additionally with 8-OH-DPAT, but also by RU 24969 in 2/2 cells tested. Twelve cells were tested with both RU 24969 and 8-OH-DPAT in a random order. In 10/12 cells tested with RU 24969, the effect was a selective inhibition of the pinch response. In two cells, however, increasing the ejection currents abolished this selectivity. In 9 of the 10 cells displaying selective inhibition in response to RU 24969, 8-OH-DPAT caused a non-selective inhibition of all evoked responses. An example of this differential effect of agonists on the same cell is shown in Fig. 3. In one out of 10 cells, 8-OH-DPAT had no effect. One cell was unaffected by either agonist, while another showed the same non-selective inhibition in response to both agonists.

The effect of 5-HT antagonists

Two compounds were used, ketanserin, a selective 5-HT_2 receptor antagonist[42,43] and cyanopindolol, a selective 5-HT_1 antagonist (which also has high affinity to β-adrenoceptors[32]). Cyanopindolol has little affinity for 5-HT_2 sites, but high affinity for the 5-HT_{1B} site and moderate affinity for the 5-HT_{1A} site[25,32,33,50,53]. It is useful in determining whether the effects of RU 24969 and 8-OH-DPAT are really mediated through 5-HT_1 sites.

Cyanopindolol: a 5-HT_1 receptor antagonist

Cyanopindolol was tested on 13 cells. On its own, cyanopindolol (20–80 nA) had no effect. When ejected concurrently with 5-HT (which had produced selective inhibition in two cells), cyanopindolol was successful in antagonizing the effect of 5-HT in one cell whilst failing to do so in the other. Cyanopindolol ejected together with RU 24969 on 5 cells successfully antagonized the effects of the agonist in all 5

cells (RU 24969 produced selective inhibition of pinch in 4/5 cells and non-selective inhibition in one cell). The effects of 8-OH-DPAT were also antagonized in 5/6 cells (4 showed non-selective inhibition in response to the agonist, whilst one cell displayed excitation).

Ketanserin: a 5-HT$_2$ receptor antagonist

This compound was tested on 8 cells and ejected at currents of 10–50 nA concurrently with one or the other of the 5-HT$_1$ agonists. The ejection current could not be increased above 50 nA as severe spike reduction occurred. Ketanserin failed to antagonize either agonist effects in 7/8 cases. In one case only, ketanserin diminished the selective inhibitory effect of RU 24969 on the noxious input. On its own, ketanserin (20–40 nA) had no effect on the evoked responses of 7 cells, but in 3 cases, it decreased spontaneous activity. An example of the ability of cyanopindolol, but not ketanserin, to effectively antagonize the selective antinociceptive effect of RU 24969 is shown in Fig. 4.

DISCUSSION

A number of electrophysiological and behavioural studies have implicated a role for 5-HT in modulating neuronal activity in the spinal cord, as well as in behavioural analgesia. The discovery of multiple receptor sites for 5-HT in the central nervous system[5] led us to investigate which site(s) mediate(s) the action of 5-HT on nociceptive processing in the dorsal horn.

The present results confirm the heterogeneity of the effects of ionophoretically-applied 5-HT on dorsal horn neurones observed in other studies[2,26]. Three different actions of 5-HT on evoked neuronal responses were observed: selective inhibition of the nociceptive response, non-selective inhibition of all responses tested and non-selective excitation. This heterogeneity of responses to 5-HT may be a reflection of differential distribution of 5-HT receptor sites. In the majority of cells tested (10/18), the effect of 5-HT was to selectively inhibit the response to noxious cutaneous stimulation. In contrast, Headley et al.[26], found that ejecting 5-HT near cell bodies in laminae IV and V of cat dorsal horn produced non-selective inhibition of cells. In their study, however, they used much higher 5-HT currents (mean 98 ± 13 nA) than those employed in the present study (mean 12 ± 5 nA). Although direct comparison of ejection currents employed is difficult (since the concentration of drug reaching receptors is unknown) it was noted here in 3 examples that increasing 5-HT currents beyond levels producing selective antinociception abolished this selectivity.

One model which may explain the observed phenomenon of antinociceptive selectivity centres around 5-HT having a presynaptic action upon terminals of primary afferent fibres. Indeed there is some electrophysiological evidence in support of an action of 5-HT on primary afferents[6,13,31]. Furthermore, serotonergic axo-axonic contacts have been observed in the marginal zone of the rat dorsal horn[45].

