STUDIES ON CHARACTERIZED

NUCLEUS GIGANTOCELLULARIS NEURONES

by

DAVID PLATT HARRIS

B.Sc., the University of Victoria

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Division of Pharmacology and Toxicology in the

Faculty of Pharmaceutical Sciences)

We accept this thesis as conforming

to the required standards

THE UNIVERSITY OF BRITISH COLUMBIA

MAY 1980

© DAVID PLATT HARRIS, 1980

In presenting this thesis in partial fulfilment of the requirements for

an advanced degree at the University of British Columbia, I agree that

the Library shall make it freely available for reference and study.

I further agree that permission for extensive copying of this thesis

for scholarly purposes may be granted by the Head of my Department or

by his representatives. It is understood that copying or publication

of this thesis for financial gain shall not be allowed without my

written permission.

Division of Pharmacology and Toxicology

Faculty of Pharmaceutical Sciences

The Univesity of British Columbia

2075 Wesbrook Place

Vancouver, Canada

V6H 1W5

April 23, 1980.

ABSTRACT

Periaqueductal gray (PAG) stimulation produced antinociception may be mediated by inhibition of an ascending nociceptive pathway at the brainstem level and/or the activation of descending inhibition on spinal nociceptor-driven neurones. Experiments were performed on urethane anaesthetized rats to establish if there is a monosynaptic pathway from the PAG to the nucleus gigantocellularis (nGC). Single units in the PAG were able to be antidromically activated by stimulation of the nGC, indicating that a direct pathway does exist.

To further clarify nGC involvement in the two possibilities stated above, single units in the nGC were characterized according to their responses to natural stimuli. About one-half of the neurones tested were excited by noxious stimuli. Similarly, about one-half of those tested with PAG stimulation were inhibited. Of those neurones affected by both noxious peripheral stimuli and PAG stimulation, the largest group (37%) were excited by the noxious stimuli and inhibited by the PAG stimulation. Intravenous and iontophoretic fluoxetine, a specific serotonin uptake blocker, enhanced the period of inhibition and reduced the excitation of nGC neurones due to PAG stimulation. Furthermore, inhibition of nGC neurones by iontophoretic serotonin or fluoxetine was correlated with the PAG stimulation produced inhibition of these neurones, suggesting that this inhibition was mediated by serotonin.

These data suggest that many nGC neurones are in an ascending nociceptive pathway and under inhibitory control of the PAG.

John G. Sinclair, Ph. D.,

Supervisor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

<u>ACKNOWLEDGEMENT</u>

To my wife, Liza, and my mother, Ruth.

INTRODUCTION

In 1969 Reynolds reported that electrical stimulation of the periaqueductal gray matter produced sufficient analgesia to allow laparotomies to be performed on unanaesthetized rats. This paper caused an abundance of research on stimulation produced antinociception from different brain regions, including the periaqueductal gray (Reynolds, 1969; Mayer et al., 1971; Oleson et al., 1978) and the nucleus reticularis gigantocellularis (nGC) (Takagi et al., 1975; McCreery and Bloedel, 1975). In particular, stimulation of the periaqueductal gray produces both a reduction in behavioural responses (Oleson et al., 1978) and reflex responses (Mayer et al., 1971; Akil et al., 1976; Yaksh et al., 1976c; Yeung et al., 1977; Oleson et al., 1978; Rhodes and Liebeskind, 1978) to noxious stimuli. In addition, this reduction was reported to last up to five minutes beyond the period of stimulation (Mayer et al., 1971; Rhodes and Liebeskind, 1978). However, the mechanism of stimulation produced antinociception in general, and specifically that of the periaqueductal gray, is still not known in any detail.

Periaqueductal gray stimulation produced antinociception may be mediated by the inhibition of an ascending nociceptive pathway at any level of the nervous system, including the spinal cord or brainstem levels. Noxious peripheral information travels through the dorsal horn and eventually reaches the thalamus, either directly via the spinothalamic tract, or indirectly via the spinoreticular tract, the spinocervical tract, or propriospinally. The spinoreticular tract,

propriospinal fibres, and probably collaterals of the spinothalamic tract are also known to terminate in the nucleus reticularis gigantocellularis (Mehler et al., 1960; McCreery and Bloedel, 1975).

This thesis started with the premise that periaqueductal gray stimulation produced antinociception may be mediated by the nucleus reticularis gigantocellularis. This nucleus may, on one hand, be involved in this antinociception by inhibition of an ascending nociceptive pathway at the spinal cord level. Electrical stimulation of the periaqueductal gray has been reported to inhibit spinal cord nociceptor-driven neurones (Oliveras et al., 1974a). However, the periaqueductal gray has few direct axonal projections to the spinal cord, therefore, these inhibitory effects must be mediated by other brainstem nuclei. The most often suggested nucleus for this role is the nucleus raphé magnus (cf. Mayer and Price, 1976), but the nGC is also a possibility. The nucleus gigantocellularis is known to project to the spinal cord (Wolstencroft, 1964) and electrical stimulation of the nGC inhibits spinal cord nociceptor-driven neurones (Takagi et al., 1975). Therefore, the nucleus gigantocellularis may mediate periaqueductal gray stimulation produced antinociception by inhibiting an ascending nociceptive pathway at the spinal level.

Conversely, the nucleus gigantocellularis may mediate the antinociception by inhibition of the ascending pathway at the brainstem level. The nGC is known to receive noxious input from the spinal cord (Schiebel and Schiebel, 1968; Wolstencroft, 1964; Casey, 1969, 1971a). The nucleus gigantocellularis is

also known to have axonal projections to the thalamus (Bowsher et al., 1968), suggesting that nGC neurones may be in an ascending nociceptive pathway. Also, periaqueductal gray stimulation has been reported to inhibit nucleus gigantocellularis neurones (Morrow and Casey, 1976). Therefore, periaqueductal gray stimulation produced antinociception may be mediated by the nucleus gigantocellularis by inhibition of ascending nociceptive information at the brainstem level.

This thesis involved experiments in which neurones in the vicinity of the periaqueductal gray were antidromically activated by stimulation of the nucleus gigantocellularis and experiments correlating the responses of nGC neurones to various tests. These tests included noxious and non-noxious peripheral stimuli, stimulation of the PAG, and intravenously and iontophoretically administered serotonin and fluoxetine, a specific serotonin uptake blocker. As well, the effects of intravenous and iontophoretic fluoxetine on the responses of nGC neurones to noxious peripheral stimuli and periaqueductal gray stimulation were studied.

The studies reported in this thesis were directed at establishing evidence for a monosynaptic pathway from the PAG to the NGC and for the involvement of the nGC in the mediation of the antinociceptive effects of periaqueductal gray stimulation via ihibition of nociceptive information in either the spinal cord or the nucleus gigantocellularis.

BACKGROUND

A. Nociceptors and their Afferent Fibres

Nociceptors are defined as receptors which respond to noxious stimuli, that is, stimuli which are potentially or actually damaging to the tissue stimulated. Nociceptors are classified by their responses to various stimuli, the afferents supplying them, and their location. Although the different classes of nociceptors have distinctive responses to noxious stimuli they all appear to have free endings and to be supplied by small-sized afferent fibres. These fibres include myelinated A-delta and unmyelinated C fibres (Paintal, 1960; Burgess and Perl, 1973). The major cutaneous nociceptors include the A-delta mechanical, A-delta heat, and C polymodal receptors. A-delta mechanical nociceptors are excited best by damaging mechanical stimuli and do not respond to noxious heat or algesic chemicals (Burgess and Perl, 1967; Perl, 1968; Burgess et al., 1968). A-delta heat nociceptors are excited by noxious heat (> 45 °C) as well as intense mechanical stimuli (Beck et al., 1974; Georgopoulos, 1976, 1977). The third major class of nociceptors are the C polymodal nociceptors. These are abundant and respond to noxious mechanical, thermal, and chemical stimuli (Bessou and Perl, 1969). Muscle nociceptors are also activated by noxious mechanical, thermal, or algesic chemical stimuli (Paintal, 1960; Stacey, 1969).

B. Spinal Cord Substrates of Nociception

Laminae of the Spinal Cord Gray Matter

The anatomy and to a large extent the electrophysiology of
the spinal cord is described in the terms introduced by
Cajal (1909) and Rexed (1952). Rexed divided the spinal cord
gray matter into nine discrete laminae. These are described in
terms of their cell types, inputs, and outputs in the following
sections.

Lamina I - the Marginal Layer

The most prominent feature of lamina I is the 'marginal
cell' of Waldeyer (1888). These sparse large neurones have
extensive overlapping dendritic trees made up of relatively long
unbranched axons. Most of the axons remain inside the marginal
layer but a few penetrate into deeper laminae (Waldeyer,1888;
Cajal, 1909; Scheibel and Scheibel, 1968). In addition to these
cells there are two other types of neurones in this lamina: very
small and medium sized cells. The latter have large dendritic
tufts projecting into the substantia gelatinosa and axons
projecting to the fasciculus proprius or Lissauer's tract
(Cajal, 1909).

The marginal layer receives fine afferents from the tract
of Lissauer, some of which may be primary afferents, but most
are probably propriospinal (Kerr, 1975a). Peripheral nerve
stimulation studies in cats and monkeys have indicated lamina I
neurones receive input from both A-delta and C fibres

(Christensen and Perl, 1970; Kumazawa et al., 1975). Recent autoradiographical studies in the cat and monkey (Réthelyi et al., 1979) and horseradish peroxidase (HRP) studies in the cat (Light et al., 1979; Light and Perl, 1979a,b) also indicate lamina I receives small diameter myelinated nociceptive fibres. Many neurones in lamina I respond specifically to noxious mechanical and/or noxious thermal stimuli (Christensen and Perl, 1970; Cervero et al., 1976, 1979), but a few can be classified as wide-dynamic range neurones as they respond to non-noxious as well as noxious stimuli (Cervero et al., 1976).

Marginal layer neurones probably contribute to the spinothalamic tract. They have been antidromically activated by stimulation of the spinothalamic tract from the diencephalic level in the cat (Willis et al., 1974) and cervical level in the cat and monkey (Kumazawa et al., 1975). Horseradish peroxidase studies have also located neurones in this lamina in the cat (Trevino and Carstens, 1975; Carstens and Trevino, 1978) and the monkey (Trevino and Carstens, 1975; Willis et al., 1979). In addition, marginal layer neurones may project propriospinally to more rostral sites via the tract of Lissauer or the lateral fasciculi proprius (Schiebel and Schiebel, 1968; Cervero et al., 1979).

Laminae II and III -the Substantia Gelatinosa

The neurones in lamina II are small but have quite extensive radial dendritic trees extending from their apical and basal poles (Cajal, 1909; Schiebel and Schiebel, 1968). The dendrites reach fairly extensively dorsomedially but usually do

not leave laminae II or III. The neurones in lamina III are slightly larger and more dispersed and have larger and more complex dendritic arborizations (Schiebel and Schiebel, 1968).

Lamina II and III appear to be distinguishable by their afferent input. Lamina II neurones receive fine fibres, most of which are collaterals of axons running in the tract of Lissauer or the nearby white matter, although other areas might contribute to them (Cajal, 1909; Szentagothai, 1964; Schiebel and Schiebel, 1968). These fibres are probably nociceptive unmyelinated fibres as lamina II neurones are reported to receive this class of fibres (Kurazawa and Perl, 1976; Yaksh et al., 1977; Light et al., 1979a,b). Conversely, lamina III neurones are reported to receive coarse collaterals of primary afferent origin from large fibres which pass ventrally along the medial edge of the dorsal horn before turning and entering lamina III from below in a flame-shaped arborization (Cajal, 1909; Szentagothai, 1964). These were reported to be innocuous myelinated afferent fibres in studies involving: electrical stimulation of nerves in the monkey (Kumazawa and Perl, 1976, 1978) and the cat (Yaksh et al., 1977; Wall et al., 1979); autoradiography in the cat and monkey (Réthelyi et al., 1979); and HRP in the cat (Light and Perl, 1979a,b).

The axonal projections of the neurones in these laminae either never leave the substantia gelatinosa or temporarily run in the tract of Lissauer or fasciculi proprius before terminating back in the substantia gelatinosa (Cajal, 1909; Szentagothai, 1964; Schiebel and Schiebel, 1968). This suggests that the substantia gelatinosa is a closed system. However,

outflow from the substantia gelatinosa probably exists via lamina IV antenna neurones which have dendrites projecting into the substantia gelatinosa (Szentagothai, 1964; Réthelyi and Szentagothai, 1973) or by way of axons from lamina III neurones, some of which are reported to dip into deeper laminae before returning to the substantia gelatinosa (Mannen and Sugiura, 1976).

Neurones in the substantia gelatinosa respond to noxious as well as innocuous cutaneous stimuli. Many are activated by polymodal or mechanical nociceptors (Kumazawa and Perl, 1976, 1978), by innocuous stimuli or by both (Yaksh et al., 1977; Wall et al., 1979). Also, some neurones display extended discharges after only brief stimuli (Yaksh et al., 1977; Cervero et al., 1977; Wall et al., 1979).

Laminae IV, V, and VI - the Nucleus Proprius

These laminae have neurones of varied size interspersed between myelinated fibres (Cajal, 1909). Lamina IV neuronal dendrites are orientated longitudinally, whereas the dendrites of neurones in lamina V are orientated in the dorsoventral plane (Réthelyi and Szentagothai, 1973). The dorsal dendrites of many of these neurones, especially those in lamina IV, penetrate into the substantia gelatinosa and therefore probably receive afferent input similar to that of substantia gelatinosa neurones (Szentagothai, 1964; Réthelyi and Szentagothai, 1973). These neurones are termed antenna neurones and are suggested to be the major neuronal outflow of the substantia gelatinosa (Réthelyi and Szentagothai, 1973).

Recent HRP studies have reported nociceptive afferent fibres terminating in these laminae. Light and Perl (1979a,b) report that mechanical nociceptive slowly conducting afferent fibres end in lamina V, and Réthelyi et al. (1979) report that small to medium afferents responding to mechanical noxious stimuli end in the lateral parts of lamina V. Many neurones in these laminae are wide-dynamic range neurones but narrow range neurones responding to just innocuous or just noxious stimuli have also been reported (Wall, 1960, 1967; Mendell, 1966; Willis et al., 1974; Fields et al., 1977a).

The axonal projections of these laminae have been suggested to contribute to the spinothalamic, spinoreticular, spinocervical, and propriospinal systems (Rexed, 1952; Réthelyi and Szentagothai, 1973). Indeed, antidromic activation from the brainstem (Willis et al., 1974; Giesler et al., 1977; Fields et al., 1977b) and HRP studies in the rat (Giesler et al., 1977) and the monkey (Willis et al., 1979) both indicate that these laminae are the origin of the spinothalamic and spinoreticular tracts.

Ascending Nociceptive Pathways

Many ascending pathways exist in the spinal cord which convey nociceptive information. In man, the major pathway for pain is the crossed spinothalamic tract (Kuru, 1949; White and Sweet, 1969), however, other pathways are undoubtedly involved because surgical procedures which lesion this tract produce only temporary cessation of pain sensation (White and Sweet, 1969). There is also a wide variation in the distribution of

nociceptive pathways among species. For instance, the cat has a relatively poorly developed spinothalamic tract. The following sections will discuss the major nociceptive pathways in greater detail.

The Spinothalamic Tract

The spinothalamic tract is the classical pathway for carrying pain in man. Neurones of origin for this tract are located in the gray matter of the spinal cord. Their axons cross the spinal cord via the ventral white commissure and run rostrally in the ventrolateral quadrants. Although there appears to be a difference in the route taken by the lateral versus the ventral spinothalamic tracts, their final destinations in the thalamus appear to be identical in the monkey (Kerr, 1975b). These two tracts terminate in the ventrobasal, posterior, and intralaminar nuclei of the thalamus in the monkey (Mehler et al., 1960; Kerr and Lippman, 1974; Boivie, 1979) and the cat (Holloway et al., 1978; Fox et al., 1980). The intralaminar termination is suggestive of a functional role in arousal mechanisms.

The origin of the spinothalamic tract has been studied extensively with retrograde and electrophysiological methods. The retrograde studies indicate the greatest concentration of spinothalamic tract neurones in monkeys and rats to be in the dorsal horn laminae I, IV, and V, with lesser concentrations in the more ventral parts of the gray matter (Kerr, 1975b; Trevino and Carstens, 1975; Carstens and Trevino, 1978; Giesler et al., 1977). However, HRP studies in the cat indicated most

spinothalamic tract neurones of origin were in the ventral horn (Trevino and Carstens, 1975). Similarly, electrophysiological studies involving the antidromic activation of spinal cord neurones by electrical stimulation of the spinothalamic tract at either its terminus or at more caudal sites substantiate the retrogade findings in the cat (Dilly et al., 1968; Trevino et al., 1972), rat (Dilly et al., 1968; Giesler et al., 1977), and the monkey (Trevino et al., 1973; Albe-Fessard et al., 1974; Willis et al., 1974).

Spinothalamic tract neurones have been shown to respond to non-noxious and/or noxious stimuli. Effective noxious stimuli include mechanical, thermal, and chemical algesics. Nociceptive-specific spinothalamic neurones are located mainly in lamina I, but some occur in the deeper laminae (Dilly et al., 1968; Trevino and Carstens, 1975; Willis et al., 1974; Kumazawa et al., 1975; Levante et al., 1975). Foreman et al. (1979) report that mechanical and algesic chemical (bradykinin, serotonin, and KCl) stimulation of fine muscle afferents excited many spinothalamic tract neurones but did not affect those in lamina I. Most spinothalamic neurones are reported to be wide-dynamic range neurones, that is, they respond to both innocuous and noxious stimuli (Willis et al., 1974; Albe-Fessard, 1974; McCreery and Bloedel, 1975).

The Spinoreticular Tract

The spinoreticular tract arises in the spinal cord gray matter and projects ipsilaterally to the brainstem in the lateral and ventral funiculi. It is divided into two parts: the first terminates in the lateral reticular nucleus and is part of the spinoreticularcerebellar pathway (Oscarrson, 1973); the second part terminates in the medial reticular formation and will be dealt with further below.

The terminations in the medial reticular formation include the nucleus medullae oblongatae centralis, nucleus reticularis gigantocellularis (nGC), nuclei reticularis pontis caudalis and oralis, nuclei paragigantocellularis dorsalis and lateralis, nucleus subcoeruleus, and nuclei raphé pallidus and magnus (Mehler et al., 1960; Bowsher and Westman, 1970; Kerr, 1975b; Brodal et al., 1960). Although this pathway is reported to be uncrossed by studies involving commissural myelotomy in the monkey (Kerr and Lippman, 1974), it appears to be bilateral in the cat (Fields et al., 1975, 1977b).

Antidromic activation of spinoreticular tract neurones by stimulation of the reticular formation indicates a concentration of cells of origin in laminae VI to VIII of the spinal cord (Levante and Albe-Fessard, 1972; Fields et al., 1975, 1977b). Fields et al. (1975, 1977b) noted that stimulation in the nGC resulted in the antidromic activation of neurones in the more superficial layers of the dorsal horn, particularly lamina I. The neurones projecting to the nGC often only responded to stimulation of deep structures, although some responded to both innocuous and noxious or only noxious cutaneous stimuli (Fields

et al., 1975). These neurones displayed contra-, ipsi-, and bilateral receptive fields. The neurones responding to noxious stimuli were often located in the ventral horn in laminae IV to IX (Fields et al., 1975).

The Spinocervicothalamic Tract

The spinocervical tract (SCT) ascends ipsilaterally in the dorsolateral fasciculus of the spinal cord to terminate in the lateral cervical nucleus (Cajal, 1909; Ha and Liu, 1966). Axonal projections from the lateral cervical nucleus (LCN) decussate and ascend to the ventroposterolateral, posterior, and medial geniculate nuclei of the thalamus (Boivie, 1970).

Rexed (1951) identified the lateral cervical nucleus in a wide range of species, including the cat and monkey, but concluded that it does not exist as such in the rat. Recent HRP studies by Giesler et al. (1979) have demonstrated a diffuse group of cells in the rat which is shifted somewhat dorsally and laterally from the position of the well-defined LCN of the cat. This cell group in the rat may be equivalent to the LCN as it responds to similar stimuli. However, Giesler et al. (1979) noted that the conduction velocity for the cervico-thalamic tract in the rat was about a quarter of that in the cat and they suggested that this pathway may serve a different function from that in the cat.

The cells of origin of the spinocervical tract in the cat and monkey have been located both electrophysiologically (Bryan et al., 1973; Brown et al., 1976; Cervero et al., 1977) and by labelling studies using procion yellow or HRP (Craig, 1976; Snow

et al., 1976). Most cells bodies were located in lamina IV, with some also in laminae I and V to VIII. Most SCT neurones responded to A fibre stimulation with or without responding to C fibre stimulation (Mendell, 1966; Brown et al., 1973a, 1975). Some spinocervical tract neurones also respond to group III and IV muscle afferent stimulation (Kniffi et al., 1977; Hamann et al., 1978). Most SCT neurones respond to hair movement, but many also respond to noxious mechanical, thermal, or algesic chemical stimuli (Brown et al., 1975; Cervero et al., 1977; Kniffi et al., 1977; Giesler et al., 1979).

The Spinotectal Tract

The term spinotectal tract is applied generally to spinal cord projections to the midbrain which ascend in the ventral quadrants (Anderson and Berry, 1959; Mehler et al., 1960). This tract terminates in the superior colliculus, the external nucleus of the inferior colliculus, the periaqueductal gray matter, and the midbrain reticular formation (Anderson and Berry, 1959; Mehler et al., 1960; Schroeder and Jane, 1971; Kerr, 1975b). Trevino (1976) reported cells of origin in laminae I and IV to VII in the monkey. Other species have not been studied but it is assumed that the origin of this tract is similar to that of the spinothalamic and spinoreticular tracts. No recordings from spinotectal tract neurones have been done but responses of midbrain reticular and PAG neurones suggest that this pathway is activated best by A-delta and C fibres, with additional input from A-alpha and A-beta fibres and group II and III muscle afferent fibres (Colins and Randt, 1960; Pompieano

and Swett, 1963).

C. Mesencephalic Nuclei

The Periaqueductal Gray Matter

The periaqueductal gray matter (PAG) consists of a volume of fairly small neurones free from myelinated fibres that surrounds the aqueduct of Sylvius. It contains a high density of opiate receptors (Pert and Yaksh, 1974) and has been implicated in antinociception. Analgesia has been induced both by electrical stimulation of and by microinjection of opiates and opioids into the PAG (Reynolds, 1969; Mayer et al, 1971; Pert and Yaksh, 1974; Yaksh et al., 1976a-c).

The PAG receives peripheral sensory input either directly or via other brainstem nuclei. Observation of denervation of the PAG after cordotomies suggests that the PAG receives ascending afferents directly via the spinotectal tract (Mehler et al., 1960; Kerr, 1975b). The recording of evoked potentials also implies a peripheral sensory input to the PAG. Liebeskind and Mayer (1971) reported that stimulation of the skin of any limb, the tail, or the face caused short latency evoked potentials in the PAG of rats under deep Nembutal anaesthesia. Similarly, recordings made in the PAG of curarized, artificially respirated rats showed evoked potentials from electrical stimulation of the sciatic nerve (Palmer and Klemm, 1976). The PAG also receives afferents from other brain areas. Beitz (1979) reports a major afferent input to the PAG from the hypothalamus, with additional minor projections from the caudal

raphé.

The periaqueductal gray matter has axonal projections both rostally to the diencephalon and caudally to the brainstem. Autoradiographical studies indicate projections to the reticular formation and the nucleus raphé magnus (Ruda, 1975). Horseradish peroxidase (HRP) studies by Gallager and Pert (1978) report projections to the nucleus reticularis gigantocellularis as well as to the caudal raphé. Electrophysiological studies have also been completed which indicate input to the nucleus raphé magnus from the PAG. Pomeroy and Behbehani (1979a,b) electrically stimulated in the PAG and recorded activation of single units in the nucleus raphé magnus. Although this study could not indicate if this was a direct pathway, antidromic activation of PAG neurones by stimulation of the nucleus raphé magnus indicate there is a monosynaptic connection (Dostrovsky and Shah, 1979). Although electrical stimulation of the PAG has been reported to inhibit spinal cord neurones, the PAG has not been reported to have direct projections to the cord by either denervation studies (Nyberg-Hansen, 1965), HRP studies (Kuypers and Maisky, 1975; Basbaum and Fields, 1977b), or autoradiographical studies (Ruda, 1975).

There have been numerous reports of electrical stimulation of the PAG causing analgesia. The first report of PAG stimulation produced antinociception (SPA) was published by D. V. Reynolds in 1969. He reported that laparotomies could be performed on unanaesthetized rats without any overt reactions to the procedure while the PAG was being focally stimulated. Antinociception induced by stimulation in the PAG has been found

by other workers in the rat (Mayer et al., 1971; Akil and Mayer, 1972; Mayer and Liebeskind, 1974; Mayer and Hayes, 1975; Akil et al., 1976; Oleson et al., 1978), in the cat (Liebeskind et al., 1973; Oliveras et al., 1974a,b), and in other species including man (Richardson and Akil, 1977a,b).

Periaqueductal gray SPA has been shown to reduce both the reflex and behavioural responses to noxious stimuli. Stimulation of the PAG increases the latency of the tail-flick reflex (Akil and Mayer, 1972; Mayer and Liebeskind, 1974; Akil et al., 1976; Yaksh et al., 1976c), the threshold for jump to electric shock (Mayer and Liebeskind, 1974), leg flexion to footpad shock (Oleson et al., 1978), and the jaw-opening reflex to tooth pulp stimulation (Oliveras et al., 1974b).

The periaqueductal gray SPA is reported to include large areas of the body, usually at least one quadrant and often the whole body (Mayer et al., 1971; Liebeskind et al., 1973; Richardson and Akil, 1977b). The analgesia produced is approximately equivalent to that produced by 10 - 50 mg/kg of morphine administered intravenously (Mayer and Liebeskind, 1974; Oleson et al, 1978). While this amount of morphine is sufficient to produce obvious side effects, such as extensor rigidity, the action of PAG stimulation appears to be specific for analgesia. During stimulation, animals respond normally to fright evoking stimuli or to noxious stimuli applied to non-analgesic areas of the body (Mayer et al, 1971; Mayer and Liebeskind, 1974). However, in man stimulation of the PAG produces sufficient side effects to disallow its chronic use. More rostral areas, such as the periventricular gray, are

usually used (Richardson and Akil, 1977a). In rats and cats the best area of the periaqueductal gray for SPA in terms of efficacy and lack of side effects is reported to be the ventral PAG, in or around the dorsal raphé nucleus.

Studies have been done to examine the effects of PAG stimulation on single and multiple units in the central nervous system. Stimulation of the PAG (excluding the dorsal raphé) has been reported to reduce noxious stimuli evoked potentials in the dorsal and median raphé nuclei and the nucleus raphé magnus (Oleson et al, 1978). Similarly, 55% of all medial reticular neurones (probably in the nucleus reticularis gigantocellularis) which were driven by tail shock and 40% of neurones that responded to noxious stimuli were reported to be inhibited by PAG stimulation (Morrow and Casey, 1976). Mohrland and Gebhart (1979) also reported the inhibition of nGC neurones by PAG stimulation.

Similarly, neurones in the dorsal horn of the spinal cord responding to noxious stimuli have been reported to be inhibited by PAG stimulation. Guilbaud et al. (1973a) reported that PAG stimulation inhibited most lamina V neurones, but was without effect on the majority of lamina IV neurones. Carstens et al. (1979a,b) found that neurones responding to noxious radiant heat in or near lamina V were inhibited by stimulation of the PAG. This inhibition has been reported to be selective on wide-dynamic range neurones over neurones responding only to non-noxious stimuli (Bennett and Mayer, 1979). Conversely, Duggan and Griersmith (1979) report that stimulation near the dorsal raphé nucleus inhibits excitation of lamina I, IV, and V dorsal

horn neurones by both noxious and non-noxious stimuli.

Although PAG stimulation clearly affects spinal cord
neurones as noted above, the PAG has no reported direct
connections to the cord. Therefore, these effects are probably
mediated by other brainstem nuclei. For this reason, the effect
of the PAG stimulation on likely candidate nuclei has been
studied extensively. The most often cited candidate for this
mediatory nucleus is the nucleus raphé magnus. Most studies
have found that PAG stimulation facilitates spontaneous raphé
magnus neuronal firing (Fields and Anderson, 1978; Lovick et
al., 1978a; Oleson et al., 1978) but some studies have found
inhibition or mixed effects upon the spontaneous firing rate
(Mohrland and Gebhart, 1979; Pomeray and Behbehani, 1979a,b).
Similarly, another candidate which may mediate periaqueductal
gray SPA is the nucleus gigantocellularis. Neurones within this
nucleus are reported to be excited, inhibited, or both by PAG
stimulation (Morrow and Casey, 1976; Mohrland and Gebhart,
1979).

Periaqueductal gray SPA has been antagonized by both
lesions and pharmacological manipulations. Studies have
demonstrated that analgesia due to stimulation of PAG sites in
the rat is totally abolished in the ipsilateral hindlimb caudal
to a dorsolateral funiculus lesion (Basbaum et al., 1976b).
Intravenously administered naloxone has been reported both to
inhibit and to fail to inhibit PAG stimulation produced
antinociception. Akil et al. (1976) reported partial blockade
by naloxone of the increased tail-flick latency caused by PAG
stimulation. Another study found that naloxone partially

reversed the PAG stimulation produced reduction of leg flexion and electromyograph changes due to foot shock or pinch (Oleson et al., 1978). These studies suggest that periaqueductal gray SPA is mediated by an endogenous opioid system. However, other studies have found no effect of naloxone on PAG stimulation induced increases in tail-flick latencies or reduced behavioural responses to foot pinch (Yaksh et al., 1976c), nor PAG inhibition of dorsal horn neurones excited by noxious radiant heat (Carstens et al, 1979a).

Manipulation of monoamine levels affects the efficacy of periaqueductal gray SPA. Akil and Liebeskind (1975) reported that rat-tail-flick latency is increased by: elevation of serotonin or dopamine levels; depletion of noradrenaline levels; or administration of a dopamine agonist. Conversely, the SPA was reduced by depletion of serotonin or blockade of dopamine with an antagonist. Lysergic acid diethylamide (LSD), which presumably alters serotonin synaptic transmission although the mechanism is unclear, has been reported to block the inhibition of spinal cord nociceptor-driven neurones produced by stimulation near the dorsal raphé (Guilbaud et al., 1973a). Increased tail-flick latencies in rats due to PAG stimulation have also been found to be antagonized by LSD suggesting that periaqueductal gray SPA is mediated by serotonin and/or dopamine.

As well as periaqueductal gray SPA there have been many reports that microinjection of drugs into the PAG produces analgesia. The most commonly studied drug is morphine and microinjection studies have been done in the mouse (Criswell,

1976; Yaksh et al., 1976b), the rat (Sharpe et al., 1974; Lewis
and Gebhart, 1977; Yaksh and Tyce, 1979; Levy and Proudfit,
1979) and primates (Pert and Yaksh, 1974). As with
periaqueductal gray SPA, microinjection-produced antinociception
can cause whole body or partial body analgesia and is reported
not to have side effects such as hyperreactivity (Yaksh and
Tyce, 1979). The findings that there are opiate receptors in
the PAG and that the analgesia is a stereospecific effect
indicate that the analgesia is due to a specific interaction
with opiate receptors. For example, dextrorphan is ineffective
in producing analgesia when microinjected into the
periaqueductal gray (Pert and Yaksh, 1974). Again, the effect
appears to be specific, like SPA, to antinociception. In
monkeys trained to discriminate between two aversive
electrocutaneous stimuli, microinjected morphine produced a
reduction in perceived pain without reducing the monkeys'
discriminative abilities (Bennett et al., 1976).

Like PAG stimulation studies, the effect of morphine
microinjection has been studied on single units. Nucleus
gigantocellularis and nucleus raphé magnus neuronal responses to
noxious stimuli are reduced by morphine microinjection into the
PAG (Mohrland and Gebhart, 1979). Bennett and Mayer (1976,
1979) reported that 55% of wide-dynamic range spinal cord
neurones responding to noxious stimuli were also inhibited by
morphine injected into the PAG. Microinjection of morphine into
the PAG also increases the spontaneous firing of neurones in the
nucleus raphé magnus (Behbehani and Pomeroy, 1978; Behbehani,
1979; Mohrland and Gebhart, 1979) and nucleus gigantocellularis

(Mohrland and Gebhart, 1979).

As with PAG stimulation produced antinociception, morphine microinjection analgesia can be antagonized by lesions or chemical manipulations. Murfin et al. (1976) reported dorsolateral funicular lesions abolished the lengthening of tail-flick latencies by microinjected morphine. The inhibition of spinal cord nociceptor-driven neurones by morphine microinjection into the PAG was reversed by intravenously administered naloxone (Bennett and Mayer, 1979). As well, cinanserin and methysergide, two putative serotonin antagonists, are reported to antagonize analgesia due to PAG microinjected morphine (Yaksh et al., 1976a).

Microinjection of other drugs into the PAG has been reported to produce analgesia. The narcotic etorphine is more potent than morphine and it inhibited 80% of spinal cord neurones excited by noxious stimuli (Bennett and Mayer, 1979). Baclofen allegedly increases tail-flick latencies in rats when microinjected into the PAG (Levy and Proudfit, 1979) and glutamate is reported to increase the threshold for foot pad shock evoked leg flexion (Behbehani and Fields, 1979). This effect of glutamate was reversed by intravenously administered naloxone or by nucleus raphé magnus lesions. Microinjection of glutamate into the PAG also facilitated the spontaneous firing of the majority of nucleus raphé magnus neurones.

Microiontophoresis of morphine onto PAG neurones was reported to inhibit about one-half and to excite about one-fifth of the neurones tested while etorphine inhibited four-fifths of the neurones tested (Wolstencroft et al., 1977). However, none

of these effects were blocked by naloxone suggesting a non-specific mode of action for these two drugs. In agreement with these results, Haigler (1978) reported that iontophoresis of morphine onto dorsal and medial raphé neurones inhibited 34% of the neurones. However, this inhibition was also not stereospecific and was rarely blocked by naloxone.

Lesions of the periaqueductal gray were found by Deakin and Dostrovsky (1978) to reduce the lengthening of tail-flick and hot plate test latencies due to intravenously administered morphine. Similarly, lesions of the caudal PAG increased thresholds for caudal thalamus SPA and decreased the baseline latencies for the tail-flick reflex (Rhodes and Liebeskind, 1978).

The Dorsal and Medial Raphé

The dorsal raphé nucleus (DR) and the medial raphé nucleus (MR) are two midline nuclei in the mesencephalon. The dorsal raphé is contained in the ventral most part of the periaqueductal gray; the medial raphé is located ventrally to the dorsal raphé and runs caudally from about the level of the PAG. Both nuclei contain many serotonergic neurones (Dahlstrom and Fuxe, 1964; Poitras and Parent, 1978) but they also contain catecholaminergic neurones. Cell bodies and axonal terminations containing met-enkephalin were observed within these nuclei and described by Hokfelt et al. (1977).

Dorsal raphé neurones receive afferent input from other dorsal raphé neurones and medial raphé neurones (Mosko et al., 1977). This nucleus is reported to also receive input from the

spinal cord, although it may not be direct. Aghajanian et al.
(1978) report that low frequency peripheral nerve stimulation
results in inhibition of neurones identified as serotonergic but
in excitation of those neurones which were non-serotonergic.
Stimulation of the pontine reticular formation has been found to
inhibit dorsal raphé neurones (Wang et al., 1976). Bobillier et
al. (1975, 1976) report a low amount of labelling in both
nuclei when ^{14}C-leucine was microinjected into the nucleus raphé
centralis superior in the rat.

These two nuclei predominantly send axonal fibres, with
large numbers of terminal collaterals, to the diencephalic and
telencephalic forebrain regions via the medial forebrain bundle.
Destinations include the hypothalamus, striatum, septum, and
cortex (Dahlstrom and Fuxe, 1964; Anden et al., 1966; Pierce et
al., 1976). Descending axonal projections terminate in the
substantia nigra (Dray et al., 1976; Pierce et al., 1976), the
caudal raphé, and the nucleus gigantocellularis (Conrad et al.,
1974; Pierce et al., 1976; Bobillier et al., 1976, 1979). The
two mesencephalic nuclei do not appear to have direct
projections to the spinal cord (Conrad, 1974).

As the dorsal raphé is so intimately associated with the
periaqueductal gray, and indeed is part of it, most of the
reports dealing with the effects of stimulation of and
microinjection into the dorsal raphé were included in the
section on the PAG. Microiontophoretic serotonin was reported
to be inhibitory on almost all raphé neurones in studies by
Bramwell and Gonye (1976). This is contrasted with the
excitatory effect that serotonin had on the non-raphé neurones

in these studies.

Lesioning in the vicinity of the dorsal raphé was reported to reduce the efficacy of intravenously administered morphine analgesia (Samanin et al., 1970). A recent study by Chance et al. (1978) found morphine analgesia was also antagonized by lesioning of the medial raphé nucleus. However, in the same study, methadone-induced analgesia was unaffected by similar lesions, suggesting that methadone has a different site of action.

D. Medullary Nuclei

The Nucleus Reticularis Gigantocellularis

The nucleus reticularis gigantocellularis (nGC) is a large ill-defined nucleus in the medullary reticular formation lateral to the caudal raphé. As its name implies, the cells within the nGC are very large. It has been suggested that the nGC may be a relay nucleus for ascending information destined for the centre median nucleus of the thalamus (Bowsher et al., 1968). As the nGC also projects to the spinal cord and has been shown to inhibit spinal cord neurones responding to noxious stimuli (McCreery and Bloedel, 1975), it may also be involved with descending inhibitory control of nociceptive pathways at the spinal cord level. Like the periaqueductal gray, the the nGC has been implicated in the mechanism of antinociception by its responses to noxious stimuli and the effects that stimulation of and microinjection of drugs into the nGC have on the behavioural and reflex responses to noxious stimuli.

Anatomical studies involving either spinal cord lesions, retrograde transport of horseradish peroxidase, or electrophysiological methods have demonstrated that the nGC receives afferent input from ascending sensory pathways. Mehler et al. (1960) documented the denervation of brain areas following lesions of the ventrolateral quadrants of the spinal cord and found dense terminal degeneration in the nGC. Gallager and Pert (1978) iontophoresed horseradish peroxidase into the nGC and found HRP containing cell bodies in the dorsal and ventral gray of the cervical spinal cord. Antidromic activation of spinal cord neurones by stimulation of the nGC also indicates an input to the nGC from the cord. Fields et al. (1975) stimulated the nGC of cats with bipolar electrodes and recorded from single units deep to lamina V which could be antidromically activated. These neurones either responded to noxious stimulation of deep structures or had large cutaneous fields which were responsive to noxious stimuli.

Studies recording from single units in the nGC also support the existence of noxious afferent input to this nucleus. Casey (1969) found that two-thirds of nGC neurones responding to natural stimuli required firm pinch or heavy pressure to maximally activate them. Similarly, in subsequent experiments 61% of nGC neurones responded to noxious pinch or foot pad shock while the remainder of the units either were non-responsive to any stimuli or responded to light touch (Keene and Casey, 1970). LeBlanc and Gatipon (1974) reported that noxious electrical or mechanical stimulation of footpads caused most nGC neurones to initially increase or decrease their firing rate, followed by a

prolonged after-discharge or an extended silent period.

Similar types of responses were reported when the sciatic, radial, or trigeminal (tooth pulp) nerves were stimulated (Pearl and Anderson, 1978). Most neurones responded with a short-latency and short duration excitatory phase which was followed by a long suppression of activity. Also, most nGC neurones have been found to respond to A-delta fibre stimulation (Goldman et al., 1972). Casey (1969) reported that 70% of the maximal response to peripheral nerve stimulation remained when myelinated A fibres were blocked by anodal current.

Intra-arterial administration of the algesic agent bradykinin has also been reported to affect nGC neuronal firing. Guilbaud et al. (1973b) reported most nGC neurones in decerebrate cats were excited, a few were inhibited, and a few had mixed responses to intra-arterial bradykinin. Besson et al. (1974) recorded similar results.

The nGC also receives afferent input from other brain areas. Microiontophoretic horseradish peroxidase studies found cell bodies with axonal projections to the nGC in the superior colliculus, the dorsal and ventral tegmentum, the brainstem raphé and occasionally the PAG (Gallager and Pert, 1978). Denervation studies indicate an input from the contralateral trigeminal nucleus (Carpenter and Hanna, 1961; Stewart and King, 1963). Tracing studies using ^{14}C-leucine suggest some nGC afferent fibres originate from the medial raphé nucleus (Bobillier et al., 1976). Stimulation studies indicate that the nGC also receives afferents from the lateral hypothalamus, caudal raphé, and the PAG. Stimulation in the lateral

hypothalamus excited most nGC neurones responding to noxious stimuli whereas only a few of those which responded only to non-noxious stimuli were excited (Keene and Casey, 1970). Stimulation of the nuclei raphe magnus, obscurus, and pallidus activated most nGC neurones and this activation was reversed by LSD (Briggs, 1977). Stimulation of analgetic sites in the PAG excites, inhibits, or has mixed effects on nGC neuronal spontaneous firing but is reported to inhibit most of the responses of nGC neurones to noxious stimuli (Mohrland and Gebhart, 1979). Periaqueductal gray stimulation at analgetic sites were also shown by Morrow and Casey (1969) to be inhibitory on nGC neurones responding to noxious stimuli.

Neurones of the nucleus gigantocellularis project to many areas of the brain. Bowsher et al. (1968) suggested that the nGC is a relay nucleus as it projects to the centre median nucleus of the thalamus. There is substantial evidence for the existence of a descending projection from the nGC to the spinal cord. Nyberg-Hansen (1965) lesioned the nGC and followed its degenerating axon courses by the silver impregnation technique. He noted that the fibres travelled caudally in the ventral white of the spinal cord and terminated in lamina VII. A similar study using this technique together with horseradish peroxidase methods recorded descending fibres in the lateral columns to all levels of the cord (Zemlan et al., 1979). Martin (1979) reported that descending fibres from the nGC terminate on the intermedial cell column. Basbaum et al. (1976a,b, 1978), using tritiated leucine tracing techniques, reported that nGC reticulospinal fibres descend in the ventral and ventrolateral

funiculi and terminate mainly in the ventral horn in the motor neuronal layers. They concluded that any descending inhibitory influences of the nGC are limited to the motor system. However, groups studying spinal cord neurones responding to noxious stimuli, some of which are spinothalamic tract neurones, report that these neurones are inhibited by stimulation of the nGC (McCreery and Bloedel, 1975; Takagi et al., 1975).

The effects of stimulation of the nGC also imply that it is involved in analgesia. Stimulation of the nGC resulted in escape behavior in cats and rats, suggesting that the nGC receives and transmits perceivable noxious information (Casey, 1971b, Keene and Casey, 1970; Carr and Coons, 1979). These animals were trained to perform a set behavior to escape from a noxious stimulus. Stimulation of the nGC has also been reported to inhibit neurones responsive to noxious stimuli. McCreery and Bloedel (1975) recorded from spinothalamic tract neurones which responded to graded noxious mechanical stimuli. They found that these responses were inhibited by nGC stimulation. Haber and Wagman (1974), on the other hand, reported facilitation of spinal cord interneurones by nGC stimulation. However, this was probably due to their use of much higher stimulation frequencies because McCreery et al. (1979), in another study, reported that stimulation of the nGC resulted in a transient excitation followed by a subsequent inhibition of spinothalamic tract neurones. Therefore, the high stimulation frequencies may have been causing summation of the excitatory responses. In a later report, Haber et al. (1978) also found inhibition in addition to facilitation. Nucleus gigantocellularis stimulation is also

reported to inhibit bradykinin evoked activation of lamina V
neurones (Takagi et al., 1975) and to produce both synaptic
excitation and marked inhibition of some lamina V spinoreticular
neurones (Fields et al., 1975).

Similarly to the PAG, microinjection of opiates into the
nGC results in analgesia. Microinjection of morphine (Takagi et
al., 1976, 1977; Kuraishi et al., 1979) and met- and leu-
enkepalin (Takagi et al., 1978) into the nGC reduced the
behavioural responses of rats to tail pinch and this inhibition
was reversed by intravenously administered naloxone. Also, Chan
(1979) reports that injection of morphine into the nGC reduced
tooth pulp evoked responses in the spinal trigeminal nucleus.
However, at least two groups have found little analgetic effect
from microinjected morphine (Dickenson et al., 1979; Levy and
Proudfit, 1979). Levy and Proudfit (1979), however, did report
that microinjection of baclofen into the nGC did cause analgesia
because it increased rat tail-flick latencies.

Microiontophoresis of morphine or met-enkephalin onto nGC
neurones results in an excitation of about one-half of the
neurones which is reversible by microiontophoresed naloxone
(Mohrland and Gebhart, 1979). These workers rarely saw
inhibition of nGC neuronal responses to noxious stimuli. In
contrast, other workers (Gent and Wolstencroft, 1976;
Wolstencroft et al., 1977) reported that iontophoresed morphine,
met- or leu-enkephalin, or endorphin in the cat resulted in
inhibition of excitation induced in nGC neurones by noxious
stimuli. However, they reported that these effects were not
antagonized by naloxone. Satoh et al. (1979) have also

reported excitation of nGC neurones by iontophoresed morphine and endorphin in the rat.

Lesions of the nGC are reported to affect the behavioural responses to noxious stimuli. Halpern and Halverston (1967, 1974) reported that lesioning of the nGC, nucleus paragigantocellularis, and/or the magnocellular tegmental field of cats resulted in an increase in latency and threshold for escape from foot shock. Similar results were published by Anderson and Pearl (1975); lesioning of the nGC abolished escape responses evoked by noxious stimuli.

The Caudal Raphé

The caudal raphé includes the nuclei raphé magnus, pallidus, and obscurus in the rat. These nuclei are a caudal extension of the mesencephalic raphé nuclei and similarly contain a large proportion of serotonergic neurones (Taber et al., 1960; Dahlstrom and Fuxe, 1964; Poitras and Parent, 1978). However, as opposed to the mesencephalic raphé nuclei, these nuclei have quite low opiate receptor binding levels (Atweh and Kuhar, 1977; Pert et al., 1976).

Although the caudal raphé nuclei only receive a sparse direct afferent input from ascending sensory pathways, as demonstrated by microiontophoretic horseradish peroxidase studies (Gallager and Pert, 1978), there are many reports of neurones in these nuclei responding to peripheral stimuli. Approximately one-half of raphé magnus neurones, including many raphéspinal neurones, are excited by noxious peripheral stimuli, and many are activated by non-noxious forms of stimuli (Lobatz

et al., 1976,1977; Moolenaar et al., 1976;, Anderson et al., 1977; Fields and Anderson, 1978; Zaretsky et al., 1979)., Zaretsky et al., (1979) reported two types of neuronal responses to noxious radiant heat: the first was excitation beginning just before the occurrence of a tail-flick in response to heat which was not present if a tail-flick did not occur; the second response was inhibition of the neurone during the period of application of radiant heat.

There have been numerous studies of the PAG afferent input to the caudal raphé. Oleson and Liebeskind (1975, 1976) reported that stimulation of the PAG resulted in the reduction of electrical potentials and multiunit firing in the raphé magnus evoked by noxious stimuli. Furthermore, periaqueductal gray stimulation facilitated spontaneous raphé magnus neuronal activity. This has been confirmed by other workers (Fields and Anderson, 1978; Hayes et al., 1977). Although excitation was the major response seen, periaqueductal gray stimulation induced inhibition or mixed responses on raphé magnus neuronal firing were also seen (Pomeroy and Behbehbani, 1979a,b; Mohrland and Gebhart, 1979).

Evidence to prove that these effects are mediated by a direct monosynaptic pathway is provided by horseradish peroxidase and antidromic studies. Gallager and Pert (1978) observed HRP containing cell bodies in the periaqueductal gray after iontophoretic application of HRP into the caudal raphé. They also observed labelling of nGC neurones. In addition, Dostrovsky and Shah (1979) antidromically activated neurones in the PAG by electrical stimulation of the raphé magnus.

Of the caudal raphé nuclei, the raphe magnus has the largest axonal projection to the spinal cord. Dahlstrom and Fuxe (1965) reported that lesions of the caudal raphé caused denervation of the dorsal, intermediate, and ventral horns of the spinal cord. Antidromic activation of raphé magnus neurones from the spinal cord, together with discrete lesions in the rostral cord, verified the existence of a raphéspinal pathway descending via the dorsolateral funiculus (DLF) and terminating in laminae I, V, VI, and VIII (Akil and Liebeskind, 1975). Comfirmation of this pathway was obtained from additional studies involving: spinal lesioning (Basbaum and Fields, 1977a,b; Willis et al., 1977), tritiated leucine tracing studies (Basbaum et al., 1976a,b; Fields and Basbaum, 1978), horseradish peroxidase tracing studies (Basbaum and Fields, 1977a,b; Leichnetz et al., 1978; Watkins et al., 1980), and antidromic activation studies (West and Wolstencroft, 1977).

An equivalent projection to the spinal trigeminal nucleus has been reported by Lovick et al. (1978a,b). They demonstrated that the nucleus raphé magnus projects to both the lumbar cord and the trigeminal nucleus via branched axons by colliding antidromic action potentials originating from either site. Other areas receive axonal input from the caudal raphé. Briggs (1977) found that raphé magnus stimulation caused the activation of nucleus gigantocellularis neurones which was correlated with their responses to iontophoretically applied serotonin, and which could be blocked by iontophoretic LSD. Mapping studies with ^{14}C-leucine indicate a major projection from the raphé magnus to the intralaminar nuclei of the

thalamus, and minor projections to the nGC and the dorsal and medial raphé nuclei (Bobillier et al, 1976).

Stimulation of the nucleus raphé magnus has been widely reported to produce analgesia. It has reduced behavioural responses to noxious stimuli in the cat (Oliveras et al., 1975, 1977), increased tail-flick latencies in the rat (Oleson et al., 1975, 1978), and reduced reflex responses to noxious stimuli in the cat (Engberg et al., 1968; Oliveras et al., 1977) and the rat (Oleson and Liebeskind, 1975). Naloxone was reported to antagonize the reduction of behavioural responses to pinch and the reduction of the jaw-opening reflex produced by stimulation of the raphé magnus (Oliveras et al., 1977). An equivalent inhibition of the spinal trigeminal nucleus has been found in decerebrate cats (Lovick and Wolstencroft, 1979). Raphé magnus stimulation abolished the response of spinal trigeminal neurones to noxious stimuli while having a weak or insignificant effect on responses to non-noxious inputs. This depression paralleled the increase in threshold of the jaw-opening reflex.

Nucleus raphé magnus stimulation has also been reported to inhibit the responses of spinothalamic tract neurones to mechanical and thermal noxious stimuli (Beall et al., 1976; Willis et al., 1976, 1977), and to differentially inhibit spinal cord neuronal responses to noxious over non-noxious stimuli (LeBars et al., 1976; Fields et al., 1977a; Guilbaud et al., 1977; Duggan and Griersmith, 1979). Duggan and Griersmith (1979) found that this inhibition, in the cat, was not reversible by intravenously administered naloxone, suggesting that the inhibition is not mediated by endogenous opioids.

Conversely, Pearson et al. (1979) reported that the inhibition was not preferential for noxious stimuli responses in the rat. They found, in fact, that the threshold for inhibition of the non-noxious responses was less than that required to inhibit the responses to noxious stimuli. Their data also suggest that the inhibition was post-synaptically mediated as glutamate evoked unit firing could also be inhibited by stimulation of the raphé magnus.

Two groups have reported raphé magnus stimulation induced depolarization of primary afferent fibres (Proudfit and Anderson, 1974a,b, 1975; Martin et al., 1979). The depolarization was blocked by intravenously administered cinanserin, methysergide, and LSD (Proudfit and Anderson, 1974a,b), suggesting that it is serotonergically mediated. The inhibitory effect was not preferential for noxious responses, and Martin et al. (1979) suggested that the preferential effects reported above must occur at sites post-synaptic to the afferent terminal.

Intravenously administered morphine has been shown to be excitatory on some nucleus raphé magnus neurones, including raphéspinal units (Fields and Anderson, 1978). This excitation was reversible by naloxone (Anderson et al., 1977; Deakin et al., 1977; Oleson et al., 1978). In fact, the naloxone inhibited the spontaneous firing of some neurones suggesting that there is tonically active opioid input to the raphé magnus (Anderson et al., 1977).

Microinjection of morphine into the nucleus raphé magnus is reported to produce analgesia: it increases tail-flick latencies

(Proudfit, 1977; Levy and Proudfit, 1979) and reduces vocalization upon tail shock (Dickenson et al., 1979). These effects could be reversed by intravenous naloxone (Proudfit, 1977; Dickenson et al., 1979) and reduced by intravenous cinanserin (Dickenson et al., 1979). Also, microinjection of naloxone into the raphé magnus reversed the analgesia induced by intravenously administered morphine (Proudfit, 1977; Dickenson et al., 1979). Levy and Proudfit (1979) also reported that microinjection of baclofen into the raphé magnus did not produce analgesia as it had done when it was injected into the nGC. Phentolamine, on the other hand, increased tail-flick and hot plate latencies when microinjected into the raphé magnus, suggesting tonically active adrenergic input onto raphé magnus neurones (Hammond and Levy, 1979).

There have been conflicting reports of the effects of microiontophoresed morphine on the spontaneous activity of nucleus raphé magnus neurones. It was reported to either not affect the activity of most neurones (Haigler, 1977) or to inhibit about one-half of them (Gent and Wolstencroft,1976; Lobatz et al., 1976, 1977; Wolstencroft et al., 1977). However, the inhibition of spontaneous activity was not reversed by naloxone in these studies and therefore these effects are unlikely to be mediated by opiate receptors. Although approximately one-half of all raphé magnus neurones were reported to be inhibited by iontophoretic morphine, the majority of neurones identified as raphéspinal were unaffected by it (Gent and Wolstencroft, 1976; Wolstencroft et al., 1977). In addition, although Lobatz et al., (1976, 1977) found that about

one-half of the neurones responding to noxious stimuli were inhibited by iontophoretic morphine, Mohland and Gebhart (1979) rarely found inhibition of raphé magnus neurones which responded to noxious stimuli. To test the possibility that morphine was working by modification of the action of acetylcholine, Lobatz et al. (1977) also tested raphé magnus neurones with iontophoretic acetylcholine. They found that 85% of the neurones were excited by the acetylcholine but that this excitation was unaffected by iontophoretic morphine.