A number of reports, however, suggest that 5-HT acts postsynaptically on dorsal horn neurones. Postsynaptic potentials were recorded from impaled dorsal horn neurones upon stimulation of the nucleus raphe magnus (NRM)[18,44,68], an area with a large serotonergic projection to the dorsal horn[3,4]. The effect of stimulating this area upon transmission in the dorsal horn is thought to be mediated at last partially by 5-HT[51,63,64,70]. Also, anatomical studies[30,35,51] suggest that in the superficial laminae of cat dorsal horn, serotonergic axonal contacts occur predominantly on dendritic shafts and somata of neurones rather than axons, providing evidence for a postsynaptic site for 5-HT action. It may be possible for a selective antinociceptive effect to be achieved through a postsynaptic mode of action if the relevant receptor sites were concentrated selectively on dendrites receiving nociceptive inputs. If the postulated 5-HT contacts were to interdigitate with contacts arriving from a primary afferent relaying nociceptive information to a dendrite, it may be possible to inhibit a cell's response to afferent input by preventing spatial summation of that input[51]. No firm conclusion on the mode of action of 5-HT can yet be drawn for this or other studies and multiple mechanisms may in fact be working.

Ionophoresis of 5-HT at low currents on a subpopulation of neurones in this study (4/18) resulted in non-selective inhibition of all responses tested. This type of action can be explained by a postsynaptic location for the 5-HT receptors on or near the cell body. The selectivity observed during the ejection of low currents of 5-HT was abolished in some cases, upon increasing the current, suggesting spill-over

from receptors restricted to the nociceptive pathway onto the postsynaptic sites on or near the somata of these cells. The type of action exerted by 5-HT on a cell may depend upon the activation of anatomically distinct components of the serotonergic input or on the general level of activation. Non-selective inhibition of all inputs to certain cells may function in vivo as a generalized mechanism of information discrimination or filtering. There is some evidence for serotonin involvement in diffuse noxious inhibitory controls[8,10,11,38,40,41]. It should be noted, however, that in some neurones, the selective inhibitory effect on the nociceptive response was not abolished by increasing currents of 5-HT, suggesting that generalized inhibition in response to 5-HT is not ubiquitous.

It is clear from our results with the agonists that the 5-HT_1 site is the important site for 5-HT action in the dorsal horn. The selective 5-HT_2 agonist, DOI, failed to affect nociceptive or non-nociceptive responses. In contrast, the 5-HT_1 agonists mimicked the actions of 5-HT. The two 5-HT_1 receptor subtype agonists, however, had qualitatively different effects; RU 24969, the 5-HT_{1B} receptor subtype agonist produced selective inhibition of nociceptive responses in the majority of cells tested, whilst 8-OH-DPAT, the 5-HT_{1A} receptor subtype agonist, had a predominantly non-selective inhibitory effect. The results suggest that the 5-HT_{1B} site mediates the selective inhibitory effects of 5-HT on the nociceptive input, whilst the 5-HT_{1A} site mediates the non-selective effects of 5-HT.

A comparison of the effects of more than one compound on the same neurone supports this hypothesis. In a number of cases, the non-selective inhibitory effects of 5-HT were reproduced by 8-OH-DPAT, and the selective inhibitory action of 5-HT on the pinch response was mimicked by RU 24969. The non-selective inhibition observed in a significant subpopulation of cells with RU 24969 may be explained by the moderate affinity of this compound for the 5-HT_{1A} site.

The present results also point to the existence of a minor population of cells which is unaffected by 5-HT. There have been reports in the literature on the inability of 5-HT to affect the responses of some neurones in the dorsal horn. The results obtained with the antagonists confirm and complement the results obtained with 5-HT agonists: ketanserin (a 5-HT_2 antagonist), failed to antagonize the effects of either RU 24969 or those of 8-OH-DPAT. Cyanopindolol (a 5-HT_1 antagonist), however, antagonized the various effects of 5-HT_1 agonists on neuronal responses whilst having no effect when ejected on its own. The important sites for 5-HT action in modulating sensory transmission in the dorsal horn therefore seem to be the subtypes of the 5-HT_1 receptor.