MATERIALS AND METHODS

A. Surgical Preparation

Male albino Wistar rats (150-300 g) were used in these
experiments. Each animal was anaesthetized with an
intraperitoneal injection of urethane (1.4 g/kg, Sigma)
administered as a 20% solution by weight in distilled water.
The rectal temperature was maintained within physiological
limits with an automatic animal heater-controller driving a
plexiglass and wire heating pad. This temperature was also
monitored independently on a Yellow Springs Telethermometer.

To allow subsequent intravenous injections to be made, the
right femoral vein was cannulated with No. 10 polyethylene
tubing (Clay Adams) prefilled with normal saline. If the blood
pressure was to be recorded the right femoral artery was
cannulated with No. 50 polyethylene tubing prefilled with a
dilute saline-heparin (Sigma) solution and attached to a
pressure transducer (Stratham Medical Instruments, Inc., model
P23AC). The resulting electrical signal was recorded on one
channel of a four channel polygraph (Grass Instruments, model
79D).

The sciatic nerve was prepared for electrical stimulation
by attaching a bipolar electrode. This electrode was
constructed from two lengths of 0.35 mm silver wire wrapped
around a 2 cm long piece of No. 90 polyethylene tubing and
connected to two insulated wires which were led to the exterior.
The electrode was held in place and isolated from the
surrounding tissue by a 10 mm piece of rubber tubing placed

around the electrode and the nerve and secured with surgical
thread. The protruding wires were further secured by being tied
to a skin flap with thread.

The animal's head was then positioned in a stereotaxic
animal frame (Narashige Scientific Instrument Laboratory). The
toothbar was positioned such that its top surface was level with
the centre of the earbars, according to the method of Abad-
Alegria (1971).

The skin over the skull was cut and reflected, the skull
scraped clear of tissue, and a burrhole drilled with a Mototool
(Dremel). The hole, about 5 mm in diameter, was placed
approximately 9 mm left of the sagittal suture and level with
the skull landmark 'lambda' in the anterior-posterior plane.
This location avoided damage to the dural venous sinuses. A
further small section of skull over the cerebellum was removed
with a pair of rongeurs to allow an electrode to be placed into
the nucleus gigantocellularis.

B. Electrode Preparation and Positioning

Preparation

Stimulating electrodes were constructed by inserting a
length of thin insulated copper wire into a 1.5 mm glass
capillary tube (Kimble Products) and pulling the electrode with
a microelectrode puller (Narashige). The rate of pull was
slowed by hand to achieve a long well-formed electrode. The
wire protruding from the blunt end was glued in place with a
spot of epoxy, the tip broken and the exposed wire stripped of

insulation and trimmed with a scalpel blade leaving 0.1 mm of stripped wire. If a bipolar electrode was being contructed then a shorter piece of capillary tube bent into the shape of a hockey stick was glued parallel to the first and a wire inserted into each tube. Also, prior to pulling, the electrode was heated and twisted 180° to bind the capillaries together and ensure that the two barrels would form one tip when the electrode was pulled.

Single barrel recording electrodes were made from 1.5 mm diameter glass micropippettes containing a single strand of fibreglass to ease the filling, by capillary action, of the resulting electrodes (Glass Company of America, Omega Dot). The electrodes were pulled in the conventional manner on a commercial electrode puller (Narashige) and the tips broken against a glass rod under a microscope to about one μm. They were then filled with 2% pontamine sky blue 6BX (ICN Pharmaceuticals, Inc.) in 0.5 M sodium acetate, allowing both recording and the histological marking of recording sites by iontophoretic ejection of the dye (Hellon, 1971). Electrode resistances were deemed acceptable if they were between 10-30 megohms.

Multibarrel electrodes were constructed by glueing a number of short hockey stick shaped capillary tubes (Omega Dot) around a full length micropipette. The resulting multibarrel assemblage was placed in the microelectrode puller, heated, and twisted 180° to bond the barrels together. After a delay to allow for cooling, the electrode was pulled in the conventional manner. The tip was broken back to a diameter of about 10 μm to

prevent blockage of the drug barrels during iontophoresis. The centre barrel was filled with dye solution, as was done above for the single barrel electrodes, and the surrounding barrels were filled with test drugs (see below).

The various drugs were made up in solutions at optimum pH or with a polar vehicle in order to achieve satisfactory release of the drug during iontophoresis. The pH was selected such that the drug was in a highly polar state. For drugs which are non-polar the solution was made with a polar solute, usually sodium chloride, as a vehicle to enable the drug to be released by electro-osmosis.

The drugs used in these experiments were: serotonin creatinine sulfate (Sigma), 0.04 M; fluoxetine (Lilly), 0.1 M in 200 mM sodium chloride, pH 6.5; glutamate (Sigma), 0.2 M, pH 7.5; and sodium chloride (BDH Chemicals), 2.0 M. The solutions were filtered with a 0.45 um Millipore filtering system and stored frozen in 1.0 ml lots between experiments.

Positioning

The electrode to be positioned in the periaqueductal gray matter, whether it was a stimulating or recording electrode, was held by a standard electrode carrier (Narashige) with an additional sliding rack and pinion attached. The added rack was inclined 30° left of vertical in the lateral plane to allow the PAG to be reached without damaging the dural venous sinuses. The electrode tip was centred on a stereotaxic calibrator frame (Narashige) in order to record the anterior-posterior and vertical zero coordinates. The lateral zero coordinate was

obtained by direct comparison of the electrode tip to the saggital suture of the rat's skull. This allowed for any assymetry in the lateral placement of the head in the stereotaxic frame. The final coordinates were calculated using the coordinates of the PAG specified by a stereotaxic atlas (Abad-Alegria, 1971): 0.0-0.5 mm posterior; 0.6-0.9 mm lateral; and 4.0-5.0 mm dorsal.

The electrode positioned in the nucleus reticularis gigantocellularis was held in another standard carrier inclined 25° posterior of vertical in the saggital plane. It was mounted on the arm of the stereotaxic frame opposite that of the first carrier to avoid collision of the two and to allow free access of a stereoscope (Olympus). Calibration and positioning of this electrode was similar to that for the PAG electrode, except that the atlas coordinates used were: 3.0-4.0 mm posterior; 0.6-1.0 mm lateral; and ±0.5 mm dorsal.

C. Equipment Interconnection

The sciatic nerve electrode and the brain stimulating electrode were connected via a switch box to a constant voltage stimulator (Ortec) allowing selection of either site for electrical stimulation. A noxious radiant heat stimulator (see Appendix) was focused on a small area of the tail (4x4 mm). This stimulator was under temperature feedback control via a thermocouple which regulated both the noxious and basal non-noxious temperatures of the surface of the tail. The basal temperature, which was kept constant between noxious trials, was set at 30-32 °C and the noxious applications were set at 48-
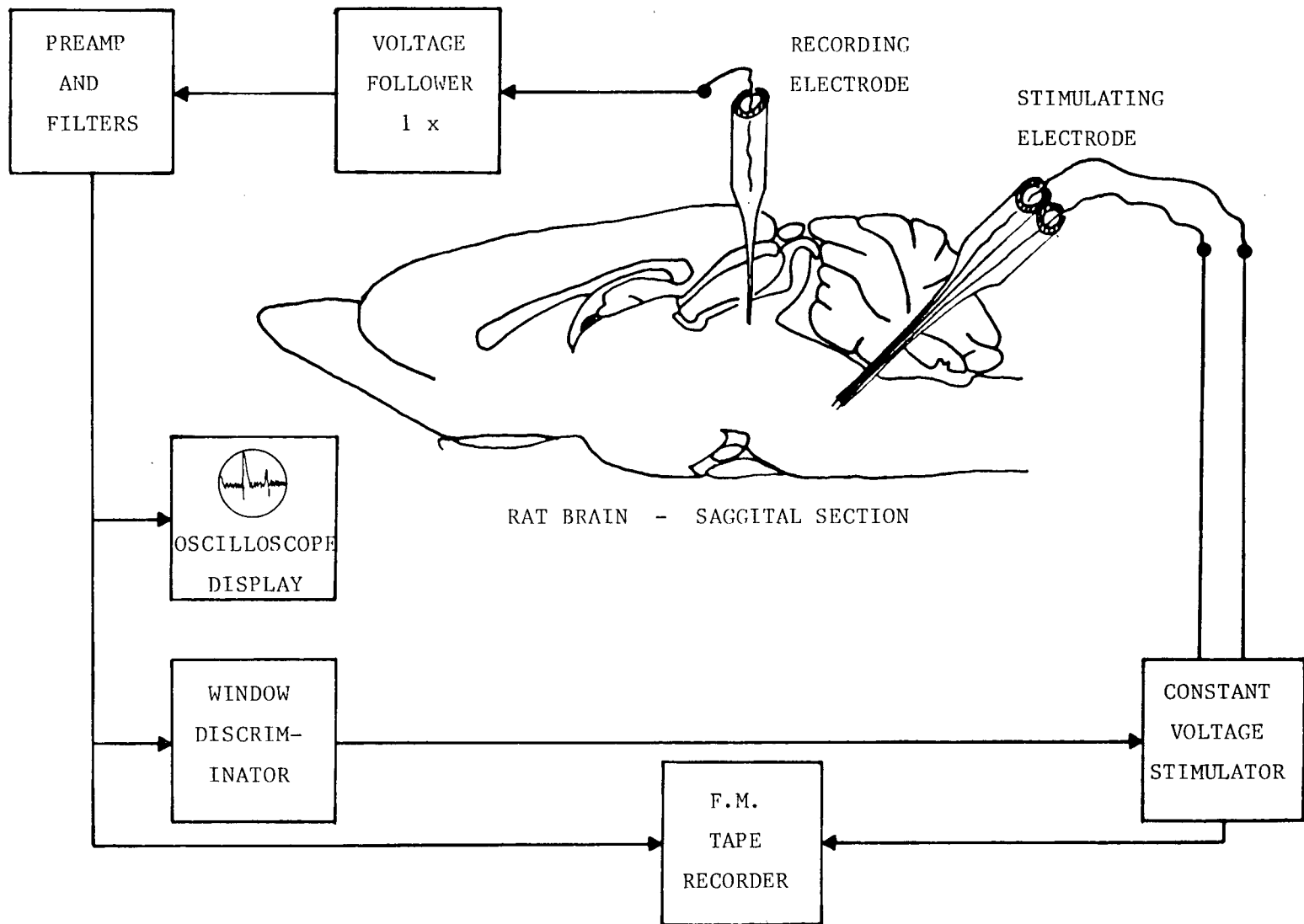
54 °C. The noxious trials were automatically limited to 15 s to prevent skin trauma. The temperature of the irradiated thermocouple was recorded on one channel of the polygraph.

Single unit activity was recorded by inserting a silver wire into the recording electrode with the other end attached to the probe of a unity gain voltage follower (W. P. Instruments, Inc.). The resulting low level signal was fed differentially to one channel of a storage oscilloscope (Tektronix) which amplified and filtered the signal in addition to displaying it on the screen. The differential connection and the filters were used to reduce unwanted noise. The low-pass filter's 3 db cutoff was set at 1 kHz and the high-pass filter's 3 db cutoff was set at 1 kHz. The output of the oscilloscope preamplifier was fed to an FM tape recorder (Hewlett-Packard) allowing the recording and subsequent playback of unit activity. A trigger pulse from the stimulator was recorded on another channel of the tape recorder for syncronizational purposes. The preamplifier output was also fed to a window discriminator (see Appendix) that allowed the selection of spikes of interest while excluding background noise and unwanted spikes.

Periaqueductal Gray Neurones - Antidromic Activation

In the experiments performed to test for antidromic activation of PAG neurones by stimulation of the nucleus gigantocellularis the following interconnections were made in addition to those listed above (see Fig. 1). The output from the window discriminator was connected to the trigger input of a constant voltage stimulator (Ortec), which itself was connected

Figure 1: Schematic diagram of equipment interconnection used in experiments studying the effect of electrical stimulation of the nucleus gigantocellularis on periaqueductal gray neurones. The stimulator was connected so that it could be triggered by spontaneously or evoked action potentials. After a suitable delay following triggering, the stimulator would discharge.

RAT BRAIN - SAGGITAL SECTION

PREAMP AND FILTERS

VOLTAGE FOLLOWER 1 x

RECORDING ELECTRODE

STIMULATING ELECTRODE

OSCILLOSCOPE DISPLAY

WINDOW DISCRIMINATOR

CONSTANT VOLTAGE STIMULATOR

F.M. TAPE RECORDER

to the bipolar stimulating electrode positioned in the nGC.

The stimulator could be used in two ways: in a free-running mode with a stimulation every 2 s, or in a triggered mode with the stimulator being triggered irregularly by the discriminator output. The triggered mode allowed the stimulation to be positioned at a selected time interval after a spontaneous action potential. See the protocol section for further details.

The taped responses were processed with the following equipment setup illustrated in Figure 2. The output of the tape recorder was filtered and passed to the window discriminator and a delay line (see Appendix). The discriminator allowed the stimulator to be triggered by either the recorded trigger pulses, the artifacts, or the recorded action potentials. The stimulator was used to select every nth pulse which then triggered the raster scan unit (see Appendix). The delay line was used to allow the triggering signal to be placed in the centre of the oscilloscope screen. The delayed signal was also passed to a another discriminator whose output fed the signal inputs of the raster scan unit.

The raster scan unit constructed a display on the oscilloscope consisting of n trials. Each trial consisted of one oscilloscope trace positioned at a progressively lower position on the oscilloscope screen. Each sweep could be either the original signal or that signal processed into a series of dots where each dot represented an action potential or a stimulus artifact.

Figure 2: Schematic diagram of the equipment setup used to process the recorded neuronal unit activity. Note the use of a delay line to allow the triggering to be from signals in the middle of a sweep.
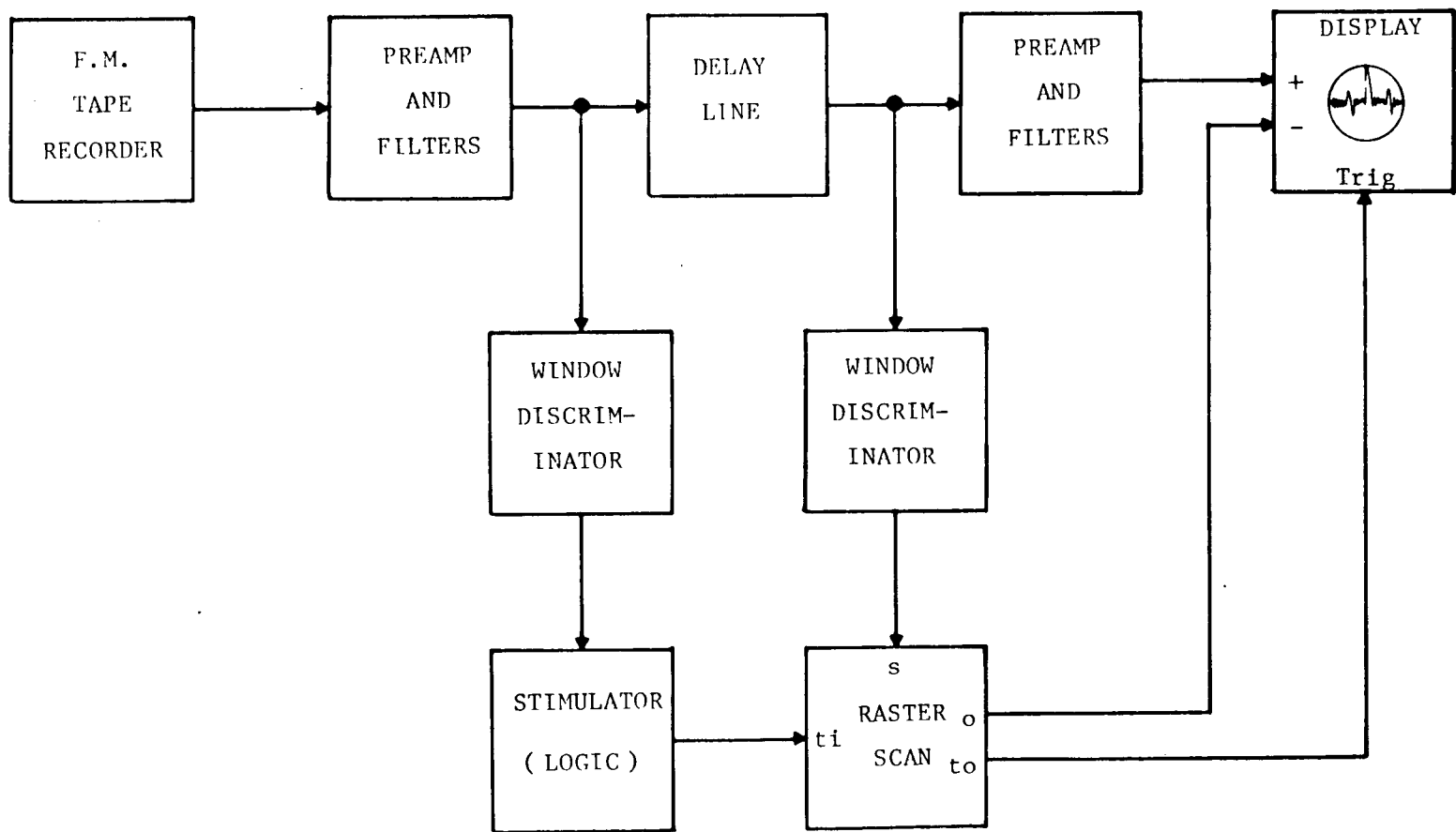
Nucleus Gigantocellularis Neurones - Effect of PAG Stimulation

The equipment interconnection for the experiments studying
the effect of PAG stimulation on nGC neurones in addition to
that above is discussed in this section (see Fig. 3). The
output of the discriminator was connected to: a spike integrator
(see Appendix) which allowed the cell firing rate during preset
time epochs to be recorded on one channel of the polygraph; and
to a time histogram analysis module (Ortec) that allowed the
construction of peristimulus time histograms (PSTHs) and
interspike interval histograms (ISI). The resulting histograms
were viewed on a dual beam oscilloscope (Tektronix) and hard
copies of them made on a X-Y plotter (Houston Instruments).

The drug barrels of multibarreled electrodes were connected
to a six channel programmable microiontophoretic current
controller (Dagan). The applications of drugs were monitored on
one channel of the polygraph. Taped responses were processed by
connecting the tape recorder's output to the first
oscilloscope's preamplifier input.

D. Protocol

Periaqueductal Gray Neurones - Antidromic Activation

A search was made for single units which responded to
stimulation (1-5 V, 0.1 ms, 2.0 Hz) of the nucleus
gigantocellularis. Once such a neurone was found it was
classified according to its response to noxious and non-noxious
peripheral stimuli and electrical stimulation of the nGC. The

Figure 3: Schematic diagram showing the equipment interconnection used in experiments studying nucleus gigantocellularis neurones.

SIX CHANNEL PROGRAMMABLE IONTOPHORESIS UNIT

FIVE BARREL RECORDING ELECTRODE

TWO BARREL GLASS COATED WIRE STIMULATING ELECTRODE

VOLTAGE FOLLOWER 1 x

(PLAYBACK)

PREAMP AND FILTERS

F.M. TAPE RECORDER

SAGGITAL SECTION OF RAT BRAIN

SWITCH BOX

SCIATIC NERVE BIPOLAR ELECTRODE

WINDOW DISCRIM-INATOR

DISPLAY

t

STIMULATOR

PRESSURE TRANSDUCER

POLYGRAPH

SPIKE INTEGRATOR

PERI-STIM-ULUS TIME HISTOGRAM UNIT

X-Y PLOTTER

FOCUSED LIGHT SOURCE

RADIANT HEAT CONTROLLER

noxious peripheral stimuli included: noxious pinch and pressure
to the tail, hind paws, and ears; electrical stimulation of the
sciatic nerve (1-25 V, 0.1 ms, 0.5-2.0 Hz); and radiant heat
focused on the tail. These stimuli were clearly noxious to the
experimenter. Non-noxious stimuli included airpuffs to or
gentle mechanical movement of the animal's fur and joint
movement.

The response of a cell to stimulation of the nGC was
classified as "suspected antidromic" or "orthodromic". The
response was classified as orthodromic if it failed to fulfil
one or more of the following criteria:

(1) demonstration of constant latency at threshold;

(2) demonstration that the response was able to follow
stimulation frequencies to greater than 200 Hz; and

(3) demonstration of collision between the evoked action
potential and an orthodromic action potential within the
critical time period: the latency plus the refractory
period,

or if the latency of the response was greater than 7 ms. The
value of 7 ms was chosen because the direct distance,
stereotaxically, between the two nuclei is about 3.5 mm.
Therefore, accepting the slowest conduction velocity of
unmyelinated fibres to be 0.5 m/s, an action potential would
travel between the nuclei in less than 7 ms. A paired pulse was
used to test the following frequency in most cases but longer
trains were sometimes used. Responses were classified as

'suspected antidromic' if they demonstrated a constant latency of less than 7 ms and if they passed all of the criteria for which they were tested.

Collision was tested in two ways. If the neurone's spontaneous activity was fairly high then the nGC was stimulated regularly at 0.5 Hz and the interaction of the evoked potentials with the spontaneous potentials was observed. If the spontaneous activity was slow or non-existant then an attempt was made to increase it, if possible, with noxious stimuli. The other method used was to trigger the stimulator from spontaneous spikes. This allowed the stimulation to be placed at varying periods after the spontaneous spike, allowing collision to be studied more closely and having the added advantage of making each spontaneous action potential count.

Nucleus Gigantocellularis Neurones - Effect of PAG Stimulation

While searching for cells in these experiments iontophoresis of glutamate was frequently used to excite cells not spontaneously active. Neurones were then characterized by their responses to peripheral stimuli, iontophoresis of drugs, and to stimulation of the PAG.

The effects of iontophoresis were recorded as changes in the spontaneous or glutamate evoked firing rate as well as changes in the PSTHs constructed from responses to electrical stimulation of the PAG or the sciatic nerve. To check that the responses were not due to the iontophoretic current, control applications of NaCl, at currents greater than those used in the drug applications, were performed. If the multibarrel

electrodes were unsatisfactory, i.e. they became blocked or had poor recording characteristics, the experiment was continued with single barrel electrodes.

Towards the end of most experiments a neurone was selected for testing with an intravenously administered drug. The effects of the drug were noted on the spontaneous firing rate of the neurone and the response of the neurone to PAG stimulation as recorded in PSTHs.

E. Histology

Recording sites of interest were marked by passing up to 15 µA of direct negative current through the recording barrel for up to 15 min. This procedure left a spot of the blue dye contained in the recording barrel at the recording site. These spots were subsequently located after the brain had been sectioned on a microtome. Stimulation sites were marked by lesioning the tissue at the electrode tip with direct current (0.3-0.9 mA, 30 s) passed through the electrode. At the end of the experiment the animal's chest was opened and a needle, attached to a 50 ml syringe filled with normal saline, was introduced into the left ventricle of the heart. The right ventricle was then slit and the animal perfused with the saline. The animal was decapitated with a guillotine, the brain removed and stored in 10% formaldehyde solution for at least three days. The brain was then sliced into 40 µm sections with a freezing microtome (Damon/IEC Division) at -20 °C and mounted onto glass slides using an alcohol-gelatin (2 g of gelatin in 2 l of 40% alcohol) solution. The brain slices were then used to map the

recording and stimulation sites onto composite diagrams.
Recording sites that were not marked were interpolated from the
locations of marked sites in the same electrode track.
Responses to electrical stimulations in which the stimulation
site was located outside its target area were discarded.

RESULTS

A. Periaqueductal Gray Neurones

A total of forty-five experiments were performed to study the responses of neurones in the vicinity of the periaqueductal gray to noxious peripheral stimuli and to electrical stimulation of the nucleus gigantocellularis. The results of these experiments are presented in the following two sections.
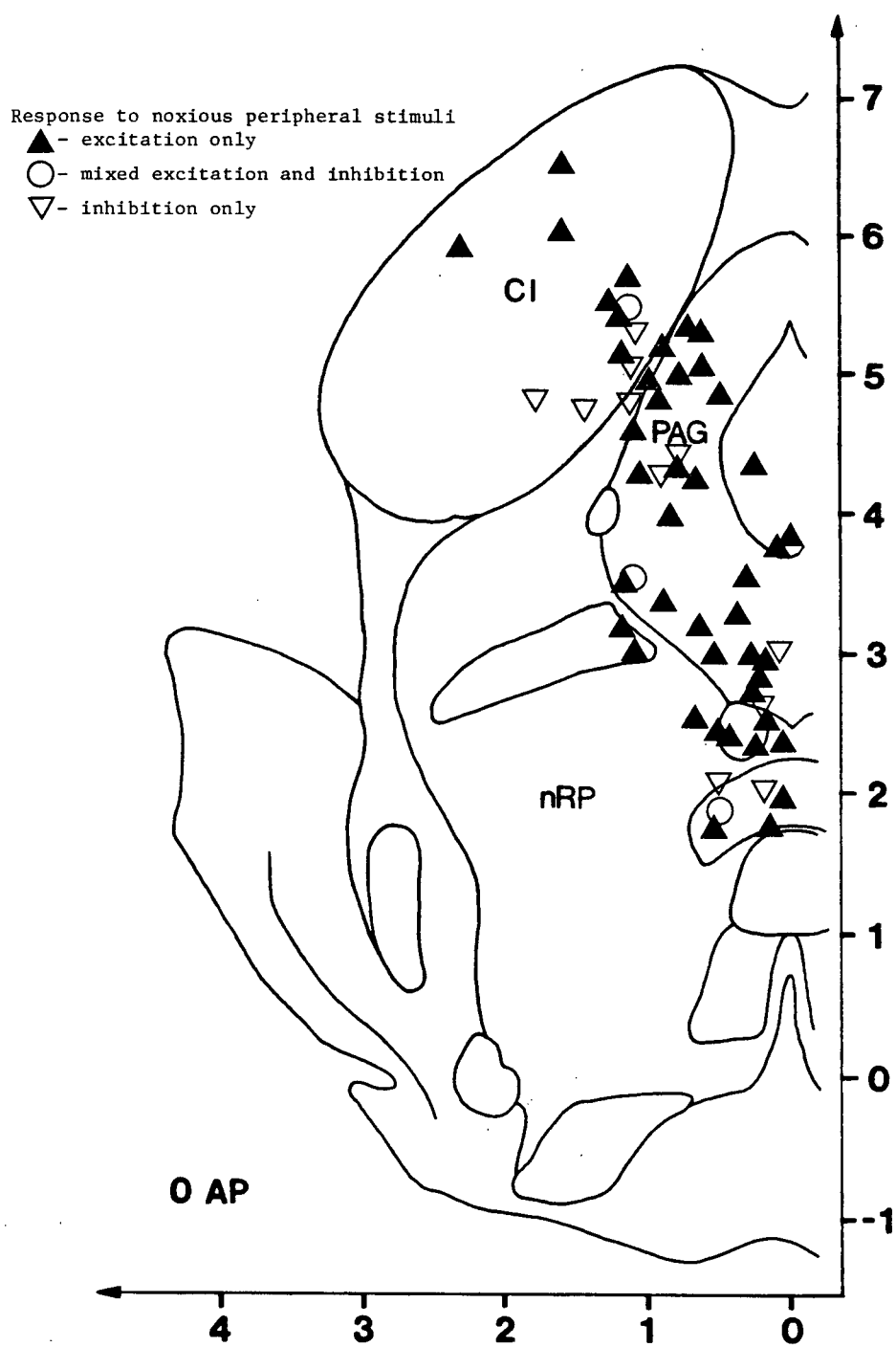
The recorded action potentials were probably from cell bodies rather than axons as they could be recorded for a distance of 40-100 μm and the action potentials were biphasic. Most units had slow spontaneous firing rates of less than 15 Hz although rates as high as 80 Hz were also seen.

Responses to Noxious Peripheral Stimuli

The noxious peripheral stimuli used to test the PAG area units included pinch and pressure applied to the paws, ears, and tail, radiant heat applied to the tail, and electrical stimulation of the left sciatic nerve. About one-third of the neurones were affected by the noxious stimuli. Of the 61 units affected, 46 were excited and 12 were inhibited. In addition, 3 neurones were excited by some stimuli but inhibited by other stimuli. There did not appear to be any correlation between the neuronal responses to the noxious stimuli and their histologically determined locations. The neurones which were affected by noxious stimuli were classified by their responses to noxious stimuli (Fig. 4). The latencies of responses to electrical stimulation of the sciatic nerve were between 5 and 64 ms. The

Figure 4: Coronal section of rat brain showing location of neurones in the vicinity of the peraqueductal gray classified by their responses to: noxious pinch and pressure to the paws, ears and tail; radiant heat applied to the tail; and electrical stimulation of the sciatic nerve. The actual locations were found at ±0.5 mm anterior-posteriorly but are displayed on one section at stereotaxic zero for convenience and clarity. Symbols: CI - inferior colliculus ; nRP - n. reticularis parvicellularis; PAG - periaqueductal gray. The axes are numbered in mm. Modified from Abad-Alegria (1971).

Response to noxious peripheral stimuli
▲ - excitation only
○ - mixed excitation and inhibition
▽ - inhibition only

average latency was 18±10 ms (mean ± the standard error of the mean). The responses ranged from a single spike in silent units to an increase of firing rate lasting up to 130 ms in spontaneously active units.

Responses to Stimulation of the nGC

Ninety-five periaqueductal gray area neurones were activated by electrical stimulation of the nucleus gigantocellularis. The histologically determined sites of the stimulation electrode placed in the nGC are illustrated in Figure 5. Most units (96%) affected by nGC stimulation were activated but 4 units were inhibited. These inhibitory responses were not included with the orthodromically activated responses. The activation responses were classified as 'orthodromic' or 'suspected antidromic' based on their fulfilment of the criteria for antidromicity and their latencies as outlined in the Materials and Methods. Total numbers of units so classified are shown in Table I. A total of 59 neuronal responses were classified as orthodromic because they failed one or more of the antidromicity criteria or had latencies of greater than 7 ms. The locations of these units were diverse and included the inferior colliculus and midbrain reticular formation in addition to the PAG (see Fig. 6).

Thirty-two neuronal responses were classified as 'suspected antidromic'. They all demonstrated constant latencies at threshold of less than 7 ms and passed all of the antidromicity criteria for which they were tested. The numbers of neurones passing each test is given in Table I and the units' locations

Figure 5: Coronal section showing the histologically determined position of the stimulating electrodes placed in the nucleus gigantocellularis which were used in experiments studying the activation of PAG neurones by electrical stimulation of the nGC. The actual stimulation sites were located at 3.0±0.5 mm posterior but are displayed on one section at 3.0 mm posterior for convenience and clarity. Symbols: V, VIII, XII - nuclei of the corresponding cranial nerves; CR - caudal raphé; nGC - nucleus gigantocellularis. The axes are numbered in mm. Modified from Abad-Alegria (1971).

VIII VIII XII

V

nGC CR

-3 AP
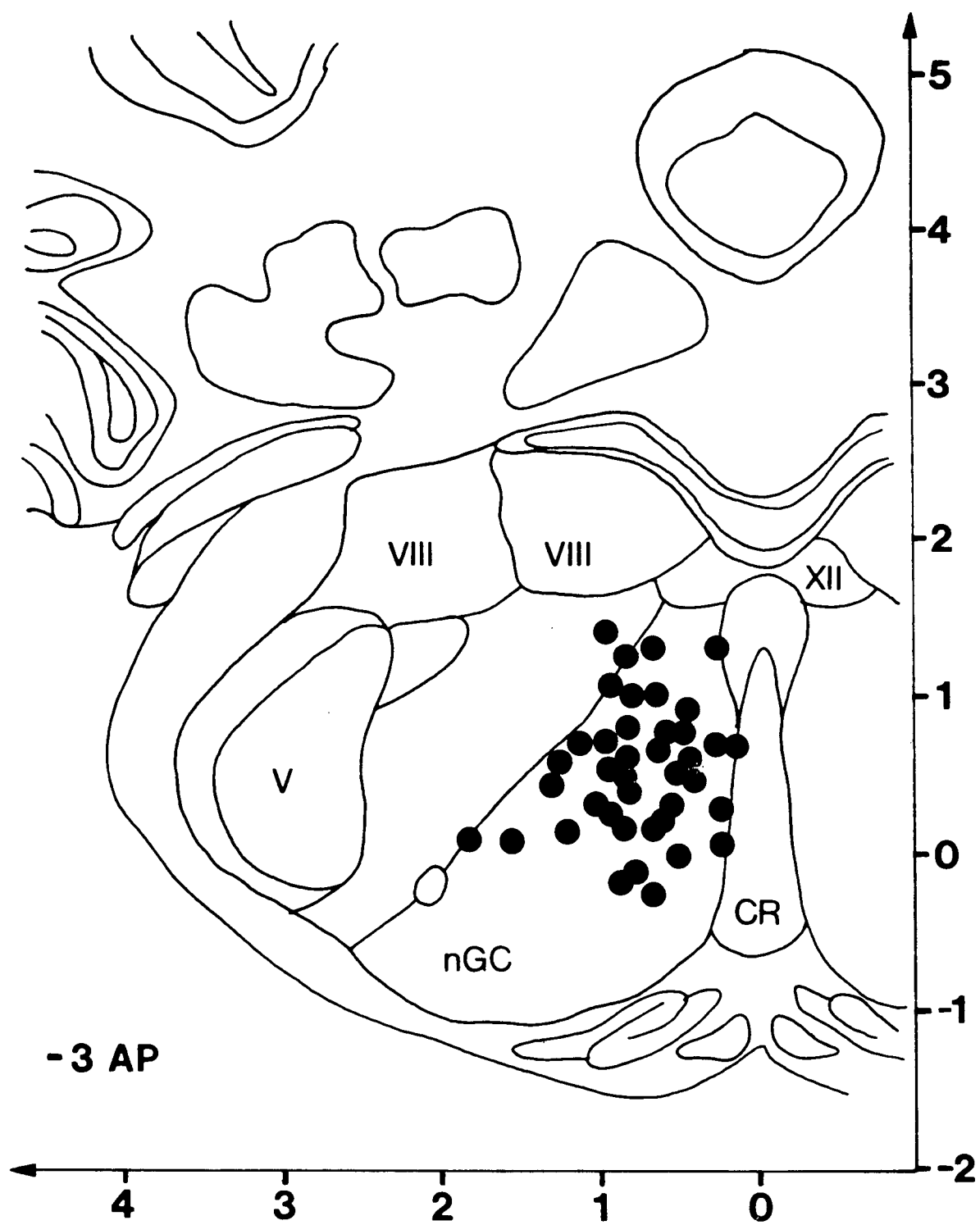
Table I: Numbers of neurones in the vicinity of the periaqueductal gray fulfilling or failing the criteria for antidromicity.

Criteria	Orthodromic		Antidromic	
	yes	no	yes	no
Collision	1	4	8	0
Following > 200 Hz	0	23	11	0
Latency $\leq$ 7 ms	30	17	32	0
Constant Latency	35	14	32	0
Total	59		32	

Figure 6: Coronal section showing the histologically determined locations of neurones in the vicinity of the PAG classified as being orthodromically activated by stimulation of the nGC. For classification criteria see text and Table I. Symbols and comments as in Figure 4.

CI

PAG

nRP

0 AP

7

6

5

4

3

2

1

0

-1

4

3

2

1

0
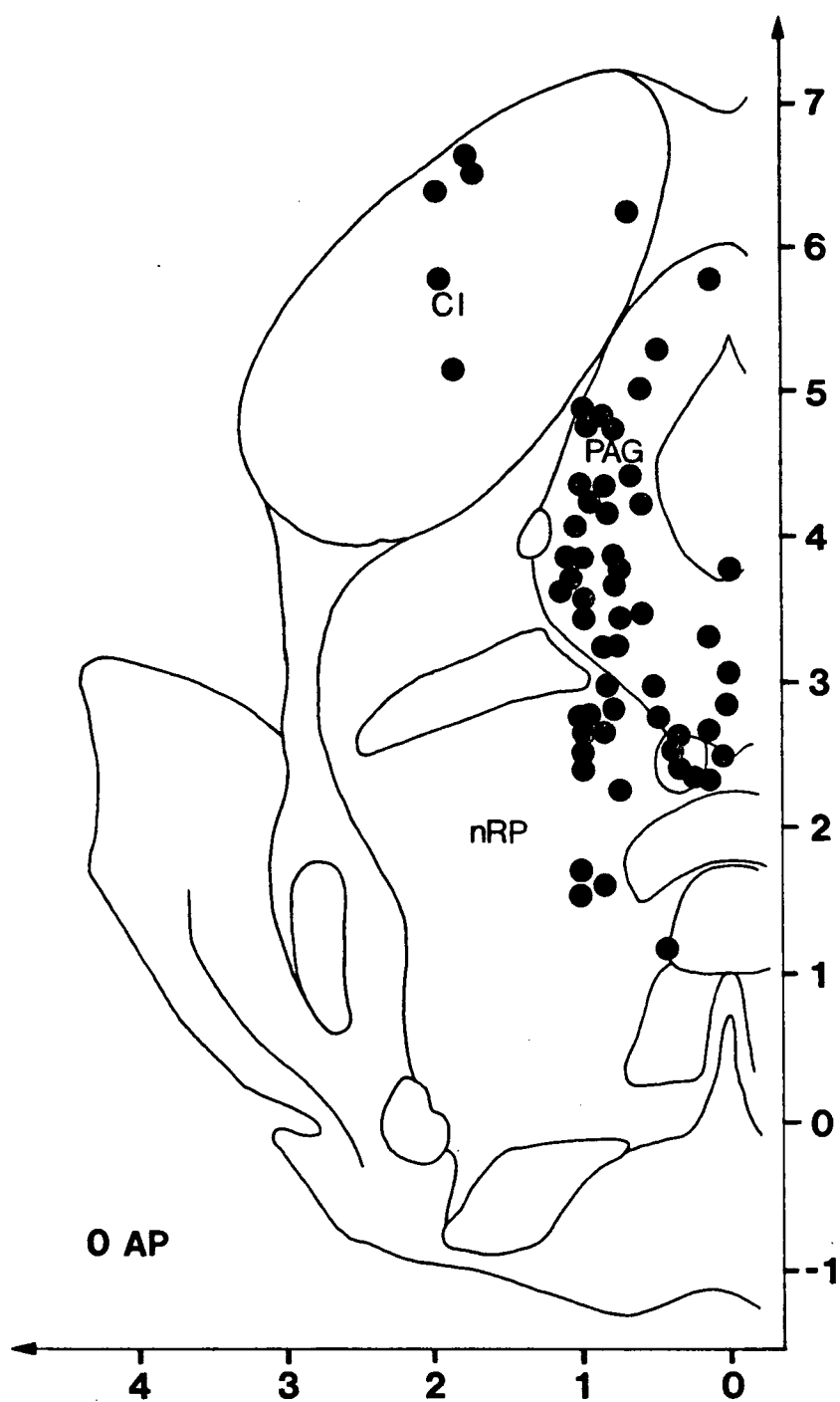
are illustrated in Figure 7.

Eight units with constant latencies of less than 7 ms demonstrated collision of evoked action potentials with spontaneously occurring orthodromic potentials. Two examples of collision are illustrated in Figures 8 and 9. One unit with a latency of 13 ms showed what appeared to be collision between a stimulation evoked action potential and a spontaneous potential. However, as it did not pass the short latency criterion its response was classified as orthodromic.

B. Nucleus Reticularis Gigantocellularis Neurones

A total of forty experiments were performed to study the effect of iontophonetic serotonin, iontophoretic and intravenous fluoxetine, which is a specific serotonin uptake blocker, noxious peripheral stimuli, and PAG stimulation on the activity of nGC neurones. As well, the effects of serotonin and fluoxetine on the nGC neuronal responses to stimulation of the sciatic nerve and the PAG were studied. The results of these experiments are described below.

The recordings made were almost certainly from cell bodies and not axonal fibres because 97% of the neurones tested with iontophoreses glutamate were excited. Glutamate is reported to produce excitatory effects specifically on cell bodies (Curtis et al., 1960; Krnjevic and Phyllis, 1963). Also, the recorded action potentials had constant size and were biphasic. Most neurones could be recorded over a distance of 50-100 μm.

Figure 7: Coronal section showing the histologically determined locations of PAG area neurones which were suspected of being antidromically activated by nGC stimulation. See Table I and text for classification descriptions. Symbols: ○ - displayed a contant latency of less than 7 ms; □ - followed stimulation to a frequency greater than 200 Hz and had constant latency of less than 7 ms; ◆ - demonstrated collision and had a constant latency of less than 7 ms. See Figure 4 for the other symbols and for comments.
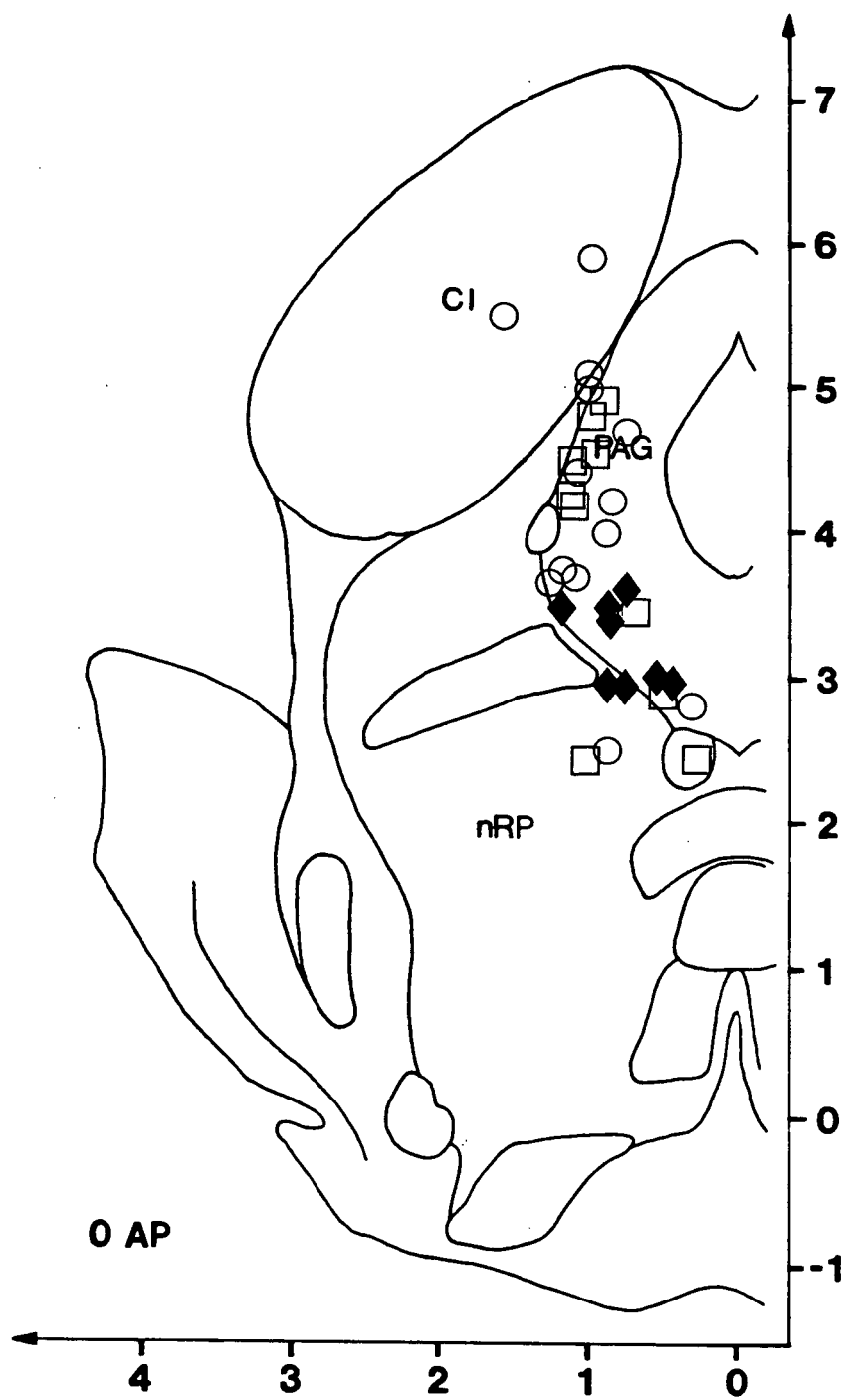
Figure 8: Oscilloscope tracings of a periaqueductal gray neurone showing collision of spontaneous action potentials and action potentials evoked by stimulation of the nucleus gigantocellularis. (a) Raster scan of consecutive stimulations of the nGC, at regular intervals, which caused the activation of the neurone to the right of the stimulation artifact (below the dot). Note that collision apparently occurred when a spontaneous action potential occurred within a certain distance of the artifact. (b) This trace is identical to the first trace above except it was at a higher amplification and had less filtering. Notice the prominent 'initial segment' of the evoked potential.
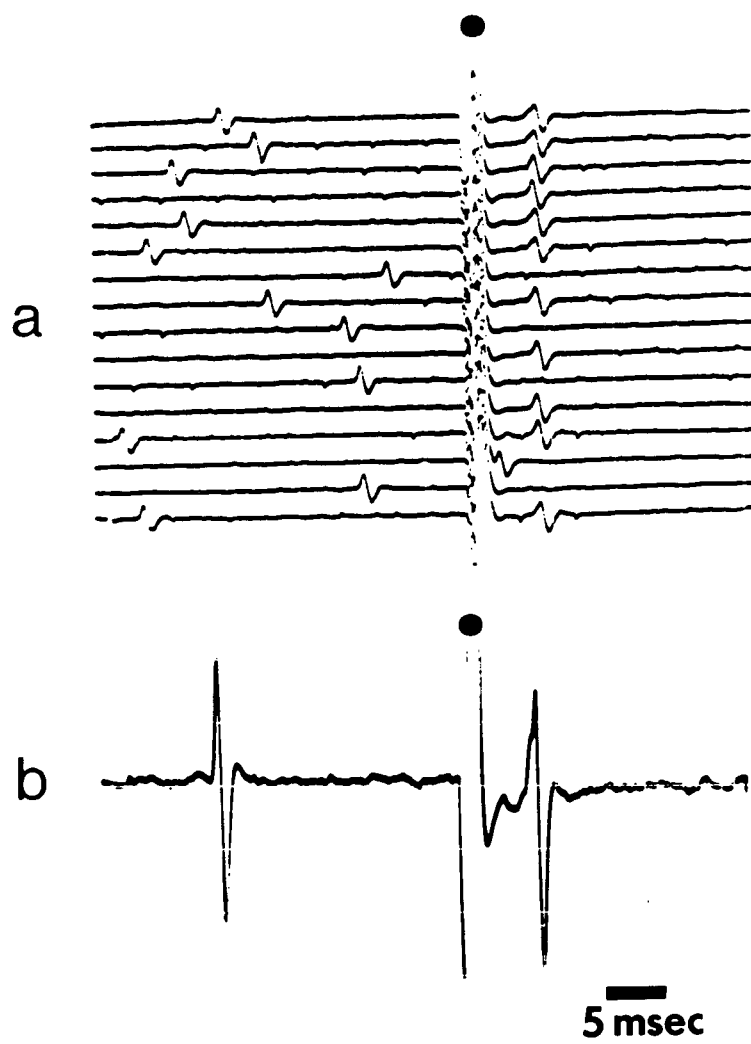
a

b

5 msec

Figure 9: Oscilloscope tracings showing spontaneously occurring action potentials colliding with and cancelling action potentials activated by triggered stimulation in the nGC. The stimulator was triggered by spontaneous potentials, and, after a selected delay, stimulated the nGC electrically. (a) A trace showing a spontaneous potential to the left of the stimulation artifact (indicated by the dot) which triggered the stimulator after a delay of 6.6 ms. (b) Similar to above but with a delay of 6.8 ms, an activated potential is seen at right as no collision with the spontaneous potential occurs as in part (a). (c) A raster scan of 32 sweeps or trials. Each dot represents an action potential or a stimulation artifact (under the dot). The stimulator was triggered by the spontaneously occurring potential to the left, and the delay until stimulation set successively to 6.6, 6.7, and 6.8 ms, but failed at the 6.6 ms setting because of collision.

a

b

c

6.6▶

6.7▶

6.8▶

2 msec
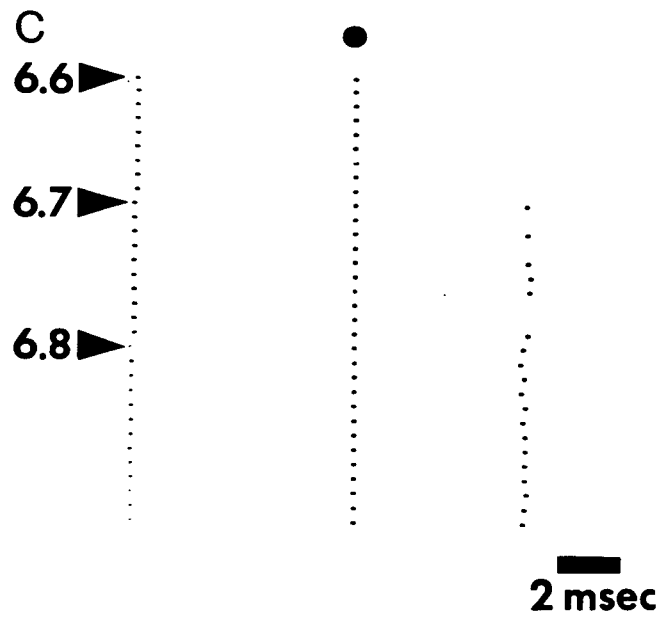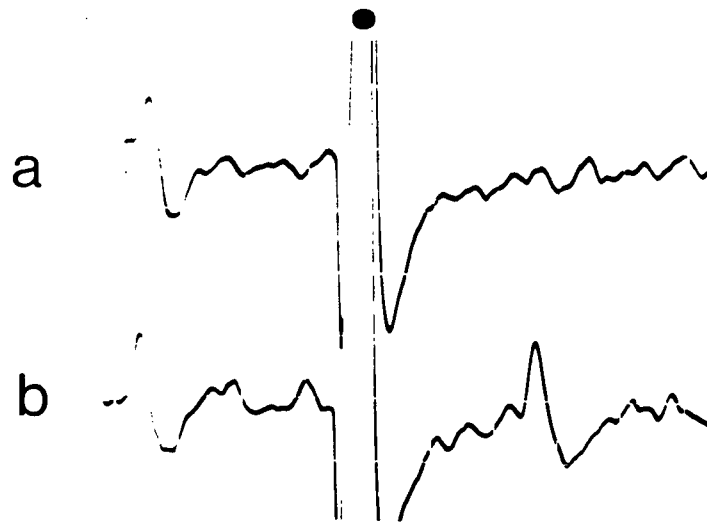
Effect of Drugs on Unit Activity

The effect of intravenous fluoxetine (3-10 mg/kg) was tested on 16 nGC neurones. Nine of these were unaffected by the drug. Of the three neurones inhibited by the fluoxetine, the inhibition was minimal in one case. Two of the 4 excitatory responses were also slight and another was transitory.

Microiontophoretic application of glutamate excited all but two, which were not affected, of the 59 neurones on which it was tested. Forty-two neurones were tested with iontophoresed serotonin. Approximately two-thirds of these responded to this treatment: 45% were inhibited and 21% excited. Similarly, 50% of the 28 neurones tested with iontophoresed fluoxetine were inhibited while 14% were excited and the remaining 36% were unaffected. These responses are listed in Table II and an example of a neurone's response to iontophoretic serotonin, fluoxetine, and glutamate is included in Figure 10.

Peripheral Stimuli

Twenty-nine units were tested with non-noxious forms of peripheral stimuli. These included light touch and air-puffs applied to the fur of the animal or gentle joint movement. Only 18% of the neurones tested were affected and these were excited (see Table II).

In contrast, 75% of the neurones tested with noxious stimuli were affected. Over one-half of the 224 neurones were excited (53%) by noxious stimuli which included pinch and pressure applied to the paws, ears and tail, radiant heating of

Table II: Responses of neurones in the nucleus gigantocellularis to various tests.

Test	Response				
	Excitation	Mixed	Inhibition	None	Total
sciatic nerve[1]	43	7	9	46	105
noxious[2]	118	15	35	56	224
non-noxious[3]	7	0	0	32	39
u[4] serotonin	9	–	19	14	42
u fluoxetine	4	–	14	10	28
u glutamate	57	–	0	2	59
PAG[5]	17	12	58	41	128

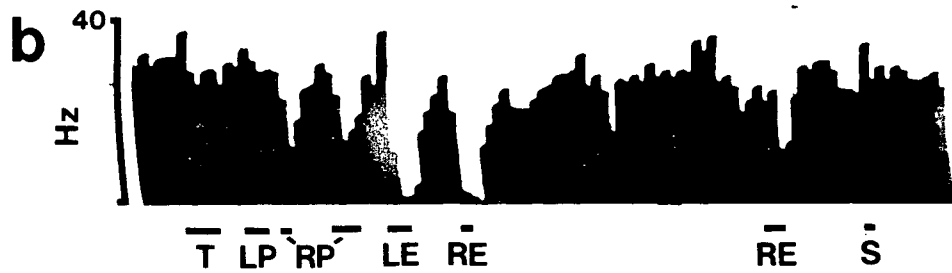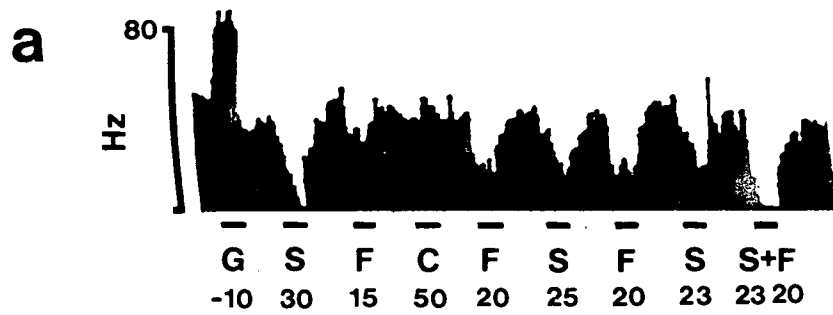1 - electrical stimulation of the sciatic nerve
2 - included pinch and pressure to the paws, ears, and tail, radiant heating of tail, and electrical stimulation of the sciatic nerve
3 - included air-puff and stroking applied to the animal's hair and gentle joint movement
4 - microiontophoretic application of ...
5 - electrical stimulation of the periaqueductal gray matter

Figure 10: Examples of nucleus gigantocellularis neuronal responses to iontophoresis and peripheral stimuli. (a) Ratemeter output displaying a nGC neurone's firing rate changes in response to iontophoretic serotonin (S), Fluoxetine (F), glutamate (G), and NaCl, used as a current control, (C). The abscissa represents time and the bars and numbers below it represent the periods of and the currents (nA) used for the drug applications, respectively. The ordinate represents unit activity in spikes per epoch, where each epoch is 1.0 s in duration. Notice the cell was excited by the glutamate and was inhibited by the serotonin and fluoxetine but was not affected by the current control. Also note that the inhibition by serotonin was enhanced by the simultaneous ejection of fluoxetine. (b) Activity of another nGC neurone in response to peripheral stimuli. It was excited by noxious pinch applied to the right paw (LP) and both ears (LE, RE) but not to the left paw (LP) or tail (T) nor by non-noxious stroking of the fur (S). (c) The top trace shows the temperature (ordinate) of a thermocouple mounted on the surface of the tail in the focus of the radiant heat source. The bottom trace shows another nGC neurone which was activated by noxious radiant heat pulses (47 ^{o}C, 15 s).
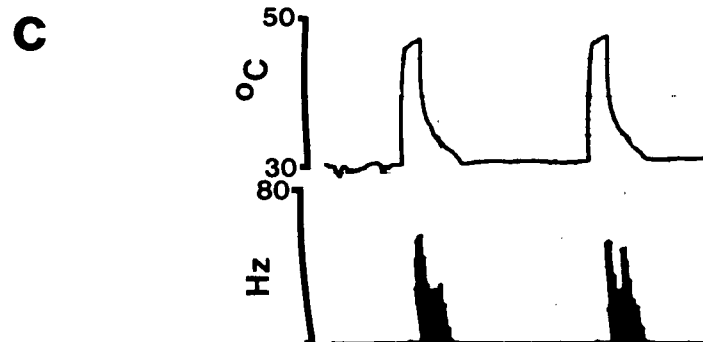
the tail, and electrical stimulation of the left sciatic nerve. In addition, some units (16%) were inhibited and others responded with mixed excitation-inhibition (7%). Examples of two neurones' response to peripheral stimuli are illustrated in Figure 10. The histologically determined locations of the neurones did not appear to be correlated to their responses to noxious peripheral stimuli as can be seen in Figure 11.