The present results show that there is a complex spectrum of 5-HT actions on dorsal horn neurones. Serotonin has a wide ranging effect in modulating both nociceptive and non-nociceptive transmission (with the possibility of discriminative effects according to the level of serotonergic activation). The action of 5-HT in the dorsal horn appears to be mediated through the 5-HT_1 sites. Different subtypes of the 5-HT_1 receptor appear to mediate the different effects of 5-HT. Our evidence indicates that whilst the 5-HT_{1A} site mediates the non-selective effects of 5-HT, the 5-HT_{1B} site mediate the selective inhibitory effect of 5-HT on the cutaneous nociceptive input to dorsal horn neurones.

ACKNOWLEDGEMENTS

This work was supported by the Faculty of Veterinary Medicine, Edinburgh University. We are very grateful to Dr. P.J. Hope for valuable help and support throughout our work. We thank Sandoz, Janssen Pharmaceuticals and ICI for generous gifts of compounds. Thanks also go to Professor A. Iggo for helpful comments on the manuscript, to Mrs. A. Leask, Mrs. H. Anderson, Mr. C. Warwick and Miss F. Manson for skilled technical assistance, and to Mrs. S. Wilson for secretarial assistance and to the Wellcome Animal Research Unit of the Faculty of Veterinary Medicine for the provision of facilities.

REFERENCES

1 Arvidsson, L.E., Hacksell, V., Nilsson, J.L.G., Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D. and Wikstrom, H., 8-Hydroxy-2-(di-*n*-propylamino) tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist, *J. Med. Chem.*, 24 (1981) 921–923.

2 Belcher, G., Ryall, R.W. and Schaffner, R., The differential effects of 5-hydroxytryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dor-