The responses mentioned above included stimulation of the sciatic nerve, however, stimulation of the sciatic nerve alone produced similar results. Of the 105 units tested with sciatic nerve stimulation, 41% were excited while approximately the same proportion were unaffected. A few neurones were inhibited (9%) or had mixed responses (7%). The responses are enumerated in Table II and three examples of typical responses are illustrated in Figure 12(d-f).

The excitatory responses had latencies ranging from 5 to more than 200 ms and durations ranging over 4-52 ms. The excitatory responses with latencies of less than 200 ms could be divided into two groups: short-latency responses having a mean latency of 10.2±1.3 ms and long-latency responses having a mean latency of 70±11 ms. The inhibitory responses' latencies and durations ranged over 5-35 ms and 4-85 ms, respectively. The mean inhibitory response latency was 13.8±4.7 ms, while the mean duration of the inhibitory responses was 30±11 ms.

In those units tested with both noxious peripheral stimuli and iontophoretic serotonin and/or fluoxetine there did not appear to be a correlation between the responses to the two tests. For example, of the 14 neurones which were excited by

Figure 11: Coronal section section showing histologically determined locations of nGC area units which responded to noxious peripheral stimuli. Stimuli included pinch and pressure to the paws, ears, and tail, radiant heating of the tail, and electrical stimulation of the left sciatic nerve. Symbols and comments as in Figure 5.

Response to noxious peripheral stimuli

▲- excitation only

○- mixed excitation and inhibition

▽- inhibition only
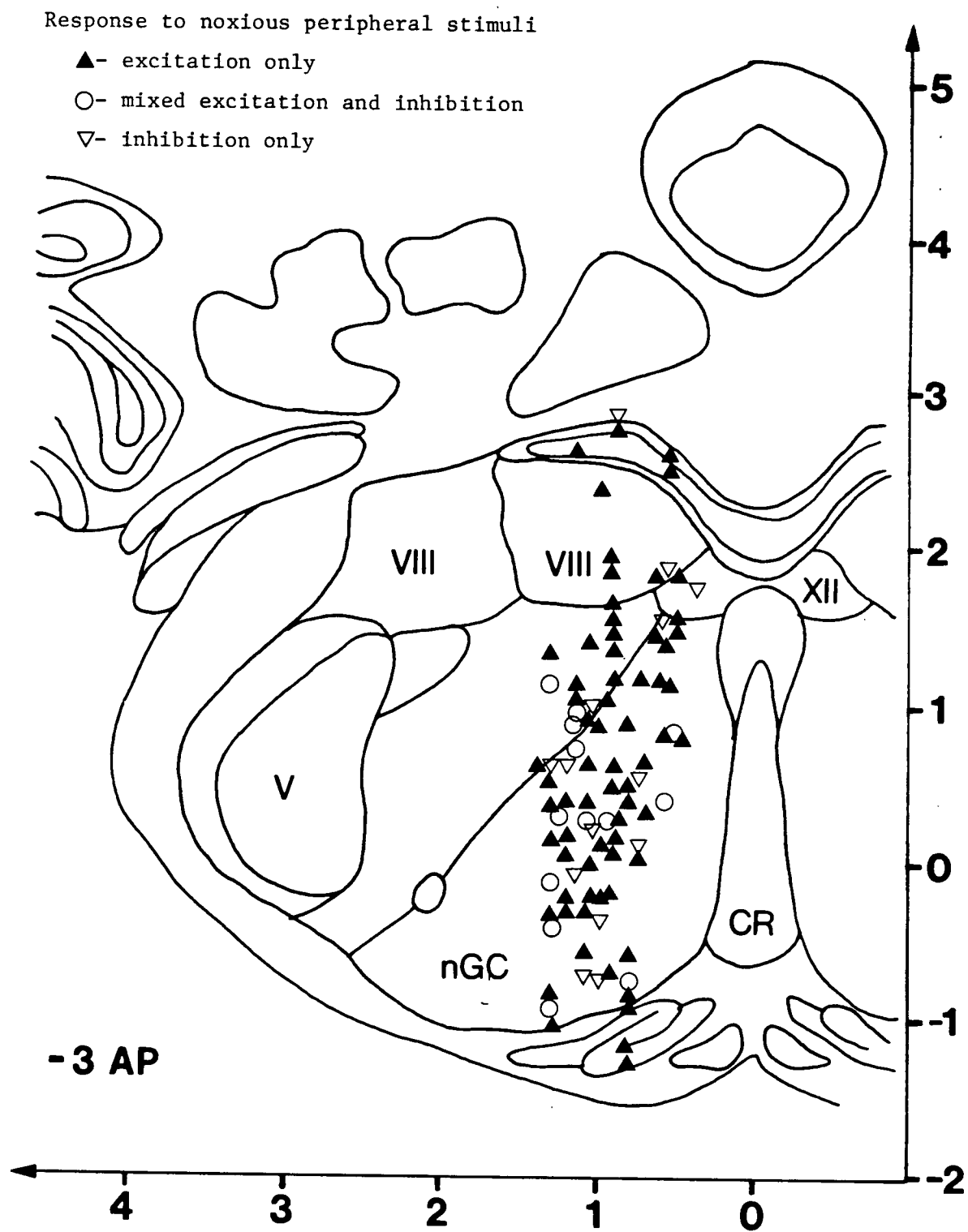
Figure 12: Examples of nucleus gigantocellularis neuronal responses to electrical stimulation of the periaqueductal gray (a-c) and the sciatic nerve (d-f). The responses are in the form of peristimulus time histograms (PSTH) and demonstrate neurones which responded with excitation only (a,d), mixed excitation-inhibition (b,e), and inhibition only (c,f). The abscissae represent time and the ordinates the number of spikes per bin (or epoch). The bars above the responses indicate the periods of stimulation and the large peaks below them are truncated stimulation artifacts. Stimulation of either a single pulse (a,e,f) (0.1 ms, 2-5 V) or a train of pulses (b-d) (0.05 ms, 2-5 V) and. Each PSTH is constructed from 32 or 64 sweeps. Bin width = 1.0 ms (a-e) or 2.0 ms (f).
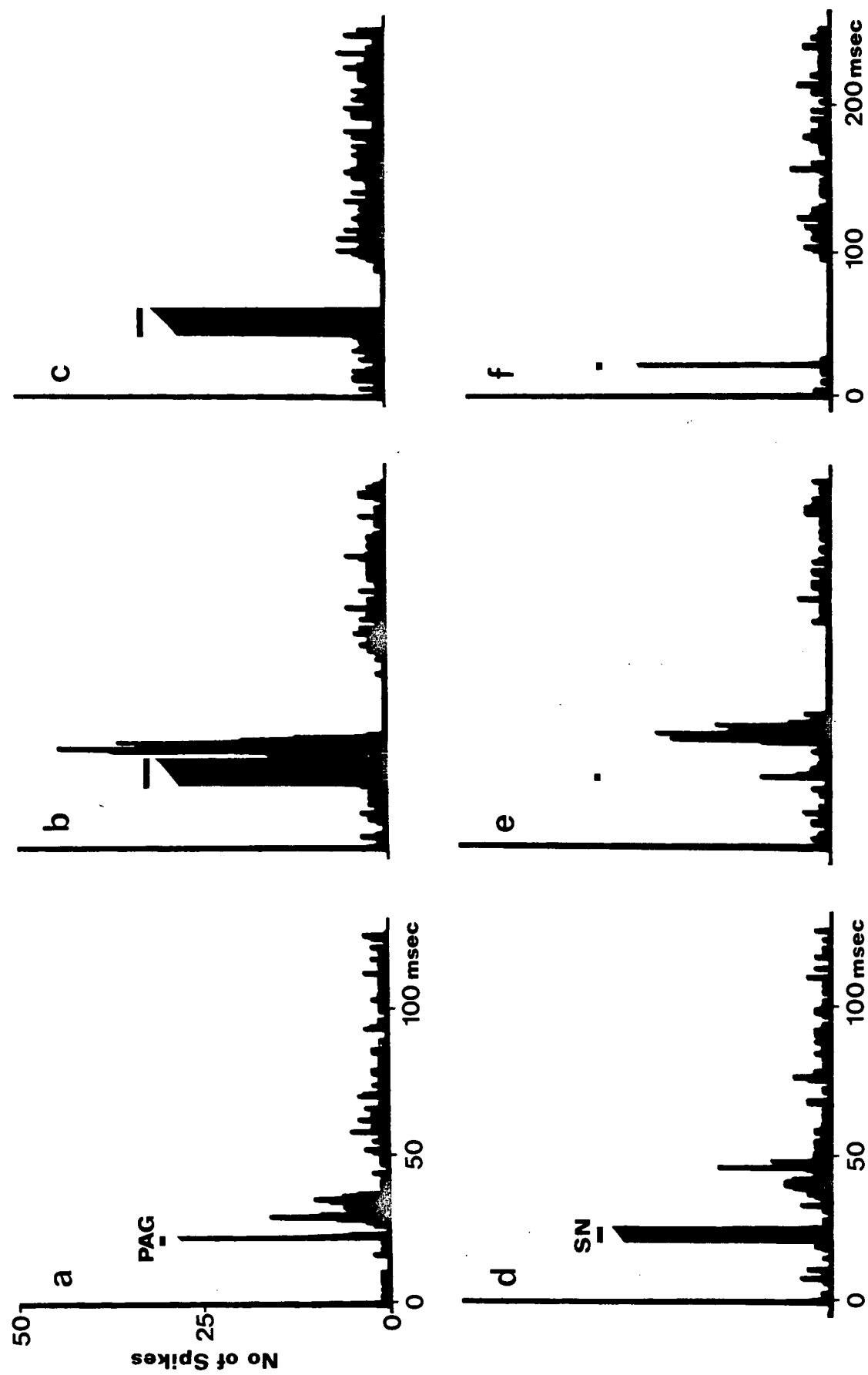
noxious stimuli only 4 were excited by serotonin (Table III).

Six units were tested with both intravenously administered fluoxetine and sciatic nerve stimulation. In 4 of these, drug administrations did not affect the response of the neurone to nerve stimulation. In the other two trials the excitatory response of the units was increased by the fluoxetine, as can be seen in Table IV.

Stimulation of the Periaqueductal Gray

The predominant response of the 128 nucleus gigantocellularis neurones tested with stimulation of the periaqueductal gray was inhibition (45%). Some excitatory (13%) and mixed excitatory-inhibitory (9%) effects were also seen while approximately a third of the neurones were non-responsive (see Table II). An example of each type of response is included in Figure 12 (d-f) and the histologically determined locations of the stimulation sites in the periaqueductal gray are mapped in Figure 13.

The excitatory responses of nGC neurones to PAG stimulation had latencies of 0-75 ms and durations of 0-110 ms. Their mean latency and duration were 23±6 ms and 33±8 ms, respectively. The inhibitory responses' latencies and durations ranged over 0-45 ms and 15-200 ms, respectively. The mean inhibitory response was 11.6±1.3 ms, while the mean duration of the inhibitory responses was 51±8 ms.

To see if there was a correlation between a neurone's response to noxious peripheral stimuli and its response to PAG stimulation, the numbers of neurones responding in each way to

Table III: Comparison of the responses of nucleus gigantocellularis neurones to noxious peripheral stimuli[1] and iontophoretic application of serotonin or fluoxetine.

Response to noxious peripheral stimuli	Responses to iontophoretic application of:					
	Serotonin			Fluoxetine		
	Excited	Inhibited	None	Excited	Inhibited	None
Excitation	4	9	1	1	7	1
Mixed	0	1	0	1	0	0
Inhibition	0	1	1	0	2	0
None	0	1	0	0	1	0
Column total	4	12	2	2	10	1

1-- included pinch and pressure to paws, ears, and tail; radiant heating of tail; and electrical stimulation of the sciatic nerve.

Table IV: The effect of intravenously applied fluoxetine
on the responses of nucleus gigantocellularis neurones to
electrical stimulation of the periaqueductal gray of the
sciatic nerve.

	Response to stimulation was:			
	Enhanced	Reduced	None	Total
Response to stimulation of the periaqueductal gray:				
Excitation	0	4	0	4
Inhibition	3	0	4	7
Response to stimulation of the sciatic nerve:				
Excitation	2	0	4	6
Inhibition	0	0	0	0

Figure 13: Coronal section showing the histologically determined location of the stimulating electrodes used in experiments which studied the effect of electrical stimulation of the periaqueductal gray on the activity of nucleus gigantocellularis neurones. Symbols and comments as in Figure 4.

CI

PAG

nRP

0 AP
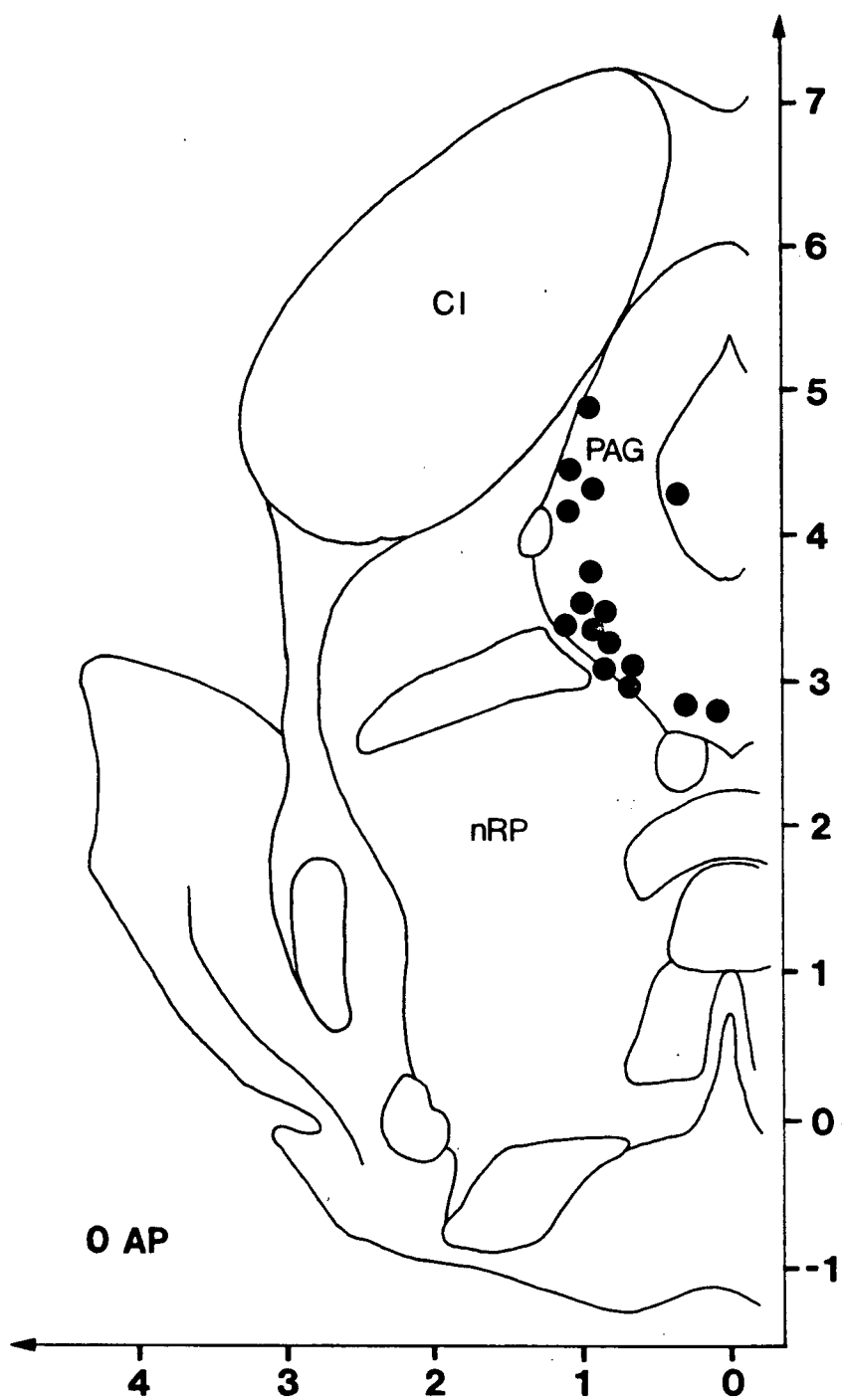
the two tests were enumerated. As can be seen from Table V the largest group of neurones (23) were excited by the noxious stimuli and inhibited by the PAG stimulation. This group was equal to 37% of those neurones affected by both treatments or 18% of the total neurones tested (128).

Although few neurones were tested with both iontophoreretic serotonin or fluoxetine and PAG stimulation, a comparison of the responses to these two tests reveals a modest correlation (see Table VI). Both of the units excited by PAG stimulation were also excited by iontophoretic serotonin. Similarly, 5 of the 7 neurones inhibited by PAG stimulation were also inhibited by serotonin.

The effect of intravenous and iontophoretic fluoxetine on the responses of nGC neurones to PAG stimulation were also tested. The results of intravenous fluoxetine are shown in Table IV. The inhibitory response produced by PAG stimulation in 4 units was enhanced by the fluoxetine. Another seven units were excited by PAG stimulation. In all 3 of these excitatory responses which were modified by the fluoxetine, the responses were reduced. Three examples of the effect of fluoxetine, both iontophoretic and intravenously administered, on the response of nGC neurones to PAG stimulation are illustrated in Figures 14 and 15.

Table V: Comparison of nucleus gigantocellularis neuronal responses to noxious peripheral stimuli[1] and stimulation of the periaqueductal gray.

Response to noxious peripheral stimuli	Response to electrical stimulation of the periaqueductal gray matter				Row Total
	Excitation	Mixed	Inhibition	None	
Excitation	14	8	23	17	62
Mixed	0	1	3	1	5
Inhibition	1	2	11	3	17
None	2	1	21	20	44
Column Total	17.7	12	58	41	128

1 - included pinch and pressure to the paws, ears, and tail, radiant heating of the tail, and electrical stimulation of the sciatic nerve.

Table VI: Comparison of the responses of nucleus gigantocellularis neurones to periaqueductal gray stimulation and iontophoretic application of serotonin or fluoxetine.

Response to periaqueductal gray stimulation	Responses to iontophoretic application of:					
	Serotonin			Fluoxetine		
	Excited	Inhibited	None	Excited	Inhibited	None
Excitation	2	0	0	1	2	0
Mixed	0	3	0	0	1	0
Inhibition	0	5	2	0	3	2
None	1	2	0	0	1	0
Column total	3	10	2	1	7	2

Figure 14: Examples of two neurones (a-b, c-f) inhibited by
periaqueductal gray stimulation and the enhancement of this
inhibition by fluoxetine, a specific serotonin uptake blocker.
Responses are shown in the form of peristimulus time histograms
with time on the abscissae and the number of spikes per bin (or
epoch) on the ordinates. The bars above the responses represent
the periods of stimulation and the large responses below them
are truncated stimulation artifacts. (a) Control response of
first unit to PAG stimulation (10 pulses, 0.05 ms, 4 V). (b)
Enhancement of the inhibitory period 15 min after intravenous
fluoxetine (3 mg/kg). (c) Control response of the second neurone
to PAG stimulation (10 pulses, 0.05 ms, 5 V). (d) Enhancement of
response during iontophoresis of fluoxetine (40 nA, 1 min). (e)
Eight min after iontophoresed fluoxetine, the response was
comparable to the control in (c). (f) Slight enhancement of
response 6 min after intravenous fluoxetine (5 mg/kg). Bin width
= 2 ms, 64 sweeps per response.
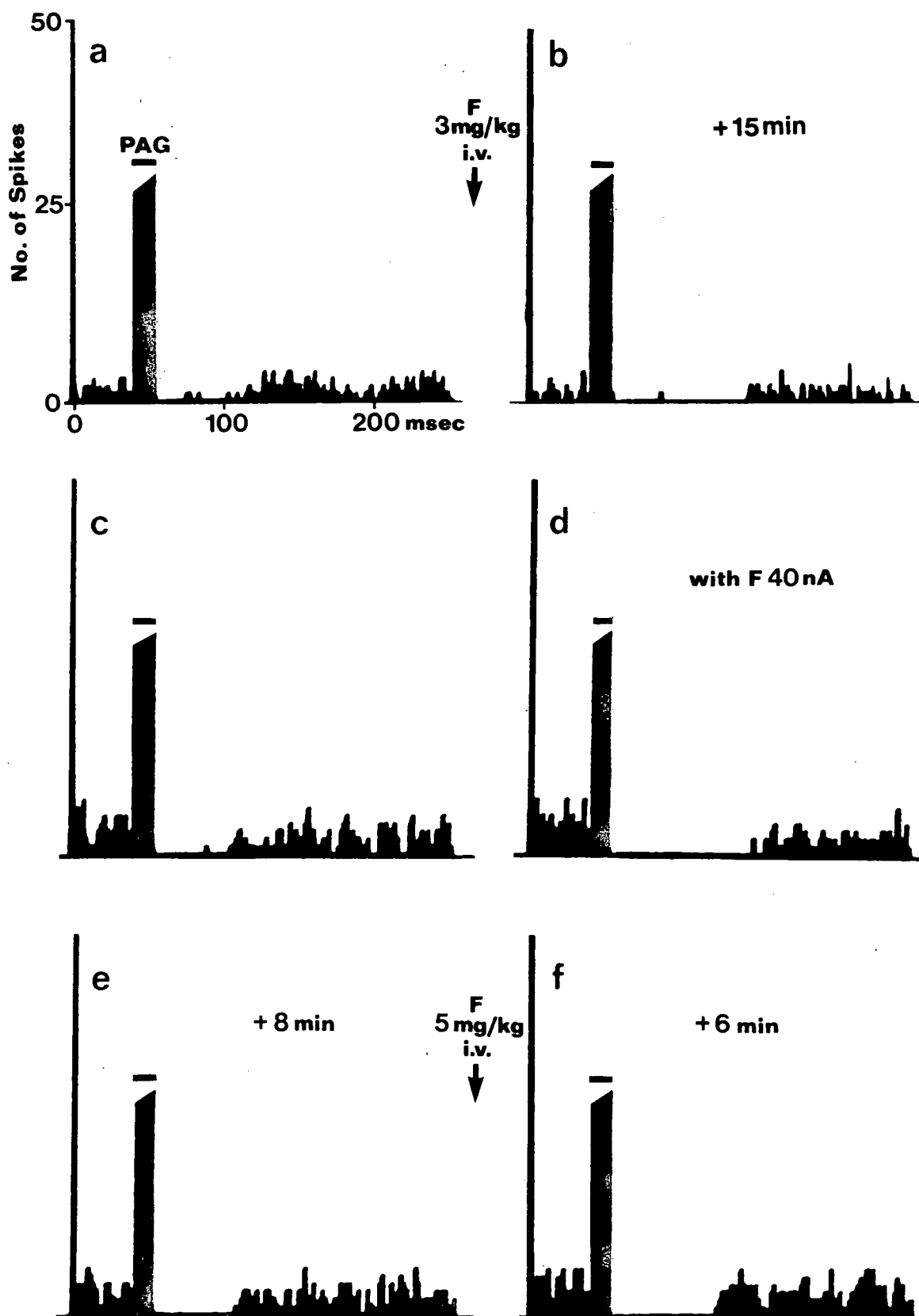
Figure 15: An example of a nucleus gigantocellularis neurone which was excited by periaqueductal gray stimulation and the reduction of this response by fluoxetine, a specific serotonin uptake blocker. See Figure 14 for a description of the peristimulus time histograms. (a) Control response. (b-d) 9.5, 23, and 25 min after iontophoresis of fluoxetine (60 nA, 1 min), respectively. (e,f) 30 and 40 min after intravenous fluoxetine (two 5 mg/kg doses). Bin width = 1.0 ms, 64 sweeps per response.
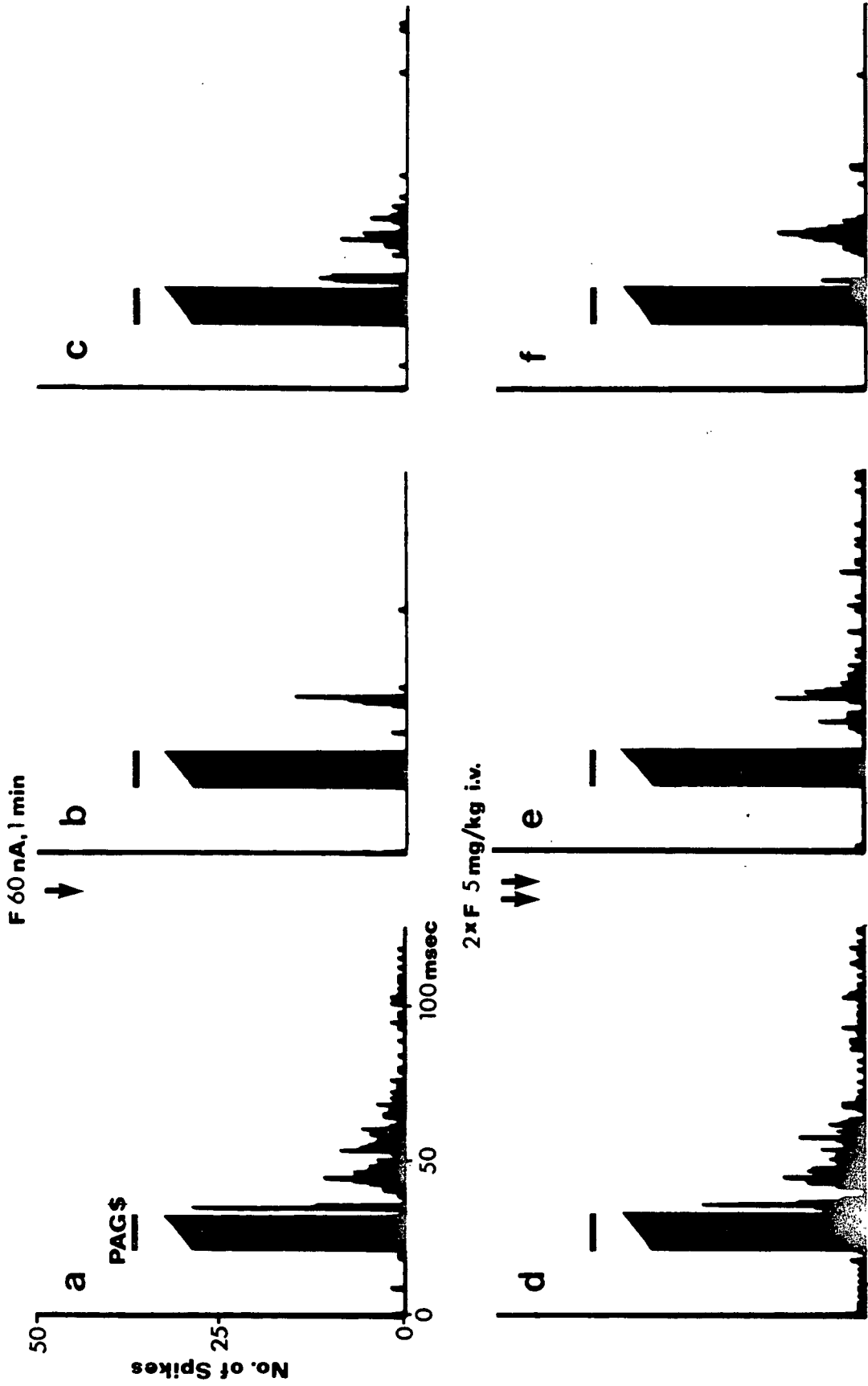
DISCUSSION

A. Periaqueductal Gray Neurones

Noxious Peripheral Stimuli

We found many neurones in the vicinity of the periaqueductal gray which responded to noxious peripheral stimuli. This was expected as the spinotectal tract is known to terminate in this area (Mehler, 1960; Kerr, 1975). Also, evoked potentials have been recorded from this area in rats in response to electrical stimulation of the skin (Mayer and Liebeskind, 1971) and the sciatic nerve (Palmer and Klemm, 1976). Recent studies, which recorded unit activity from neurones in the same area, also found excitatory and inhibitory responses to noxious stimuli which included pinch, stimulation of peripheral nerves, and radiant heat (Aghajanian et al., 1978; Haigler, 1978; Sanders et al., 1980.).