sal interneurones in the cat, *Brain Research*, 151 (1978) 307–321.
3 Bowker, R.M., Westlund, K.N. and Coulter, J.D., Origins of serotonergic projections to the spinal cord in rat: an immunocytochemical-retrograde transport study, *Brain Research*, 226 (1981) 187–199.
4 Bowker, R.M., Westlund, K.N., Sullivan, M.C. and Coulter, J.D., Organization of descending serotonergic projections to the spinal cord. In H.G. Kuypers and G.F. Martin (Eds.), Descending pathways to the spinal cord. *Progress in Brain Research, Vol. 57*, Elsevier, Amsterdam, 1982, pp. 239–266.
5 Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R., Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine, *Neuropharmacology*, 25 (1986) 563–576.
6 Carstens, E., Klumpp, D., Randic, M. and Zimmermann, M., Effect of iontophoretically applied 5-hydroxytryptamine on the excitability of single primary afferent C- and A-fibers in the cat spinal cord, *Brain Research*, 220 (1981) 151–158.
7 Chapman, C.D., Ammons, W.S. and Foreman, R.D., Raphe magnus inhibition of feline T_1-T_4 spinoreticular tract cell responses to visceral and somatic inputs, *J. Neurophysiol.*, 53 (1985) 773–785.
8 Chitour, D., Dickenson, A.H. and Le Bars, D., Pharmacological evidence for the involvement of serotonergic mechanisms in diffuse noxious inhibitory controls (DNIC), *Brain Research*, 236 (1982) 329–337.
9 Dahlstrom, A. and Fuxe, K., Evidence for the existence of monoamine-containing neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems, *Acta Physiol. Scand.*, Suppl. 247 (1965) 1–36.
10 Dickenson, A.H., Rivot, J.P., Chaouch, A., Besson, J.M. and Le Bars, D., Diffuse noxious inhibitory controls (DNIC) in the rat with or without pCPA pretreatment, *Brain Research*, 216 (1981) 313–321.
11 Dickenson, A.H., Chitour, D. and Le Bars, D., Influence of serotonergic receptor blockade on diffuse noxious inhibitory controls (DNIC) in the rat. In J. Bonica, U. Linblum and A. Iggo (Eds.), *Advances in Pain Research and Therapy, Vol. 5*, Raven, New York, 1983, pp. 155–160.
12 Duggan, A.W. and Griersmith, B.T., Inhibition of the spinal transmission of nociceptive information by supraspinal stimulation in the cat, *Pain*, 6 (1979) 149–161.
13 Dunlap, K. and Fischbach, G.D., Neurotransmitters decrease the calcium component of sensory neurone action potentials, *Nature (Lond.)*, 276 (1978) 837–839.
14 Edeson, R.O. and Ryall, R.W., Systematic mapping of descending inhibitory control by the medulla of nociceptive spinal neurones in cats, *Brain Research*, 271 (1983) 251–262.
15 Euvrard, C. and Boissier, J.R., Biochemical assessment of the central 5-HT agonist activity of RU 24969 (a piperidinyl indole), *Eur. J. Pharmacol.*, 63 (1980) 65–72.
16 Gaddum, J.H. and Piccarelli, Z.P., Two kinds of tryptamine receptors, *Br. J. Pharmacol.*, 12 (1957) 323–328.
17 Gerhart, K.D., Wilcox, T.K., Chung, J.M. and Willis, W.D., Inhibition of nociceptive and non-nociceptive responses of primate spinothalamic cells by stimulation in medial brain stem, *J. Neurophysiol.*, 45 (1981) 121–136.
18 Giesler, G.J., Gerhart, K.D., Yezierski, R.P., Wilcox, T.K. and Willis, W.D., Postsynaptic inhibition of primate spinothalamic neurons by stimulation in nucleus raphe magnus, *Brain Research*, 204 (1981) 184–188.
19 Glennon, R.A., Titeler, M. and Young, R., Structure–activity relationships and mechanism of action of hallucinogenic agents based on drug discrimination and radioligand binding studies, *Psychopharmacol. Bull.*, 22 (1986) 953.
20 Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J. and Hamon, M., Identification of presynaptic serotonin autoreceptors using a new ligand ^{3}H-PAT, *Nature (Lond.)*, 305 (1983) 140–142.
21 Gray, B.G. and Dostrovsky, J.O., Descending inhibitory influences from periaqueductal gray, nucleus raphe magnus, and adjacent reticular formation. I. Effects on lumbar spinal cord nociceptive and non-nociceptive neurons, *J. Neurophysiol.*, 49 (1983) 932–947.
22 Griersmith, B.T. and Duggan, A.W., Prolonged depression of spinal transmission of nociceptive information by 5-HT administered in the substantia gelatinosa: antagonism by methysergide, *Brain Research*, 187 (1980) 231–236.
23 Guilbaud, G., Oliveras, J.L., Giesler, G. and Besson, J.M., Effects induced by stimulation of the centralis inferior nucleus of the raphe on dorsal horn interneurons in cat's spinal cord, *Brain Research*, 126 (1977) 355–360.
24 Hall, M.D., El Mestikawy, S., Emerit, M.B., Pichat, L., Hamon, M. and Gozlan, H., [^{3}H]8-hydroxy-2-(di-*n*-propylamino) tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain, *J. Neurochem.*, 44 (1985) 1685–1689.
25 Hamon, M., Cossery, J.M., Spampinato, U. and Gozlan, H., Are there selective ligands for 5-HT_{1A} and 5-HT_{1B} receptor binding sites in brain? *Trends Pharmacol. Sci.*, 7 (1986) 336–338.
26 Headley, P.M., Duggan, A.W. and Griersmith, B.T., Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones, *Brain Research*, 145 (1978) 185–189.
27 Heuring, R., Schlegel, J.R. and Peroutka, S.J., Species variation in RU 24969 interactions with non-5-HT_{1A} binding sites, *Eur. J. Pharmacol.*, 122 (1986) 279–282.
28 Heuring, R.E. and Peroutka, S.J., Characterization of a novel ^{3}H-5-hydroxytryptamine binding site subtype in bovine brain membranes, *J. Neurosci.*, 7 (1987) 894–903.
29 Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D., Wikstrom, H., Arvidsson, L.E., Hacksell, U. and Nilsson, J.L.G., 8-Hydroxy-2-(di-*n*-propylamino) tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT receptor stimulating activity, *J. Neural Transm.*, 55 (1982) 169–188.
30 Hoffert, M.J., Miletic, V., Ruda, M.A. and Dubner, R., Immunocytochemical identification of serotonin axonal contacts on characterized neurons in laminae I and II of the cat dorsal horn, *Brain Research*, 267 (1983) 361–364.
31 Holziv, G.G., Shefner, S.A. and Anderson, E.G., Serotonin depolarizes type A and C primary afferents: an intracellular study in bullfrog dorsal root ganglion, *Brain Research*, 327 (1985) 71–79.
32 Hoyer, D., Engel, G. and Kalkman, H.O., Characterization of the 5-HT_{1B} recognition site in rat brain: binding studies with (–) [^{125}I]iodocyanopidolol, *Eur. J. Pharmacol.*, 118 (1985) 1–12.
33 Hoyer, D., Engel, G. and Kalkman, H.O., Pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain

membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (–) [^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin, *Eur. J. Pharmacol.*, 118 (1985) 13–23.