Aghajanian et al. (1978) reported that serotonergic neurones were inhibited and non-serotonergic neurones were excited by noxious peripheral stimuli. Sanders et al. (1980) found that most neurones with excitatory responses were located in the periaqueductal gray, while those inhibited were located in the dorsal raphé. This agrees with the previous study as the dorsal raphé is a major serotonergic nucleus. We did not find any correspondence between cell locations and responses to noxious stimuli. However, dorsal raphé neurones are reported to have slow spontaneous firing rates (less than 2 Hz) which makes inhibitory responses very hard to recognize. Therefore, our

results are very probably biased towards neurones with relatively faster spontaneous firing rates and those responding with excitation. This also probably resulted in many of the inhibitory responses of dorsal raphe neurones being classified as non-responsive.

Stimulation of the Nucleus Gigantocellularis

The orthodromic responses of neurones in the vicinity of the PAG elicited by stimulation in the nGC were usually easily identified as most showed multiple firing at increased stimulus intensities, very long latencies, or very inconsistent latencies. However, distinquishing between orthodromic and antidromic responses with constant latencies of less than 7 ms was more difficult.

Constant latency is a fairly poor criterion for antidromicity except at threshold. However, even at threshold orthodromically activated neurones might have shown constant latencies if the synaptic input on to them was itself constant. In such a case the neurone would have been excitable to the same degree for each synaptically evoked response and therefore would have displayed a constant latency. Conversely, antidromically activated neurones can have latencies varying up to 0.5 ms due to delays of the action potential crossing the axonal hillock and invading the cell body (Eccles, 1955). This again depends upon the excitability of the cell body membrane and also upon the rate of increase in membrane area that the potential encounters as it travels from the axon to the cell body. In this study only 29% of the 49 neurones which were classified as

orthodromic had inconsistent latencies. Therefore, inconstant latency appears to be a good indication of orthodromic activation but constant latency not to be a good criterion for antidromic activation.

The second criterion, the maximum frequency at which the response can follow the stimulus, is often cited as good evidence for antidromicity. However, orthodromically activated neurones can sometimes follow at high frequencies (Darian-Smith et al., 1963) and, conversely, antidromically activated neurones can sometimes fail to follow high stimulation frequencies (Eccles, 1955). Again, this failure is due to the blockade of the action potential at the axonal hillock. In the present study, because the distance between the stimulation site and the recording site was so small, it was often difficult to test following frequency because of the large stimulation artifact. In addition, if long trains of stimuli were attempted, an increasingly large artifact occurred due to both summation of multi-unit evoked potentials and muscle twitch. Therefore, because of these difficulties in testing for following, some neurones which were actually antidromically activated may have been erroneously classified as orthodromic in this study. Even so, high frequency following would appear, at least in this study, to be a good indication of antidromicity because none of the responses classified as orthodromic followed the stimulation at high frequencies.

The final criterion of antidromicity, collision of orthodromic action potentials with antidromic ones, is generally considered to be the most robust of the criteria. However, some

problems do occur in actual practice. Firstly, to test for
collision, the cell under study must be spontaneously active or
capable of being activated. Activation can occur by the
iontophoretic application of an excitatory drug, such as
glutamate, onto the neurone or by increasing the excitatory
synaptic input to the cell. In this study some neurones were
activated by applying noxious pressure to the tail. Secondly,
the recorded action potential had to be large enough to give a
signal-to-noise ratio which was sufficient to allow the
consistent triggering of the stimulator.

Thirdly, the critical period is theoretically the sum of
the latency and the refractory period. However, the measurement
of these two values is subject to error. The measurement of a
refractory period involves observing the maximum frequency at
which the response consistently follows the stimulation.
Unfortunately this actually measures the 'least interval for two
propragated spikes' (cf. Fuller and Schlag, 1975) travelling
the same direction along the axon, the second spike following
immediately after the first's refractory period. For antidromic
collision the two spikes are travelling in opposite directions
and therefore the value required to calculate the critical
period will be less than that measured. The measurement of the
latency of response is subject to two errors. There is a delay
between the onset of the stimulation pulse and the activation of
the fibre. This is termed utilization time and can range over
0.2-0.5 ms (Blair and Erlanger, 1936; cf. Fuller and Schlag,
1976). There is also a delay between the arrival of the spike
at the cell body and the invasion of the soma, which is what is

usually recorded extracellularly. This delay can typically be
0.2 ms longer than the equivalent delay in orthodromic
activations (cf. Fuller and Schlag, 1976). The first two
errors can be lessened by increasing the stimulus intensity.
Unfortunately, the maximum stimulus levels which could be used
in this study were dictated by the size of the stimulation
artifact, the presence of an evoked muscle movement, and
saturation of the unity gain preamplifier.

The errors discussed in the previous paragraph are fairly
constant and do not vary with latency or refractory period.
Therefore, the error in the calculation of the critical period
for collision becomes relatively large at short latencies and is
typically 0.5-2 ms. The neurones in this study typically showed
collision in a period approximately equal to the latency. If
the error in the measurement of latency is not taken into
consideration then the refractory period would appear to be very
short or non-existent and the error to be about as large as the
refractory period. If the latency of a response is very short
(<2 ms), it would be possible to see what appears to be
collision but what is actually due to the arrival of an evoked
orthodromic potential during the refractory period of a
preceding spontaneous potential. However, for this to happen
would require the refractory period to be greater than two times
the latency.

Some anomalies were observed in the responses of some
neurones. One orthodromic neurone, classified as such because
it had a latency of 13 ms, appeared to demonstrate collision.
If this neurone was actually activated antidromically this would

imply that the conduction velocity of this neurone was about
0.3 ms. If it was in fact orthodromically activated then the
apparent collision might have been due to an extremely long
refractory period (in the order of 20 ms), recurrent inhibition,
or inhibition via a slower pathway. Some neurones demonstrated
collision where the collision interval was much greater than a
reasonable critical period. Again, this may be due to an
abnormally long refractory period or recurrent inhibition.

As well as the above criticisms and difficulties with the
individual criteria, other general difficulties must be
discussed. Because of the filtering which was necessary in
these experiments to attenuate unwanted noise, the shape of the
action potentials was distorted. This made observation of the
initial segment of the action potentials difficult except for
exceptionally large spikes. The filtering also lengthened the
time constant of the stimulus artifacts, increasing their
duration to 1-2 ms. This meant that action potentials with
latencies of less than 1 ms, or conversely, with conduction
velocities greater than 3.5 m/s, were very difficult or
impossible to distinguish from the artifact. For this reason,
the results of these experiments are biased toward long latency
responses and many antidromically activated neurones may have
been missed.

However, there are a few additional features of antidromic
activations which are useful in their identification.
Fractionation of an antidromic action potential may occur due to
blockade of the potential at the axonal hillock. The blockade
may occur at any stimulus intensity as the activated fibre

potential has an all-or-nothing nature (Bishop et al., 1962).
If the potential fails to invade the cell body then the
potential observed is reminiscent of the initial segment of a
synaptically activated potential, but is much larger and can be
distinguished easily. Also as opposed to orthodromic stimuli
which can cause multiple post-synaptic potentials from one
stimulus, antidromic stimuli cause only one activation per
stimulus.

A final difficulty in the interpretation of these results
is the possibility that the antidromic action potentials are due
to the activation of fibres which do not terminate in the nGC.
While no assurance can be given that this is not in fact the
case, anatomical studies using autoradiographical (Bobillier et
al., 1976) and HRP (Gallager and Pert, 1978) techniques also
indicate that the nGC receives direct axonal input from this
region.

While we found it difficult to test every criterion on
every neurone it is very likely that periaqueductal gray
neurones were antidromically activated from the nGC. In
addition to neurones which demonstrated collision, neurones
which met the high frequency criterion were also probably
antidromically activated. As well, some neurones classified as
orthodromic may have actually been antidromically activated as
suggested above. Some neurones may have failed the collision
criterion because of lack of spontaneous activity or action
potentials which were too small to trigger the stimulator. Some
neurones may have failed the following criterion due to the
technical difficulties. In addition, some long latency

responses may in fact be antidromic if the conduction velocities
of the fibres involved were less than 0.5 ms or if the fibres
took circuitous routes. For example, the neurone which
apparently showed collision with a latency of 13 ms did have a
constant latency and displayed fractionation. Finally,
antidromically activated neurones may have been missed because
they had very short latencies (<1 ms) or because they projected
via thin non-invadable axons (Sanders et al., 1980). The
neuronal population studied is very probably biased towards
larger neurones and this may also have influenced the numbers of
orthodromic versus antidromic responses. Therefore, it is very
likely that there is a monosynaptic pathway from the PAG to the
nGC.

B. Nucleus Gigantocellularis Neurones

Effects of Drugs

Intravenous fluoxetine increased (4), decreased (3), or did
not effect (9) the spontaneous activity of the 16 nGC neurones
on which it was tested. The effects of intravenously
administered fluoxetine on the spontaneous firing of nGC
neurones is difficult to interpret for a number of reasons.
Firstly, most of the responses were slight or transitory in
nature. Secondly, PSTHs of the responses of nGC neurones to PAG
and sciatic nerve stimulation were being constructed
concurrently with the drug administration making it difficult to
separate the effects of the stimulation on the neuronal activity
from those of the drug. Thirdly, the nGC receives diffuse input

from many areas making it impossible to know if the responses of the nGC neurones were due to direct or indirect effects.

Many other workers have reported excitatory and inhibitory responses of neurones in the vicinity of the nucleus gigantocellularis to iontophoretically applied serotonin in the cat (Bradley and Wolstencroft, 1965; Boakes et al., 1970) and the rat (Bradley and Dray, 1973; Boakes et al., 1974; Haigler and Aghajanian, 1974; Briggs, 1977). In contrast to this study these workers generally found a higher percentage of excitatory than inhibitory responses. This may have been due to the use of urethane as an anaesthetic agent in this study as Bradley and Dray (1973) reported that urethane increased the proportion of inhibitory responses to iontophoresed serotonin. Also, as creatinine is itself reported to be excitatory on some neurones, some of the excitatory responses that these workers saw may have been due to the serotonin-creatinine complex used (Bradley and Wolstencroft, 1965). Some neurones responding to serotonin with excitation were excluded from this study because they were due to the direct excitatory effect of the ejection current. Not all of the previous studies were controlled for current effects. Lastly, the population of neurones tested in this study is quite small and therefore may not be representative of the entire neuronal population of the nGC. Also, the group of neurones studied will be biased towards large neurones with stable background firing rates as these are the easiest to record and test.

Responses to Peripheral Stimuli

Most neurones in the vicinity of the nGC responding to
peripheral noxious stimuli were excited although some inhibitory
or mixed excitatory-inhibitory responses were also seen. These
results are in general agreement with the findings of other
workers. The majority of nGC neurones in the cat are responsive
to noxious peripheral stimuli. These include noxious pinch and
pressure (Wolstencroft, 1964; Casey, 1969, 1971c), intra-
arterially administered bradykinin (Guillbaud et al., 1973b;
Besson et al., 1974), peripheral nerve stimulation (Goldman et
al., 1972; Casey, 1969, 1971c; Eccles, 1975; Fox and
Wolstencroft, 1976; Pearl and Anderson, 1978), and cutaneous
stimulation (Peterson et al., 1974; LeBlanc and Gatipon, 1974).

Stimulation of the sciatic nerve was classified as a
noxious stimulus in this study. This was probably justifiable
because Goldman et al. (1972) report that the threshold of A-
delta fibre activation in the feline sciatic nerve was 0.14-
2.5 V. The stimulation intensities used in this study ranged
between 2 and 25 V with the majority lying between 5 and 10 V.
In addition, Goldman et al. (1972) stated that few neurones in
the nGC were affected by just A-beta stimulation, most also
required A-delta fibre activation. Similarly, Casey (1969)
found that 70% of the neuronal responses were due to A-delta
activation.

The proportion of excitatory, inhibitory, and mixed
responses to noxious stimuli vary among the reports, depending
upon the techniques and stimuli used, but are generally
comparable to those reported here. Multiple excitations and

inhibitions have been reported and were also observed in this study. They made the measurement and interpretation of the responses difficult as it was hard to decide where one response ended and another began. Excitatory responses are reported to have latencies of 5-20 ms and durations of 8-60 ms, which are in agreement with our findings. In addition we found some responses with durations of greater than 200 ms. Interestingly, Peterson et al. (1974) reported that depolarization of some units, which were recorded intracellularly, lasted for over 200 ms. Inhibitory responses were reported to have latencies of 5-70 ms and durations comparable to those reported here. Again, intracellular hyperpolarizations last greater than 200 ms after paw pad cutaneous shocks in the cat (Peterson et al, 1974).

Summation of excitatory responses over multiple stimuli has been observed (Goldman et al., 1972; LeBlanc and Gatipon, 1974) and may explain the differences in response durations between the responses due to natural stimuli versus electrical stimulation. Finally it must be noted that all of the above studies were done in the cat and therefore may not be directly comparable to the rat. In any event, it is reasonable to conclude that nGC neurones in the rat receive noxious peripheral input and therefore are probably involved in nociceptive processing.

The apparent enhancement of the excitatory response of the nGC neurones to stimulation of the sciatic nerve by intravenously administered fluoxetine is difficult to interpret. Because the nGC has diffuse inputs the fluoxetine may not be acting directly on the nGC neurones. To support this argument

there is no clear correlation between the response of nGC
neurones to peripheral noxious stimuli and their response to
iontophoretic serotonin or fluoxetine in those neurones tested
with both the noxious stimuli and the iontophoretic drugs.
Clearly, a greater number of neurones need to be looked at to
distinguish any relationship between these two treatments.

Responses to PAG Stimulation

Most nGC neurones were inhibited or unaffected by
periaqueductal gray stimulation. Some excitatory and mixed
excitatory-inhibitory responses were also seen. Other workers
have reported similar responses of nGC neurones to electrical
stimulation of the PAG. In preliminary experiments Casey and
Morrow (1976) found that 55% of rat nGC neurones driven by tail
shock at 10-15 ms latencies and 40% of the units responding to
noxious mechanical or thermal stimuli were inhibited by PAG
stimulation. This compares favourably with the 40% of neurones
in this study that responded to noxious peripheral stimuli and
which were also inhibited by PAG stimulation.

Morrow and Casey (1976) carried out further experiments in
awake rats. They found that 65% of neurones tested with PAG
stimulation were inhibited as compared to the 45% of the
neurones tested in this study. In addition, they reported that
stimulation of analgetic sites in the PAG, that is those sites
that supported SPA as measured by tail-flick latencies,
inhibited approximately 90% of nGC neurones which were excited
by noxious peripheral stimuli but only 48% of the neurones
excited solely by non-noxious stimuli. These results suggest a

selective inhibition of nGC nociceptive neurones over non-nociceptive neurones.

In agreement with this study Mohrland and Gebhart (1979) found that PAG stimulation produced excitatory, inhibitory, and mixed effects on the spontaneous firing rates of nGC neurones. They also found that 80% of nGC neuronal responses to noxious stimuli were inhibited by stimulation of analgetic PAG sites, but that non-analgetic sites were ineffective in this regard. We found that sites in the PAG which affected nGC neuronal spontaneous firing inhibited 58% of neurones responding to noxious peripheral stimuli were inhibited. Probably selection of PAG stimulation sites, i.e. SPA sites, in this study would have increased the percentage of neurones inhibited by PAG stimulation.

The antidromic experiments indicate that there is very likely a monosynaptic pathway from the PAG to the nGC. However, the latencies of the responses of some nGC neurones to PAG stimulation suggest that they were probably mediated by polysynaptic mechanisms. Possibly these effects of PAG stimulation are mediated by the medial raphé nucleus, the caudal raphé nucleus, or the dorsal tegmentum. All of these areas have been reported to send inputs to the nGC (Bobillier et al., 1976; Briggs, 1976; Gallager and Pert, 1978). Also, as the raphé nuclei are serotonergic, their involvement would explain the correlation observed in this study between the responses of nGC neurones to PAG stimulation and their responses to iontophoretic application of serotonin or fluoxetine.

Intravenously administered fluoxetine reduced all four PAG

stimulation induced excitations, and enhanced three of seven inhibitory responses of nGC spontaneously firing neurones. Also, there was a moderate correlation between the effects of iontophoretic serotonin and fluoxetine and the effects of PAG stimulation on nGC neuronal firing. These two results together suggest that the inhibitory affects of PAG stimulation on the nGC may be mediated by serotonin.

C. Conclusion

In the introduction we described two possible schemes by which the nGC may mediate periaqueductal SPA. The results of this study will be discussed in terms of these schemes in the following paragraphs.

Periaqueductal gray SPA may be mediated by the inhibition of nGC neurones involved in an ascending nociceptive pathway (see Fig. 16c). These nGC neurones would be predicted to be excited by noxious peripheral stimuli and to be inhibited by PAG stimulation. Indeed, in this study the majority of neurones in the nGC were excited by noxious peripheral stimuli (70% of the 168 neurones affected) and many were inhibited by stimulation of the PAG (67% of the 87 neurones affected). Of those neurones tested with both PAG stimulation and noxious peripheral stimuli, 37% were inhibited by the PAG stimulation and excited by the noxious stimuli. These data suggest that many of the nGC neurones are likely to be involved in an ascending pathway and by under the inhibitory control of the PAG.

Conversely, neurones within the nGC may be mediating periaqueductal SPA by modulating spinal cord nociceptor-driven

Figure 16: Very simplified diagrams of the nervous system
showing the proposed schemes by which the nucleus
gigantocellularis may mediate periaqueductal gray stimulation
produced antinociception. Mediation of the SPA by: (a) nGC
inhibition of spinal cord nociceptor-driven neurones; (b) tonic
nGC excitation of spinal cord neurones; or (c) nGC neurones
acting as relays in an ascending nociceptive pathway. Symbols:
nGC - nucleus gigantocellularis; PAG - periaqueductal gray; SC -
spinal cord; T - thalamus.
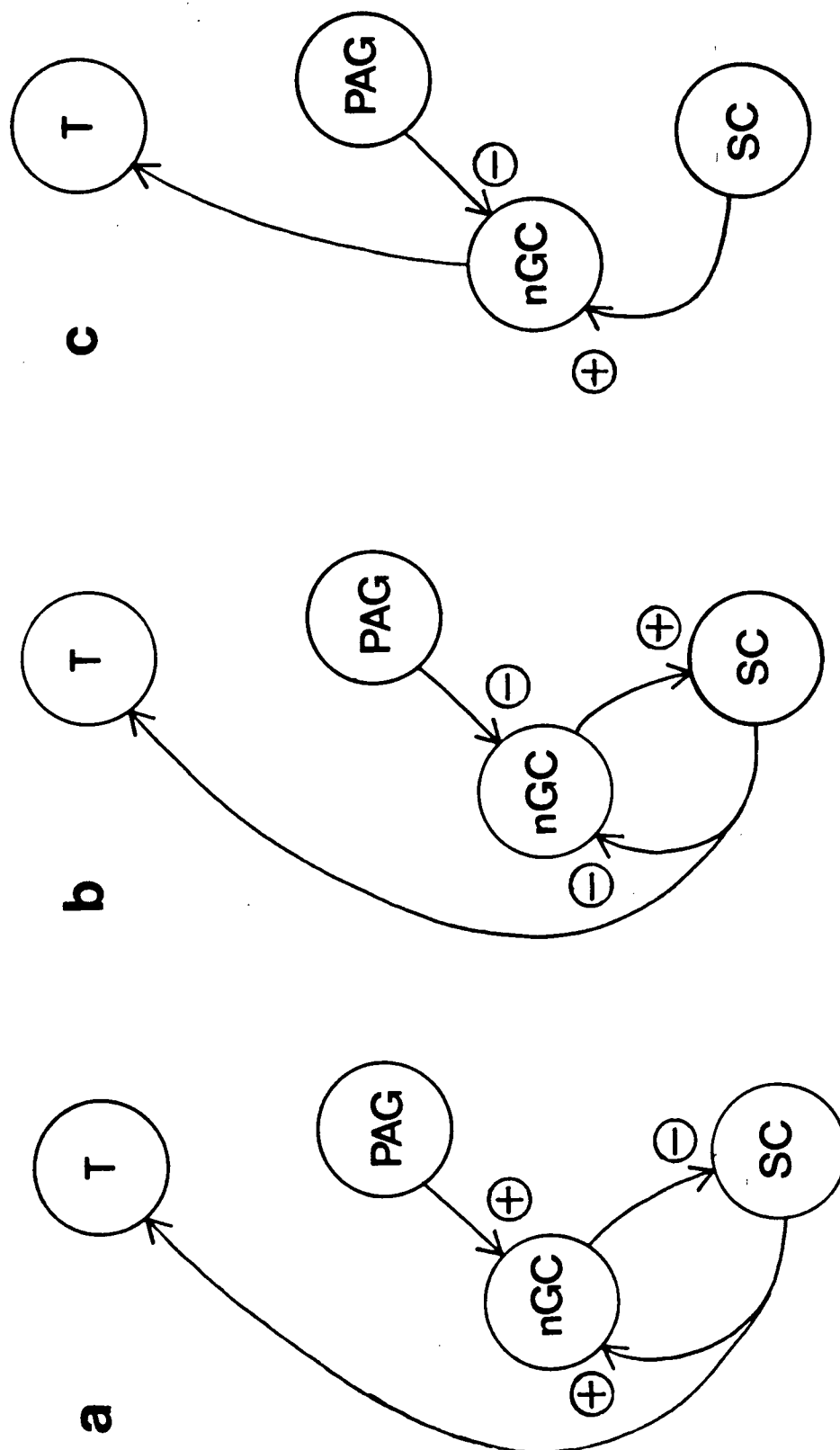
neurones. This might occur by PAG stimulation exciting nGC neurones which are inhibitory to the spinal cord neurones (see Fig. 16a). However, only a minority of nGC neurones in this study were found to be excited by PAG stimulation (19% of the 87 neurones affected). On the other hand, this modulation may occur by the PAG stimulation inhibiting tonically active nGC neurones which are excitatory to the spinal cord neurones (see Fig. 16b). However, most nGC neurones in the nGC did not have very fast spontaneous firing rates and therefore, although it is a possibility, they probably do not mediate the periaqueductal gray SPA in this way.

It has been proposed that brainstem level neurones might mediate the observation that noxious stimuli applied to one area of the body can inhibit the responses of spinal cord neurones to noxious stimuli applied to other body areas (LeBars et al., 1979). Nucleus gigantocellularis neurones involved in this effect may mediate periaqueductal gray SPA at the spinal level as well. If this was the case then one would predict that some nGC neurones should respond in similar ways to both PAG stimulation and to noxious peripheral stimuli. In fact some neurones in this study did respond this way (22% of the 63 neurones affected by both tests were excited and 17% were inhibited) and therefore there is a possibility that some nGC neurones may be involved in these effects. Clearly, experiments on neurones which are identified as to their axonal destinations will be required to further clarify the involvement of nGC neurones in these effects.

SUMMARY

(1) Antidromic activation of PAG neurones by electrical
stimulation of the nGC indicated there is a monosynaptic pathway
from the PAG to the nGC.

(2) Although the majority of nGC neurones responded with
excitation to noxious peripheral stimuli, mixed and inhibitory
responses were also seen. These results suggest the nGC plays
some role in nociceptive processing.

(3) The major effect of PAG stimulation on nGC neuronal activity
was inhibition, although mixed and excitatory responses were
also seen.

(4) The responses of nGC neurones to serotonin and fluoxetine, a
specific uptake blocker, and the effect of fluoxetine on the
responses of nGC neurones to PAG stimulation suggest that the
inhibitory effects of PAG stimulation may be mediated by
serotonin.

(5) The largest group of nGC neurones responding to noxious
peripheral stimuli and/or PAG stimulation was excited by the
former and inhibited by the latter, suggesting that many nGC
neurones are involved in an ascending nociceptive pathway and
under inhibitory control of the PAG.

(6) Other neurones in the nGC had responses to noxious
peripheral stimuli and PAG stimulation which suggest that they
may mediate PAG stimulation produced inhibition by descending
control of spinal cord neurones.

(7) The results of the study suggest that nGC neurones mediate
at least part of the antinociceptive affects of PAG stimulation.

APPENDIX

In order to do these experiments some pieces of electronic equipment were designed by the author and constructed by members of the laboratory. This procedure allowed the final designs to be fitted to the requirements of the laboratory in a fairly easy and inexpensive manner. Recent developments in integrated circuit technology allows relatively unsophisticated designs and techniques to produce professional products.