34 Hylden, J.L.K., Ruda, M.A., Hayashi, H. and Dubner, R., Descending serotonergic fibers in the dorsolateral and ventral funiculi of cat spinal cord, *Neurosci. Lett.*, 62 (1985) 299–304.

35 Hylden, J.L.K., Hayashi, H., Ruda, M.A. and Dubner, R., Serotonin innervation of physiologically identified lamina I projection neurons, *Brain Research*, 370 (1986) 401–404.

36 Jordan, L.M., Kenshalo, D.R., Martin, R.F., Haber, L.H. and Willis, W.D., Depression of primate spinothalamic tract neurons by ionophoretic application of 5-hydroxytryptamine, *Pain*, 5 (1978) 135–142.

37 Kajander, K.C., Ebner, T.J. and Bloedel, J.R., Effects of periaqueductal gray and raphe magnus stimulation on the responses of spinocervical and other ascending projection neurons to non-noxious inputs, *Brain Research*, 291 (1984) 29–37.

38 Kraus, E., Besson, J.M. and Le Bars, D., Behavioral model for diffuse noxious inhibitory controls (DNIC): potentiation by 5-hydroxytryptophan, *Brain Research*, 231 (1982) 461–465.

39 La Motte, C.C. and De Lanerolle, N., Substance P, enkephalin, and serotonin: ultrastructural basis of pain transmission in primate spinal cord. In J. Bonica, U. Lindblum and A. Iggo (Eds.), *Advances in Pain Research and Therapy, Vol. 5*, Raven, New York, 1983, pp. 247–255.

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

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

42 Leysen, J.E., Niemegeers, C.J.E., Van Neuten, J.M. and Laduron, P.M., [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites, *Mol. Pharmacol.*, 21 (1982) 301–314.

43 Leysen, J.E., De Chaffoy De Courcelles, D., De Clerck, F., Niemegeers, C.J.E. and Van Neuten, J.M., Serotonin-S$_2$ receptor binding sites and functional correlates, *Neuropharmacology*, 23 12B (1984) 1493–1501.

44 Light, A.R., Casale, E.J. and Menetrey, D.M., The effects of focal stimulation in nucleus raphe magnus and periaqueductal gray on intracellularly recorded neurons in spinal laminae I and II, *J. Neurophysiol.*, 56 (1986) 555–571.

45 Maxwell, D.J., Leranth, Cs. and Verhofstad, A.A.J., Fine structure of serotonin-containing axons in the marginal zone of the rat spinal cord, *Brain Research*, 266 (1983) 253–259.

46 McCreery, D.B. and Bloedel, J.R., Reduction of the response of cat spinothalamic neurons to graded mechanical stimuli by electrical stimulation of the lower brain stem, *Brain Research*, 97 (1975) 151–156.

47 McCreery, D.B., Bloedel, J.R. and Hames, E.G., Effects of stimulating in the raphe nuclei and in reticular formation on response of spinothalamic neurons to mechanical stimuli, *J. Neurophysiol.*, 42 (1979) 166–182.

48 Middlemiss, D.N. and Fozard, J.R., 8-Hydroxy-2-(di-*n*-propylamino)-tetralin discriminates between subtypes of the 5-HT$_1$ recognition site, *Eur. J. Pharmacol.*, 90 (1983) 151–153.

49 Middlemiss, D.N., The putative 5-HT$_1$ receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor, *J. Pharm. Pharmacol.*, 37 (1985) 434–437.

50 Middlemiss, D.N., Blockade of the central 5-HT autoreceptor by β-adrenoceptor antagonists, *Eur. J. Pharmacol.*, 120 (1986) 51–56.