The different pieces of equipment were designed in a modular format which allowed the units to be designed and built as they were required. The following pages contain the schematic diagrams of the various modules.

Figure 17: Schematic diagram of the power supply module. This module supplied +5 and ±15 V regulated D.C. to the other modules in the series.
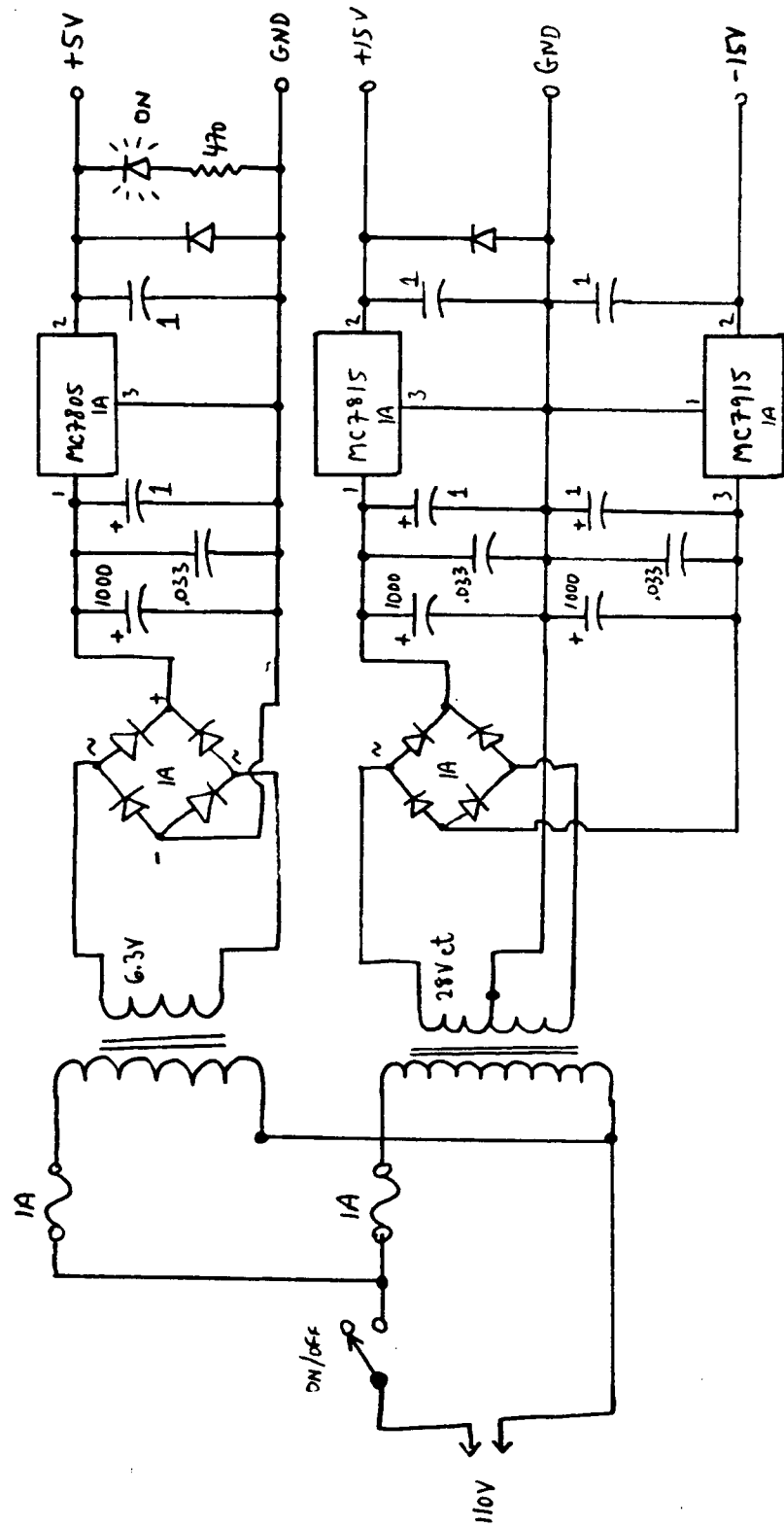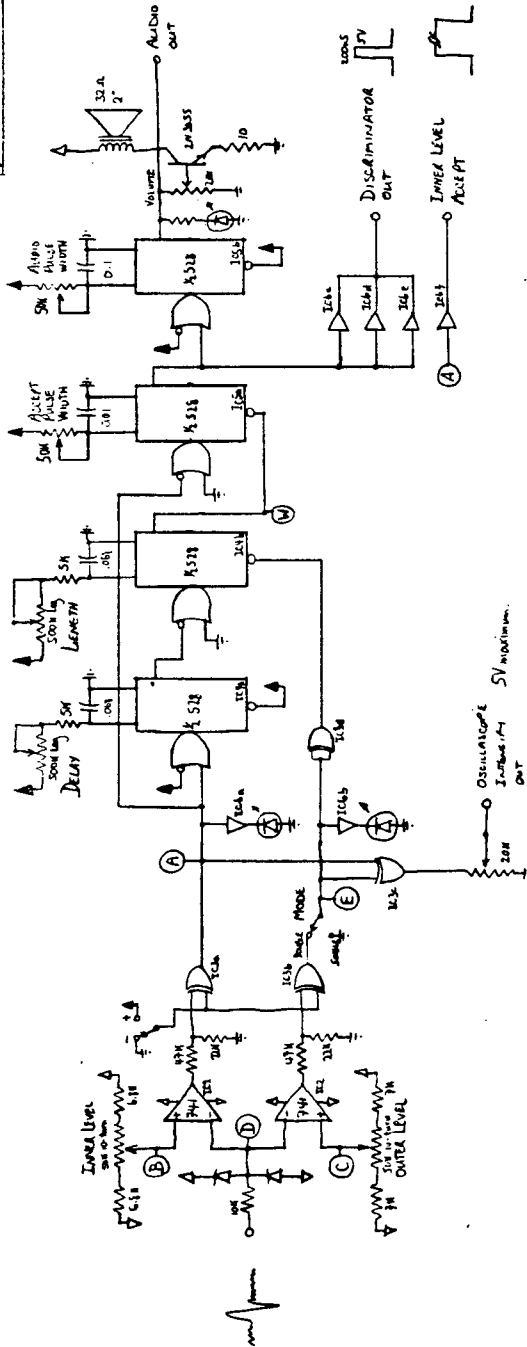
Figure 18: Schematic diagram of the window discriminator module.
This module allowed the differentiation of action potentials
from the surrounding noise and from unwanted spikes. Each
recognized spike was output from the module as a standard TTL
level pulse. The window was variable in both width and amplitude
allowing the selection of spikes on the basis of their minimum
and maximum height as well as their minimum and maximum
duration. The portion of the spike within the window could be
intensified. The window was displayed on the oscilloscope by
multiplexing the window signals with the input signal.

DISCRIMINATOR SCHEMATIC

August 22, 1979

© David Platt Harris 1979

EXAMPLE OF "WINDOW" MODE.

Figure 19: Schematic diagram of the spike integrator module. This module counted spikes during successive selectable epochs (0.5-20 s). This count was then output as an analogue signal proportional to the number of counts while the spikes were counted in the next epoch. The count of the previous epoch was also displayed digitally.

SPIKE INTEGRATOR
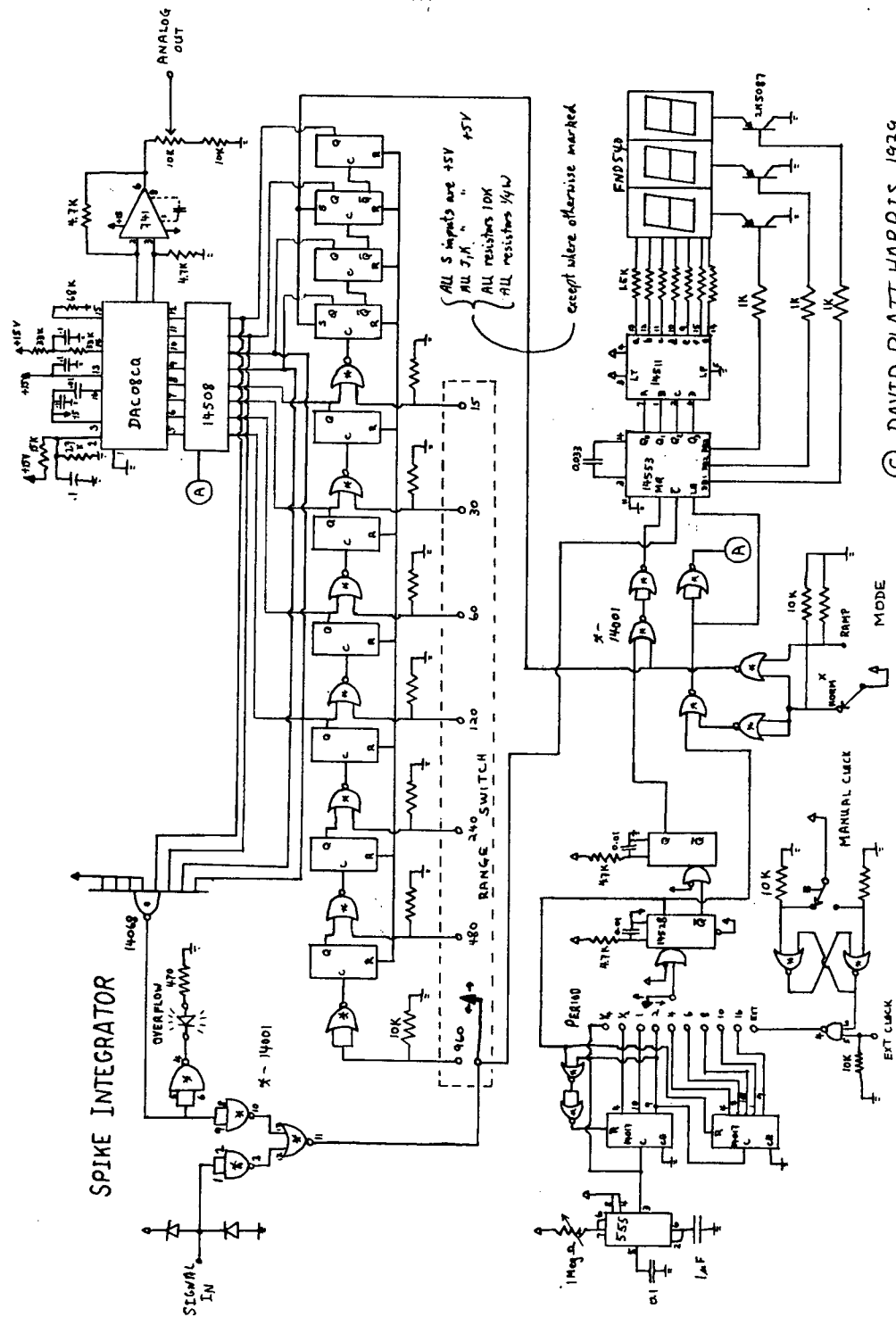
© DAVID PLATT HARRIS, 1979.

Figure 20: The schematic diagram of the delay line module. This module allowed an analogue signal to be delayed in each of two channels. If the delay is not required it could be switched out of the circuit. The circuit uses a commercially available bucket-brigade type integrated circuit and its associated development board.

INTERVAL
10M

INTERVAL
10M

	+5	GND	+15	-15
1 TTL MC4024	1,14,15	7	—	—
2,3 741	—	—	7	4
4 Reticon delay line	—	—	7	5

½ MC 4024

½ MC 4024

I_CHA I_CHB

Reticon 77 SC1024A   4

R   CLA   CLB   H

OUT CH1

IN CH1

CH 1 OUT

A   O_CHB

IN CH2   OUT

CH2 OUT

CH 1 IN

CH 2 IN

680pF
22M
22K
15K
15M
.001
470pF
10K
10K
680pF
220K
100K
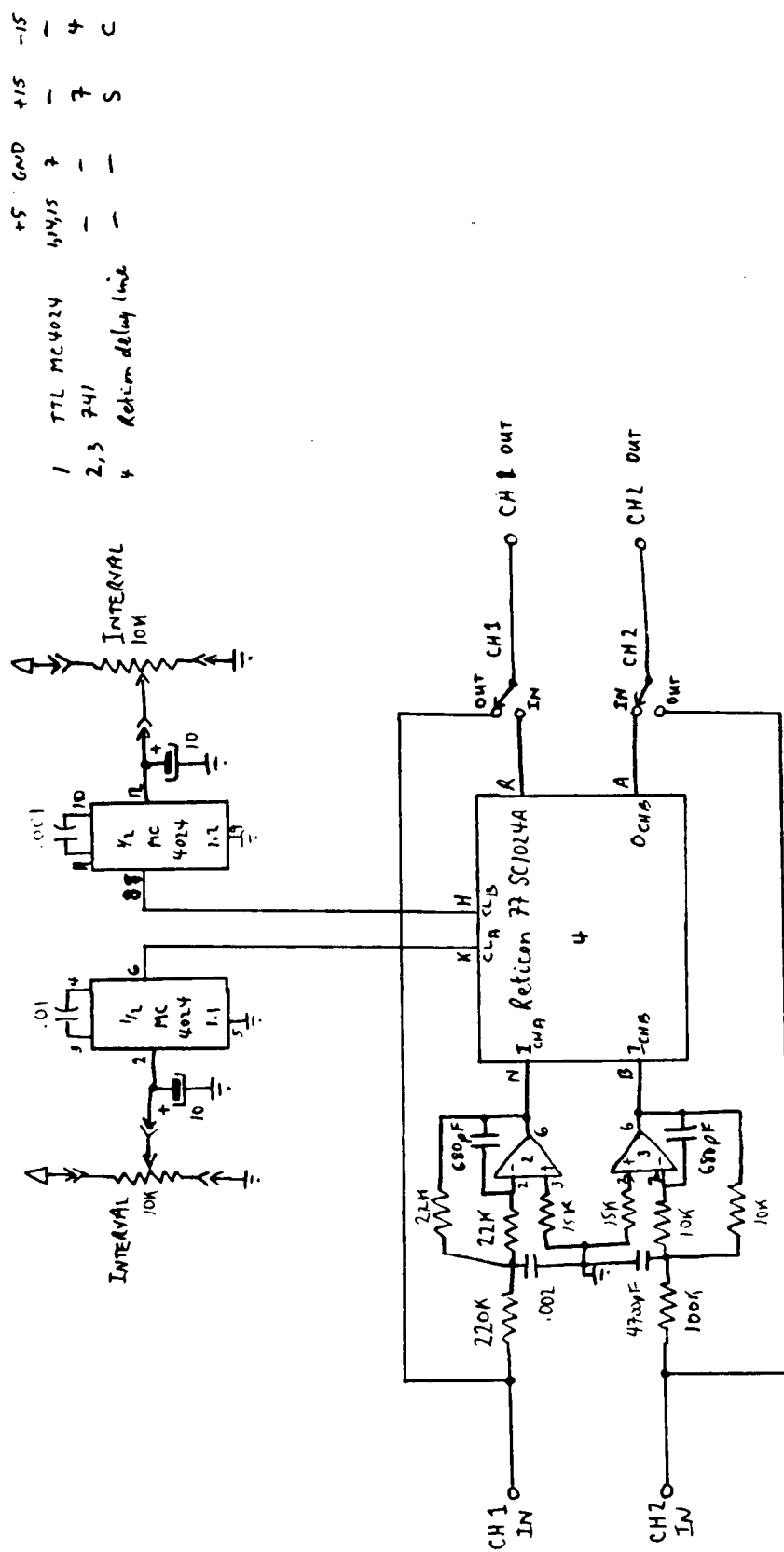
© DAVID PLATT HARRIS, 1979.

Figure 21: The schematic diagram of the raster scan module. This module allows a repetitive signal to be diplayed on an oscilloscope with each succeeding sweep displaced in the vertical direction. The module also included circuitry to allow the spikes to be displayed as dots. The unit could be set to stop after a selectable number of sweeps.

RASTER SCAN   © DAVID PLATT HARRIS, 1979.

BIBLIOGRAPHY

Abad-Alegria, F. (1971). Estereotaxis troncoencefalica de la rata, Trabojos del Inst. Cajal Invest. Biolog. 63, 103-124.

Aghajanian, G. K., Wang, R. Y., and Babaran, J. (1978). Serotonergic and non-serotonergic neurones of the dorsal raphe: reciprocal changes in firing induced by peripheral stimulation. Brain Res. 153, 169-175.

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.

Albe-Pessard, D. Levante, A., and Lamour, Y. (1974). Origin of spino-thalamic tract in monkeys. Brain Res. 65, 503-509.

Anden, N. E., Dahlstrom, A., Fuxe, K., Olson, L., and Ungerstedt, U. (1966). Ascending monoaminergic neurones to the telencephalon and diencephalon, Acta Physiol. Scand. 67, 313-326.

Anderson, F. D., and Berry, C. M. (1959). Degeneration studies of the long ascending fibre systems in the cat brainstem. J. Compar. Neurol. 111, 195-229.

Anderson, K. V., and Pearl, G. S. (1975). Long term increases in nociceptive thresholds following lesions in feline nucleus reticularis gigantocellularis. Abst. First World Congress of Pain, 70.

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

Atweh, S. F., and Kuhar, M. J. (1977). Autoradioghraphical localization of opiate receptors in rat brain. I. Spinal cord and lower medulla. Brain Res. 124, 53-67.

Basbaum, A. I., and Fields, H. L. (1977a). The dorsolateral fasciculus of the spinal cord: a major route for descending brainstem control. Soc. Neurosci. 3, 499.

Basbaum, A. I., and Fields, H. L. (1977b) Bulbospinal system in the cat: cell locations and spinal pathways. Soc. Neurosci. Abst. 3 , 499.

Basbaum, A. I., Clanton, C. H., and Fields, H. L., (1976a). Opiate and stimulus-produced analgesia: functional anatomy of a medullarospinal pathway. Proc. nat. Acad. Sci. (Wash) 73 , 4685-4688.

Basbaum, A. I., Clanton, C. H., and Fields, H. L., (1978). Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographical study of pain modulating systems. J. Compar. Neurol. 178, 207-224.

Basbaum, A. I., Marley, N. J. E., and O'Keefe, J., (1976b). Spinal cord pathways involved in the production of analgesia by brain stimulation. In: Advances in Pain Research and Therapy , Vol. 1 (J. J. Bonica and D. Albe-Pessard, ed.). Raven Press, New York, pp. 511-515.

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

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

Behbehani, M. M. (1979). Morphine tolerance decreases analgesia produced by injection of glutamate into the periaqueductal gray of the rat. Soc. Neurosci. Abstr. 5 , 605.

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

Behbehani, M. M., and Pomeroy, S. L. (1978). Effect of morphine injection in the periaqueductal gray on the activity of single units in the nucleus raphe magnus of the rat. Brain Res. 149 , 266-269.

Beitz, A. J. (1979). Ascending and descending projections to the periaqueductal gray of the rat. Soc. Neurosci. Abst. 5 , 605.

Bennett, C. T., Hulsebus, R. C., and Beran, T. E. (1976). Signal detection analysis of changes in nociception in the monkey following opiate administrationto specific brain areas. Soc. Neurosci. Abstr. 2, 565.

Bennett, G. J., and Mayer, D. J. (1976). Effects of microinjected narcotic analgesics into the periaqueductal gray (PAG) on the response of rat spinal cord dorsal horn interneurones. Soc. Neurosci. Abstr. 2 , 928.

Bennett, G. J., and Mayer, D. J. (1979). Inhibition of spinal cord interneurones by narcotic microinjection and focal electrical stimulation in the periaqueductal gray matter. Brain Res. 172 , 243-257.

Besson, J. M., Guilbaud, G., and Lombard, M. C. (1974). Effects of bradykinin intra-arterially injected into the limbs upon bulbar and reticular unit activity. In Advances in Neurology , Vol. 4 (J. J. Bonica, ed.). Raven Press, New York, pp. 207-215.

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

Bishop, P. O., Burke, W., and Davis, R. (1962). Single-unit recording from antidromically activated optic radiation neurones. J. Physiol. (Lond.) 162 , 432-450.

Blair, E. A., and Erlanger, J. (1936). On the process of excitation by brief shocks in axons. Amer. J. Physiol. (Lond.) 114 , 309-316.

Boakes, R. J., Bradley, P. B., Briggs, I., and Dray, A. (1970). Antagonism of 5-hydroxytrytamine by LSD-25 in the central nervous system: A possible basis for actions of LSD-25. Brit. J. Pharmacol. 40, 203-218.

Boakes, R. J., Bramwell, G. J., Briggs, I., and Tempesta, E. (1974). Localization with Pontamine Sky Blue of neurones in the brainstem responding to microiontophoretically applied compounds. Neuropharmacol. 13, 475-479.

Bobillier, P., Petijean, F., Salvert, D., Ligier, M., and Sequin, S. (1975). Differential projection of the nucleus raphe dorsalis and nucleus raphe centralis as revealed by autoradiography. Brain Res. 85, 205-210.

Bobillier, P., Sequin, S., Degueurce, A., Lewis, B. D., and Pujol, J. F. (1979). The efferent connections of the the nucleus raphe centralis superior in the rat as revealed by radiography. Brain Res. 166 , 1-8.

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

Boivie, J. (1970). The termination of the cervico-thalamic tract in the cat. An experimental study with silver impregnation methods. Brain Res. 19 , 333-360.

Boivie, J. (1979). An anatomical reinvestigation of the termination of the spinothalamic tract in the monkey. J. Compar. Neurol. 186 , 343-370.

Bowsher, D., Mallart, A., Petit, D., and Albe-Fessard, D. (1968). A bulbar relay to the median centre. J. Neurophysiol. 31 , 288-300.

Bowsher, D., and Westman, J. (1970). The gigantocellular reticular region and its spinal afferents: a light and electron microscope study in the cat. J. Anat. 106 , 23-36.

Bradley, P. B., and Dray, A. (1973). Modification of the responses of brainstem neurones to transmitter substances by anaesthetic agents. Br. J. Pharmacol. 48, 212-214.

Bradley, P. B., and Wolstencroft, J. H. (1965). Actions of drugs on single units in the brainstem. Brit. med. Bull. 21 , 15-18.

Bramwell, G. J., and Gonye, T. (1976). Response of midbrain neurones to microiontophoretically applied 5-hydroxytryptamine: comparison with response to intravenously administered LSD. Neuropharmacol. 15 , 457-461.

Briggs, I. (1977). Excitatory responses of neurones in rat bulbar reticular formation to bulbar raphe stimulation and to iontophoretically applied 5-hydroxytryptamine, and their blockade by LSD-25. J. Physiol. (Lond.) 265 , 327-340.

Brodal, A., Walberg, F., and Taber, E. (1960). The raphe nuclei of the brainstem of the cat. III. Afferent connections. J. Compar. Neurol. 114 , 261-279.

Brown, A. G., Hamann, W. C., and Martin, H. F. (1973a). Interactions of cutaneous myelinated (A) and non-myelinated (C) fibres on transmission through the spino-cervical tract. Brain Res. 53 , 222-226.

Brown, A. G., Hamann, W. C., and Martin, H. F. (1975). Effects of activity in non-myelinated afferent fibres on the spinocervical tract. Brain Research 98, 243-259.

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

Bryan, R. N., Trevino, D. L., Coulter, J. D., and Willis, W. D. (1973). Location and somatotopic organization of the cells of origin of the spinocervical tract. Exp. Brain Res. 17 , 177-189.

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, Somatosensory System (A. Iggo, ed.). Springer, New York, pp. 29-78.

Burgess, P. R., Petit, D., and Warren, R. M. (1968). Receptor types in cat hairy skin supplied by myelinated fibres. J. Neurophysiol. 31 , 833-848.

Cajal, S. Ramon y (1909). Histologie du Systeme Nerveaux de l'Homme et des Vertebres , Vol. 1, Maloine, Paris. (Reprinted by the C.S.I.C., Madrid, 1952.)

Carpenter, M. B., and Hanna, G. R. (1961). Fibre projection from the spinal trigeminal nucleus in the cat. J. Compar. Neurol. 117 , 117-131.

Carr, K. D., and Coons, E. E. (1979). Lateral hypothalamic gating of the adversion produced by stimulation of the nucleus gigantocellularis. Soc. Neurosci. Abst. 5 , 606.

Carstens, E., Klumpp, D., and Zimmerman, M. (1979a). The opiate antagonist, naloxone, does not affect descending inhibition from midbrain of nociceptive spinal neuronal discharges in the cat. Neurosci. Lett. 11 , 323-327.

Carstens, E., and Trevino, D. L. (1978). Laminae origins of spinothalamic projections in the cat as determined by retrograde transport of horseradish peroxidase. J. Compar. Neurol. 182 , 151-156.

Carstens, E., Yokata, T., and Zimmerman, M. (1979b). 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. (1969). Somatic stimuli, spinal pathways, and size of cutaneous fibres influencing unit activity in the medial medullary reticular formation. Exp. Neurol. 25 , 35-36.

Casey, K. L. (1971a). Responses of bulboreticular units to somatic stimuli eliciting escape behaviour in the cat. Int. J. Neurosci. 2 , 15-28.

Casey, K. L. (1971b). Escape behaviour elicited by bulbo-reticular stimulation in the cat. Int. J. Neurosci. 2 , 29-34.

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

Cervero, F. , Iggo, A., and Molony, V. (1977). Responses of spinocervical tract neurones to noxious stimulation of the skin. J. Physiol. 267 , 537-558.

Cervero, F., Iggo, A., and Molony, V. (1979). Ascending projections of nociceptor-driven lamina I neurones in the cat. Exp. Brain Res. 35 , 135-149.

Cervero, F., Iggo, A., and Ogawa, H. (1976). Nociceptor-driven dorsal horn neurones in the lumbar cord of the cat. Pain 2, 5-42.

Chan, S. H. H. (1979). Nucleus gigantocellularis: a common neural substrate for morphine and stimulation produced suppression of dental pulp evoked trigeminal responses in the cat. Soc. Neurosci. Abst. 5 , 607.

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

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

Collins, W. F., and Randt, C. T. (1960). Midbrain evoked responses relating to peripheral unmyelinated or "C" fibres in the cat. J. Neurophysiol. 23 , 47-53.

Conrad, L. C. A., Leonard, C. M., and Pfaff, D. W. (1974). Connections of the median and dorsal raphe nuclei in the rat: an autoradiogaphical and degenerative study. J. Compar. Neurol. 156 , 179-206.

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

Criswell, H. E. (1976). Analgesia and hyperactivity following morphine microinjection into mouse brain. Pharmacol. Biochem. Behav. 4 , 23-26.

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

Dahlstrom, A., and Fuxe, K. (1964). Evidence for the existence of monoamine containing neurones in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurones. Acta Physiol. Scand. 62 , Suppl. 232, 1-55.

Dahlstrcm, A., and Fuxe, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neurone systems. Acta Physiol. Scand. 64 , Suppl. 247 , 5-36.

Darian-Smith, I., Phillips, G., and Ryan, R. D. (1963). Functional orginization of the trigeminal main sensory and rostal spinal nuclei of the cat. J. Physiol. (Lond.) 168 , 129-146.

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

Deakin, J. W. F., Dickenson, A. H., and Dostrovsky, J. O., (1977). Morphine effects on raphe magnus neurones. J. Physiol. (Lond.) 267 , 43-45P.

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

Dilly, P. N., Wall, W. D., and Webster, K. E. (1968). Cells of origin of the spinothalamic tract in the cat an rat. Exp. Neurol. 21 , 550-562.

Dostrovsky, J. O., and Shah, J. (1979). Electrophysiological evidence for a direct projection from the periaqueductal gray to the nucleus raphe magnus in the cat and the rat. Soc. Neurosci. Abst. 5 , 608.

Dray, A., Gonye, T. J., Oakley, N. R., and Tenner, T. (1976). Evidence for the existance of a raphe projection to the substantia nigra in rat. Brain Res. 113 , 45-57.

Duggan, A. W., and Griersmith, B. T. (1979). Inhibition of nociceptive information by supraspinal stimulation in the cat. Pain 6 , 149-161.

Eccles, J. C., Nicoll, R. A., Schwartz, D. W. F., Taborikova, H., and Willey, T. J. (1975). Reticulospinal neurons with and without monosynaptic inputs from the cerebellar nuclei. J. Neurophysiol. 38 , 513-530.

Engberg, I., Lundberg, A., and Ryall, R. W. (1968). Reticulospinal inhibition of transmission in reflex pathways. J. Physiol. (Lond.) 194 , 201-233.

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

Fields, H. L., and Basbaum, A. I. (1978). Brainstem control of spinal pain transmission neurones. Ann. Rev. Physiol. 40 , 193-221.

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

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

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

Foreman, R. D., Schmidt, R. F., and Willis, W. D. (1979). Effect of mechanical and chemical stimulation of fine muscle afferents upon spinothalamic neurones. J. Physiol. (Lond.) 286 , 215-231.

Fox, J. E., and Wolstencroft, J. H. (1976). The reduced responsiveness of neurones in nucleus gigantocellularis following their excitation by peripheral nerve stimulation. J. Physiol. (Lond.) 258 , 687-704.

Fox, J. E., Holloway, J. A., Iggo, A., and Mokha, S. S. (1980). Spinothalamic neurones in the cat: some electrophysiological observations. Brain Res. 182, 186-190.

Fuller, J. H., and Schlag, J. D. (1976). Determination of antidromic excitation by the collision test: problems of interpretation. Brain Res. 112 , 283-298.

Gallager, D. W., and Pert, A. (1978). Afferents to brainstem nuclei (brainstem raphe, nucleus pontis caudalis, and nucleus gigantocellularis) in the as demonstrated by microiontophoretically applied horseradish peroxidase. Brain Res. 144 , 257-275.

Gent, J. P., and Wolstencroft, J. H. (1976). Actions of
    morphine, enkephalin, and endorphin on single neurones in
    the brainstem, including the raphe and the periaqueductal
    gray of the cat. In: Opiates and Endogenous Opioid Peptides
    (H. W. Kosterlitz, ed.). Pergammon Press, Oxford, pp. 217-
    224.

Georgopoulos, A. P. (1976). Functional properties of primary
    afferent units probably related to pain mechanisms in
    primate glabrous skin. J. Neurophysiol. 39 ,71-83.

Georgopoulos, A. P. (1977). Stimulation-response relations in
    high threshold mechanothermal fibres innervating primate
    glabrous skin. Brain Res. 128 , 547-552.

Giesler, G. J., Basbaum, A. I., and Menetrey, D. (1977).
    Projections and origins of the spinothalamic tract in the
    rat. Soc. Neurosci. Abst. 3 , 500.

Giesler, G. J., Urca, G., Cannon, J. T., and Liebeskind, J. C.
    (1979). Response properties of neurones in the lateral
    cervical nucleus in the rat. J. Compar. Neurol. 186, 65-77.

Goldman, P. L., Collins, W. F., Taub, A., and Fitzmartin, J.
    (1972). Evoked bulbar reticular unit activity following
    delta fibre stimulation of peripheral somatosensory neve in
    the cat. Exp. Neurol. 37, 597-606.

Guilbaud, G., Besson, J. M., Oliveras, J. L., and Liebeskind, J.
    C. (1973a). Suppression by LSD of the inhibitory effect
    exerted by dorsal raphe stimualtion on certain spinal cord
    interneurones in the cat. Brain Res. 61 , 417-422.

Guilbaud, G., Besson, J. M., Oliveras, J. L., and Wyon-Maillard,
    M. C. (1973b). Modulations of firing rate of bulbar
    reticular units (nucleus gigantocellularis) after intra-
    arterially injected bradykinin into the limbs. Brain Res.
    63 , 131-140.

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

Ha, H., and Liu, C. N. (1966). Orginization of the spino-
    cervico-thalamic system. J. Compar. Neurol. 127 , 445-470.

Haber, L.H., Martin, R. F., Chatt, A. B., and Willis, W. D.
    (1978). Effects of stimulation in the nucleus reticularis
    gigantocellularis on the activity of spinothalamic tract
    neurones in the monkey. Brain Res. 153 , 163-168.

Haber, L. H., and Wagman, I. H. (1974). Effects of stimulation of the medullary reticular formation on the activity of interneurones in lumbosaccral cord of the cat. Soc. Neurosci. 4th Ann. Meet., St. Louis.

Haigler, H. J. (1977). Morphine: effects on brainstem neurones. Iontophoresis and transmission in mammalian central nervous system, Satelite Symposium of XXVII Intl. Congr. of Phys. Sciences, Churchhill College, Cambridge, pp. 6.25-6.27.

Haigler, H. J. (1978). Morphine: effects on serotonergic nuclei and nuclei in areas with serotonergic input. Eur. J. Pharmacol. 51 , 361-376.

Haigler, H. L., and Aghajanian, G. K. (1974). Lysergic diethylamide and serotonin: a comparison of effects on serotonergic neurones and neurones receiving a serotonergic input. J. Pharmac. Exp. Ther. 188, 688-699.

Halpern, B. P., and Halverston, J. D. (1967). Elevated escape latencies after hindbrain lesions. Physiologist 10 , 193.

Halpern, B. P., and Halverston, J. D. (1974). Modulation of escape from noxious stimuli after bulbar reticular formation lesions. Behav. Biol. 11 , 215-229.

Hamann, W. C., Hong, S. K., Kniffi, K. D., and Schmidt, R. F. (1978). Projections of primary afferent fibres from muscle to neurones of spinocervical tract of cat. J. Physiol. (Lond.) 283 , 369-378.

Hammond, D. L., and Levy, R. A. (1979). Analgesia following microinjection of phentolamine into the nucleus raphe magnus. Soc. Neurosci. Abst. 5 , 610.

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

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

Hellon, R. F. (1971). The marking of electrode tip positions in nervous tissue. J. Physiol. (Lond.) 204 , 12P.

Hokfelt, T., Ljungdahl, A., Terenius, L., Elde, R., and Nilsson, G. (1977). Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P. Proc. nat. Acad. Sci. (Wash.) 74 , 3081-3085.

Holloway, J. A., Fox, R. E., and Iggo, A. (1978). Projections of the spinothalamic tract to the thalamic nuclei of the cat. Brain Res. 157 , 336-340.

Keene, J. J., and Casy, K. L. (1970). Excitatory connection from lateral hypothalamic self-stimulation sites to escape sites in medullary reticular formation. Exp. Neurol. 28 , 155-166.

Kerr, F. W. L. (1975a). Neuroanatomical substrates of nociception in the spinal cord. Pain 1 , 325-356.

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

Kerr, F. W. L., and Lippman, H. H. (1974). The primate spinothalamic tract as demonstrated by anterolateral cordotomy and commissural myelotomy. In: Advances in Neurology , Vol. 4 (J. J. Bonica, ed.). Raven Press, New York, pp. 147-156.

Kniffi, K. D., Mense, S., and Schmidt, R. F. (1977). Activation of neurones of the spinocervical tract by painful stimulation of skeletal muscle. Proc. Int. Union Physiol. Sci. 13 , 393.

Krnjevic, K., and Phyllis, J. W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. J. Physiol. (Lond.) 165 , 274-304.

Kuhar, M. J., Pert, C. B., and Snyder, S. H. (1973). Regional distribution of opiate receptor binding in monkey and human brain. Nature 245 , 447-450.

Kumazawa, T., and Perl, E. R. (1976). Differential excitation of dorsal horn and substantia gelatinosa marginal neurones by by primary afferent units with fine (A-delta and C) fibres. In: Sensory Functions of the Skin in Primates, with Special Reference to Man (Y. Zctterman, ed.). Pergammon Press, Oxford, pp. 67-88.

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

Kumazawa, T., Perl, E. R., Burgess, P. R., and Whitehorn, D. (1975). Ascending projection from marginal zone (lamina I) neurons of the spinal dorsal horn. J. Compar. 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.

Kuru, M. (1949). Sensory Paths in the Spinal Cord and Brain Stem
of Man. Sogensya, Tokyo.

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

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

LeBlanc, H. J., and Gatipon, G. B. (1974). Medial bulboreticular
responses to peripherally applied noxious stimuli. Exp.
Neurol. 42, 264-273.

Leichnetz, G. L., Watkins, I., Griffin, G., Murfin, R., and
Mayer, D. J. (1978). The projection from the 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.

Lemberger, L. Rowe, H., Carmichael, R., Crabtree, R., Horng, J.
S., Bymaster, F., and Wong, D. (1978). Fluoxetine, a
selective uptake inhibitor. Clin. Pharmacol. Ther. 23,
421-429.

Levante, A., and Albe-Fessard, D. (1972). Localisation dans les
couches VII et VIII de Rexed des cellules d'origine d'un
faisceau spino-reticulaire croise. C. R. Acac. Sci. Paris
274, 3007-3010.

Levante, A. Lamour, Y., Guilbaud, G., and Besson, J. M. (1975).
Spinothalamic cell activity in the monkey during intense
nociceptive stimulation: intra-arterial injection of
bradykinin into the limbs. Brain Res. 88, 560-564.

Levy, R. A., and Proudfit, H. K. (1979). Analgesia produced by
microinjection of baclofen and morphine at brainstem sites.
Eur. J. Pharmacol. 57, 43-55.

Lewis, V. A., Gebhart, G. F. (1977). Evaluation of the
periaqueductal central gray (PAG) as a morphine specific
locus of action and examination of morphine-induced and
stimulation produced analagesia at coincident PAG loci.
Brain Res. 124, 283-303.

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: Behavioural observations and inhibitory effects on spinal cord interneurones. Brain Res. 50 , 441-446.

Liebeskind, J. C., and Mayer, D. C. (1971). Somatosensory evoked responses in the mesencephalic central gray matter of the rat. Brain Res. 27 , 133-151.

Light, A. R., and Perl, E. R. (1979a). Reexamination of the spinal dorsal horn including observations on the differential termination of coarse and fine fibres. J. Compar. Neurol. 186 , 117-131.

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

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

Lobatz, M., Proudfit, H. K., and Anderson, E. G. (1976). Effects of noxious stimulation, iontophoresis of morphine and noradrenaline on neurones in the raphe magnus. Pharmacologist 18 , 213.

Lobatz, M., Proudfit, H. K., and Anderson, E. G. (1977). Effects of pain and iontophoresis of morphine and acetylcholine on neurones in rat raphe magnus. Soc. Neurosci. Abstr. 3 , 296.

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

Lovick, T. A., West, D. C., and Wolstencroft, J. H. (1978b). Bulbar raphe neurones with projections to the spinal trigeminal nucleus and the lumbar cord in the cat. J. Physiol. (Lond.) 277, 61-62P.

Lovick, T. A., and Wolstencroft, J. H. (1979). Inhibitory effect of nucleus raphe magnus on neuronal responses in the spinal trigeminal nucleus to nociceptive compared to non-nociceptive inputs. Pain 7 , 135-145.

Mannen, H., and Suguira, Y. (1976). Construction of neurones of the dorsal horn proper using Golgi-stained serial sections. J. Compar. Neurol. 168 , 303-312.

Martin, G. F. (1979). Evidence for four direct brainstem projections to the intermediate cell column with notes on their development. Soc. Neurosci. Abst. 5 , 726.

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

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 behavioural 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.

McCreery, D. and Bloedel, J. R. (1975). Reduction of the response of cat spinothalamic neurones to graded mechanical stimuli by electrical stimulation of the lower brainstem. Brain Res. 97 , 151-156.

McCreery, D., Bloedel, J. R., and Hannes, E. G. (1979). Effect of stimulation in raphe nuclei and in reticular formation on responses of spinothalamic neurones to mechanical stimuli. J. Neurophysiol. 42 , 166-182.

Mehler, W. R., Feferman, M. E., and Nauta, W. J. H. (1960). Ascending axonal degeneration following anterolateral cordotomy. An experimental study in the monkey. Brain 83 , 718-750.

Mendell, L. M. (1966). Physiological properties of unmyelinated fibre projection to the spinal cord. Exp. Neurol. 16 , 316-322.

Mohrland, J. S., and Gebhart, G. F. (1979). PAG morphine and stimulation and morphine iontophoretic effects on brainstem neurones in the rat. Soc. Neurosci. Abst. 5 , 613.

Moolenaar, G. -M., Holloway, J. A., and Trowth, C. O. (1976). Responses of caudal raphe neurones to peripheral somatic stimuli. Exp. Neurol. 53 , 304-313.

Morrow, T. J., and Casey, K. L. (1976). Analgesia produced by mesencephalic stimulation: effect on bulboreticular neurones. In: Advances in Pain Research and Therapy. Vol. 1 (J. J. Bonica and D. Albe-Fessard, ed.). Raven Press, New York, pp. 503-510.

Mosko, S. S., Haubrich, D., and Jabobs, B. L. (1977). Serotonergic afferents to the dorsal raphe nucleus: evidence from HRP and synaptosomal uptake studies. Brain Res. 119 , 269-290.

Murfin, R, Bennett, G. J., and Mayer, D. J. (1976). The effect of dorsolateral spinal cord lesions on analgesia from morphine micoinjection into periaqueducatal gray matter (PAG) of the rat. Soc. Neurosci. Abst. 2 , 946.

Nyberg-Hansen, R. (1965). Sites and mode of termination of reticulo-spinal fibres in the cat. An experimental study with silver impregnation methods. J. Compar. Neurol. 124 , 71-100.

Oleson, T. D., and Liebeskind, J. C. (1975). Relationship of neural activity in the raphe nuclei of the rat to brain stimulation-produced analgesia. Physiologist 18 , 338.

Oleson, T. D., and Liebeskind, J. C. (1976). Modulation of midbrain and thalamic evoked responses by analgesic brain stimulation in the rat. In: Advances in Pain Research and Therapy. Vol. 1 (J. J. Bonica and D. Albe-Fessard, ed.). Raven Press, New York, pp. 487-494.

Oleson, T. D., Twombly, D. A., and Liebeskind, J. C. (1978). Effects of pain-attenuating brain stimulation 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. (1974a). Behavioural and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. Exp. Brain Res. 20 , 32-44.

Oliveras, J. L., Hosobuchi, Y., Redjemi, F., and Besson, J. M. (1977). Opiate antagonist, naloxone, strongly reduces analgesia induced by stimulation of a raphe nucleus (centralis inferior). Brain Res. 120 , 221-229.

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

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

Oscarsson, O. (1973). Functional organization of spinocerebellar paths. In: Handbook of Sensory Physiology. Vol. II. Somatosensory System (A. Iggo, ed.), Springer-Verlag, New York, pp. 339-380.

Palmer, M. R., and Klemm, W. R. (1976). Morphine effects on centrally and peripherally evoked multiple unit activity. Soc. Neurosci. Abst. 2, 570.

Paintal, A. S. (1960). Functional analysis of group III afferent fibres of mammalian muscles. J. Physiol. (Lond.) 152, 250-270.

Pearl, G. S., and Anderson, K. V. (1978). Response patterns of cells in the feline caudal nucleus reticularis gigantocellularis after noxious trigeminal and spinal stimulation. Exp. Neurol. 58 , 231-241.

Pearson, J. A., Green, M. C., and Watson, C. (1979). Inhibition of spinal cord neurones by stimualtion raphe magnus in the rat. Soc. Neurosci. Abst. 5 , 614.

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

Pert, C. B., Kuhar, M. J., and Snyder, S. H. (1976). Opiate receptor: autoradigraphical localization in rat brain. Proc. nat. Acad. Sci. (Wash.) 73 , 3729-3733.

Pert, C. B., and Yaksh, T. (1974). Sites of morphine induced analgesia in the primate brain: relation to pain pathways. Brain Res. 80 , 135-140.

Peterson, B. W., Anderson, M. E., and Filion, M. (1974). Response of ponto-medullary reticular neurones to cortical, tectal, and cutaneous stimuli. Exp. Brain Res. 21 , 19-44.

Pierce, E. T., Foote, W. E., and Hobson, J. A. (1976). The efferent connections of the nucleus raphe dorsalis. Brain Res. 107 , 137-144.

Poitras, D., and Parent, A. (1978). Atlas of the distribution of monoamine-containing nerve cell bodies in the brainstem of the cat. J. Compar. Neurol. 179 , 699-717.

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

Pomeroy, S. L., and Behbehani, M. M. (1979b). Synaptic action and inhibition of nucleus raphe magnus (NRM) neurones following electrical stimulation of the periaqueductal (PAG) in the rat. Soc. Neurosci. Abst. 5 , 614.

Pompieano, O., and Swett, J. E. (1963). Actions of graded cutaneous and muscular afferent volleys on brainstem units in the decerebrate, cerebellectomized cat. Arch. Ital. Biol. 101 , 552-583.

Proudfit, H. K. (1977). Analgesia following microinjection of morphine into the nucleus raphe magnus of acutely decerebrate rats. Soc. Neurosci. Abst. 3 , 300.

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

Proudfit, H. K., and Anderson, E. G. (1974b). Blockade of morphine analgesia by destruction of a bulbospinal serotonergic pathway. Pharmacologist 16 , 203.

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

Réthelyi, M., and Szentagothai, J. (1973). Distribution and connection of afferent fibres in the spinal cord. In: Handbook of Sensory Physiology. Vol. II. Somatosensory System (A. Iggo, ed.), Springer-Verlag, New York, pp. 207-252.

Réthelyi, M., Trevino, D. L., and Perl, E. R. (1979). Distribution of primary fibres within the saccrococcygeal dorsal horn: an autoradiograghic study. J. Compar. Neurol. 185 , 603-621.

Reynolds, D. V. (1969). Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 164 , 444-445.

Rexed, B. (1952). The cytoarchitectonic orginization of the spinal cord in the cat. J. Compar. Neurol. 96 , 415-466.

Rhodes, D. L., and Liebeskind, J. C. (1978). Analgesia from rostral brainstem in the rat. Brain Res. 143 , 521-532.

Richardson, D. E., and Akil, H. (1977a). Pain reduction by electrical stimulation in man. I: Acute administration in periaqueductal and periventricular sites. J. Neurosurg. 47, 178-183.

Richardson, D. E., and Akil, H. (1977b). Pain reduction by electrical stimulation in man. II: Chronic self-administration in the periventricular matter. J. Neurosurg. 47 , 184-194.

Ruda, M. A. (1975). An autoradiographical study of the efferent connections of the midbrain central gray in the cat. Anat. Rec. 181 , 468.

Samanin, R., Gumulka, W., and Valzelli, L. (1970). Reduced effect of morphine in midbrain raphe lesioned rats. Eur. J. Pharmacol. 10 , 339-343.

Sanders, K. H., Klein, C. E., Mayer, T. E., Heym, Ch., and Handwerker, H. O. (1980). Differential effects of noxious and non-noxious input on neurones ascending to locations in ventral periaqueductal gray or dorsal raphe nucleus. Brain Res. 186 , 83-97.

Satoh, M., Akaike, A., and Takagi, H. (1979). Excitation by morphine and enkephalin of single neurones of nucleus reticularis paragigantocellularis in the rat: a probable mechanism of analgesic action of opioids. Brain Res. 169 , 409-410.

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

Schroeder, D. M., and Jane, J. A. (1971). Projection of dorsal column nuclei and spinal cord to brainstem and thalamus in the tree shrew, Tupaia glis . J. Compar. Neurol. 142 , 309-350.

Sharpe, L. G., Garnett, J. E., and Cicero, T. J. (1974). Analgesia and hyperactivity produced by intracranial microinjection of morphine into the periaqueductal gray matter of the rat. Behav. Biol. 11 , 303-313.

Snow, P. J., Rose, P. K., and Brown, A. G. (1976). Tracing axons and axon collaterals of spinal neurones using intracellular injection of horseradish peroxidase. Science 191 , 312-313.

Stacey, M. J. (1969). Free nerve endings in skeletal muscle of the cat. J. Anat. 105, 231-254.

Stewart, W. A., and King, R. B. (1963). Fibre projections from the nucleus caudalis of the spinal trigeminal nucleus. J. Compar. Neurol. 121, 271-286.

Szentagthai, J. (1964). Neuronal and synaptic arrangement in the substantia gelatinosa Rolandi. J. Compar. Neurol. 122 , 219-240.

Taber, E., Brodal, A., and Walberg, F. (1960). The raphe nuclei of the brainstem in the cat. I. Normal topography and cytoarchitecture and general discussion. J. Compar. Neurol. 114 , 161-182.

Takagi, H., Doi, T., and Akaike, A. (1976). Microinjection of morphine into the medial part of the bulbar reticular formation in the rabbit and rat: Inhibitory effects on lamina V cells of the spinal dorsal horn and behavioural analgesia. In: Opiates and Endogenous Opioid Peptides (H. W. Kosterlitz, ed.), North Holland, Amsterdam, pp. 1099-1109.

Takagi, H., Doi, T., and Kawasaki, K. (1975). Effect of morphine, L-dopa, and tetrabenazine on lamina V cells of the spinal dorsal horn. Life Sci. 17 , 67.

Takagi, H., Satoh, M., Akaike, A., Shibata, T., and Kuraishi, Y. (1977). The nucleus gigantocellularis of the medulla oblongata is a highly sensitive site in the production of morphine analgesia in the rat. Eur. J. Pharmacol. 45 , 91-92.

Takagi, H., Satoh, M., Akaike, A., Shibata, T, Yajima, H., and Ogawa, H. (1978). Analgesia by enkephalins injected into the nucleus gigantocellularis of rat medulla oblongata. Eur. J. Pharmacol. 49 , 113-116.

Trevino, D. L. (1976). The origin and projections of a spinal nociceptive and thermoreceptive pathway. In: Sensory Functions of the Skin in Primates, with Special Reference to Man (Y. Zotterman, ed.). Pergammon Press, Oxford, pp. 367-376.

Trevino, D. L., and Carstens, E. (1975). Confirmation of the location of spinothalamic neurones in the cat and monkey by retrograde transport of horseradish peroxidase. Brain Res. 98 , 177-182.

Trevino, D. L., Coulter, J. D., and Willis, W. D. (1973). Location of cells of origin of spinothalamic tract in lumbar enlargement of the monkey. J. Neurophysiol. 36 , 750-761.

Trevino, D. L., Maunz, R. A., Bryan, R. N., and Willis, W. D. (1972). Location of cells of origin of the spinothalamic tract in the lumbar enlargement of the cat. Exp. Neurol. 34, 64-77.

Waldeyer, H.(1888). Des Gorilla-Ruckenmark. Akad. Wissensh. Berlin, pp. 1-147.

Wall, P. D. (1960). Cord cells responding to touch, damage, and temperature of skin. J. Neurophysiol. 23 , 197-210.

Wall, P. D. (1967). The laminar organization of dorsal horn and effects of descending impulses. J. Physiol. (Lond.) 188, 403-423.

Wall, P. D., Merrill, E. G., and Yaksh, T. L. (1979). Responses of single units in lamina 2 and 3 of cat spinal cord. Brain Res. 160 , 245-260.

Wang, R. Y., Gallager, D. W., and Aghajanian, G. K. (1976). Stimulation of the pontine reticular formation suppresses firing of serotonergic neurones in the dorsal raphe. Nature (Lond.) 264 , 365-368.

Watkins, L. R., Griffin, G., Leichnetz, G. R., and Mayer, D. J. (1980). The somatotopic origin of the nucleus raphe magnus and surrounding brainstem structures as revealed by horseradish peroxidase slow release gels. Brain Res. 181 , 1-15.

West, D. C, and Wolstencroft, J. H. (1977). Location and conduction velocity of raphespinal neurones in nucleus raphe magnus and raphe pallidus in the cat. Neurosci. Lett. 5 , 147-151.

White, J. C., and Swett, W. H. (1969). Pain and the Neurosurgeon. Thomas, Springfield, Illinois.

Willis, W. D., Beall, J. E., Martin, R. F., and Applebaum, A. E. (1976). Inhibition of primate spinothalamic neurones by stimulation in nucleus raphe magnus. Soc. Neurosci. Abst. 2, 956.

Willis, W. D., Haber, L. H., and Martin, R. F. (1977). Inhibition of spinothalamic cells and interneurones by brainstem stimulation in the monkey. J. Neurophysiol. 40 , 968-981.

Willis, W. D., Kenshallo, D. R. Jr., and Leonard, R. B. (1979). The cells of origin of the primate spinothalamic tract. J. Compar. Neurol. 188 , 543-574.

Willis, W. D., Trevino, D. L., Coulter, J. D., and Maunz, R. A. (1974). Responses of primate spinothalamic tract neurones to natural stimulation of the hindlimb. J. Neurophysiol. 37, 358-372.

Wolstencroft, J. H. (1964). Reticulospinal neurones. J. Physiol. (Lond.) 174, 91-108.

Wolstencroft, J. H., West, J. C., and Gent, J. P. (1977). Actions of morphine and opioid peptides on neurones in the reticular formation, raphe nuclei, and periaqueductal gray. Iontophoresis and transmission mechanisms in mammalian central nervous system. Satelite Symposium of XXVII Intl. Congr. of Phys. Sci., Churchhill College, Cambridge, pp. 6.37-6.39.

Yaksh, T. L., DuChateau, J. C., and Rudy, T. A. (1976a). Antagonism by methysergide and cinaserin of the antinociceptive action of morphine administration into the periaqueductal gray. Brain Res. 104 , 367-377.

Yaksh, T. L., and Tyce, G. M. (1979). Microinjection of morphine into the periaqueductal gray evokes release of serotonin from the spinal cord. Brain Res. 171 , 176-181.

Yaksh, T. L., Yeung, J. C., and Rudy, T. A. (1976b). Systematic examination in the rat of brain sites sensitive to the direct application of morphine: observation of differential effect within the periaqueductal gray. Brain Res. 114 , 83-103.

Yaksh, T. L., Yeung, J. C., and Rudy, T. A. (1976c). An inability to antagonize with naloxone the elevated nociceptive thresholds resulting from electrical stimulation of the mesencephalic central gray. Life Sci. 18, 1193-1198.

Yaksh, T. L., Wall. P. D., and Merril, E. G. (1977). Response properties of substantia gelatinosa neurones in the cat. Soc. Neurosci. Abst. 3, 495.

Yeung, J. C., Yaksh, T. L., and Rudy, T. A. (1977). Concurrent mapping of brain sites for sensitivity to the direct applicationof morphine and focal stimulation in the production of antinociception in the rat. Pain 4, 23-40.

Zaretsky, M. D., Levine, J. D., and Fields, H. L. (1979). Tail-flick neurones of the nucleus raphe magnus. Soc. Neurosci. Abst. 5 , 618.

Zemlan, F. P., Kow, L.-M., Morrell, J. I., Pfaff, D. W. (1979). Descending tracts of the lateral columns of the rat spinal cord: a study using the horse-radish peroxidase and silver impregnation techniques. J. Anat. 128 , 489-512.