51 Miletic, V., Hoffert, M.J., Ruda, M.A., Dubner, R. and Shigenaga, Y., Serotonergic axonal contacts on identified cat spinal dorsal horn neurons and their correlation with nucleus raphe magnus stimulation, *J. Comp. Neurol.*, 228 (1984) 129–141.

52 Mitchell, R. and Riley, S., Characterization of receptors for 5-hydroxytryptamine in spinal cord, *Biochem. Soc. Trans.*, 13 (1985) 955.

53 Neale, R.F., Fallon, S.L., Boyar, W.C., Wasley, J.W.F., Martin, L.L., Stone, G.A., Glaeser, B.S., Sinton, C.M. and Williams, M., Biochemical and pharmacological characterization of CGS 12066B, a selective serotonin-1B agonist, *Eur. J. Pharmacol.*, 136 (1987) 1–10.

54 Oliveras, J.L., Bourgoin, S., Hery, F., Besson, J.M. and Hamon, M., The topographical distribution of serotoninergic terminals in the spinal cord of the cat: biochemical mapping by the combined use of microdissection and microassay procedures, *Brain Research*, 138 (1977) 393–406.

55 Pazos, A., Hoyer, D. and Palacios, J.M., The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site, *Eur. J. Pharmacol.*, 106 (1984) 539–546.

56 Pazos, A. and Palacios, J.M., Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors, *Brain Research*, 346 (1985) 205–230.

57 Pazos, A., Cortes, R. and Palacios, J.M., Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors, *Brain Research*, 346 (1985) 231–249.

58 Pedgio, N.W., Yamamura, H.I. and Nelson, D.L., Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain, *J. Neurochem.*, 36 (1981) 220–226.

59 Peroutka, S.J. and Snyder, S.H., Multiple serotonin receptors: differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol, *Mol. Pharmacol.*, 16 (1979) 687–699.

60 Peroutka, S.J. and Snyder, S.H., Two distinct serotonin receptors: regional variations in receptor binding in mammalian brain, *Brain Research*, 208 (1981) 339–347.

61 Peroutka, S.J., Pharmacological differentiation and characterization of 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ binding sites in rat frontal cortex, *J. Neurochem.*, 47 (1986) 429–450.

62 Randic, M. and Yu, H.H., Effects of 5-hydroxytryptamine and bradykinin in cat dorsal horn neurones activated by noxious stimuli, *Brain Research*, 111 (1976) 197–203.

63 Rivot, J.P., Chaouch, A. and Besson, J.M., Nucleus raphe magnus modulation of response of rat dorsal horn neurons to unmyelinated fiber inputs: partial involvement of serotonergic pathways, *J. Neurophysiol.*, 44 (1980) 1039–1057.

64 Rivot, J.P., Chiang, C.Y. and Besson, J.M., Increase of serotonin metabolism within the dorsal horn of the spinal cord during nucleus raphe magnus stimulation, as revealed

by in vivo electrochemical detection, *Brain Research*, 238 (1982) 117–126.

65 Schmauss, C., Hammond, D.L., Ochi, J.W. and Yaksh, T.L., Pharmacological antagonism of the antinociceptive effects of serotonin in the rat spinal cord, *Eur. J. Pharmacol.*, 90 (1983) 349–357.

66 Segu, L. and Calas, A., The topographical distribution of serotoninergic terminals in the spinal cord of the cat: quantitative radioautographic studies, *Brain Research*, 153 (1978) 449–464.

67 Sills, M.A., Wolfe, B.B. and Frazer, A., Determination of selective and nonselective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in cat frontal cortex, *J. Pharmacol. Exp. Ther.*, 231 (1984) 480–487.

68 Willis, W.D., Haber, L.H. and Martin, R.F., Inhibition of spinothalamic tract cells and interneurons by brain stem stimulation in the monkey, *J. Neurophysiol.*, 40 (1977) 968–981.

69 Yaksh, T.L. and Wilson, P.R., Spinal serotonin terminal system mediates antinociception, *J. Pharmacol. Exp. Ther.*, 208 (1979) 446–453.

70 Yezierski, R.P., Wilcox, T.K. and Willis, W.D., The effects of serotonin antagonists on the inhibition of primate spinothalamic tract cells produced by stimulation in nucleus raphe magnus or periaqueductal gray, *J. Pharmacol. Exp. Ther.*, 220 (1982) 266–277.