SPINAL INHIBITION RELATED TO NOCICEPTION

by

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Most of these experiments were performed in collaboration with Dr A. W. Duggan. The microelectrophoretic experiments and some of the stimulation-produced inhibition experiments were performed in collaboration with Dr S. M. Johnson. The experiments on reflexes and motoneurones and the final series on PAG-induced inhibition with medullary lesions were conducted with the assistance of Mr Z. Q. Zhao, and the study of the origin of tonic descending inhibition, with Dr J. G. Hall.

I was the principal investigator in the experiments on motoneurones, lateral medullary stimulation, PAG-induced inhibition with medullary lesions, and some of the experiments on the circulatory concomitants of PAG-induced selective inhibition.

C. R. Morton

During the tenure of my Australian National University Research Scholarship, the research described in this thesis has been published or submitted for publication as follows:

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Johnson, S.M., Duggan, A.W. & Morton, C.R. (1981). Differential distribution of receptors for morphine and met^5-enkephalinamide in the dorsal horn of the cat. Clin. exp. Pharmac. Physiol. 8, 661-662.

Australasian Chapter of the International Association for the Study of Pain.

Morton, C.R. & Duggan, A.W. Periaqueductal grey stimulation: selective inhibition of dorsal horn neurones is associated with an appropriate cardiovascular response. Sydney, 1982.

Zhao, Z.Q., Duggan, A.W. & Morton, C.R. The effects of coagulation of medullary raphé and lateral reticular areas on spinal inhibition from electrical stimulation of the periaqueductal grey. Perth, 1983.

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ABSTRACT

Several lines of evidence have implicated endogenous opioid peptides in the regulation of nociception in the mammalian central nervous system. In the present experiments in barbiturate-anaesthetized cats, the firing of lumbar dorsal horn neurones to noxious and non-noxious cutaneous stimuli was studied during the microelectrophoretic administration of morphine and Met^5-enkephalinamide at dorsal horn sites. Ejected in the substantia gelatinosa, both compounds selectively reduced nociceptive responses but ventral to this region, morphine was ineffective while the enkephalin effect was observed with administration at all intervening sites down to cell bodies. The results suggest that receptors in the dorsal horn for morphine and enkephalin are not identical and are distributed on different neural structures.

Inhibition of spinal reflexes by opioid peptides has been inferred from the increases observed following systemic naloxone. The present work confirmed these findings with a variety of reflexes in barbiturate-anaesthetized cats, and similar effects by the (-) but not (+)-isomer of another opioid antagonist, N-(3-furylmethyl)-α-normetazocine (FMN), provided evidence for opioid involvement in this inhibition. Naloxone and (-)FMN also blocked the inhibition of a spinal reflex by conditioning stimulation of C fibre afferents, suggesting release of opioid peptides by impulses related to pain. Intracellular recordings from motoneurones showed that naloxone administration increased depolarizing potentials from peripheral nerve stimulation by a non-somatic process.

Collectively these results suggest that opioid peptides in the spinal cord have other or additional functions besides regulation of nociceptive processes.

In anaesthetized cats, the excitation of dorsal horn neurones by noxious cutaneous stimuli or by impulses evoked electrically in primary afferent C fibres is tonically inhibited from supraspinal sites. In the present experiments, this tonic inhibition, measured with a cold block technique, was not affected by coagulation of many brain stem regions including the medullary raphé. Bilateral coagulation only in the regions of the lateral reticular nuclei in the caudal medulla reduced or abolished this inhibition.

Stimulation-produced inhibition of dorsal horn neurones was also investigated in barbiturate-anaesthetized cats. Electrical stimulation of the lateral spinal funiculi produced hyperpolarizations of these neurones, associated with inhibition of synaptic excitation by impulses in primary afferent A and C fibres. Stimulation in supraspinal regions can also inhibit spinal neurones and produce analgesia in conscious animals. The present experiments found that electrical stimulation in the midbrain periaqueductal grey selectively inhibited the C responses of dorsal horn neurones concurrently with increases in cardiac output and muscle but not cutaneous blood flow. Stimulation in the ventral tegmentum non-selectively reduced both C responses and non-noxious excitation, but without cardiovascular effects. Thus periaqueductal grey stimulation may produce inhibition of pain perception as part of an organized response to a particular environment.

Monopolar electrical stimulation in the ventrolateral caudal medulla also produced selective inhibition of C responses of dorsal horn neurones. Stimulus currents effective in this region had little effect elsewhere in the caudal medulla. Coagulation at the effective sites reduced tonic descending inhibition, suggesting that the same neurones were responsible for both inhibitions.

The final experiments compared the effects of coagulation in the medullary raphé and in lateral reticular regions on inhibition of dorsal horn neurones by periaqueductal grey stimulation. Lateral lesions produced much greater reductions in this inhibition.

List of Abbreviations

AP	:	antero-posterior
BST	:	biceps semitendinosus
CI	:	central inferior raphé nucleus
CSF	:	cerebrospinal fluid
DLF	:	dorsolateral funiculus
DLH	:	DL-homocysteate
DRG	:	dorsal root ganglion
DRN	:	dorsal raphé nucleus
EPSP	:	excitatory postsynaptic potential
FMN	:	N-(3-furylmethyl)-α-normetazocine
GABA	:	γ-aminobutyric acid
HRP	:	horseradish peroxidase
5-HT	:	5-hydroxytryptamine
IPSP	:	inhibitory postsynaptic potential
LRN	:	lateral reticular nucleus
LTM	:	low threshold mechanoreceptor
M-ENKA	:	Met^5-enkephalinamide
M-G	:	medial gastrocnemius
ML-G	:	medial and lateral gastrocnemii
NGC	:	nucleus reticularis gigantocellularis
NMC	:	nucleus reticularis magnocellularis
NPGC	:	nucleus reticularis paragigantocellularis
NRM	:	nucleus raphé magnus
PAD	:	primary afferent depolarization
PAG	:	periaqueductal grey
pCPA	:	p-chlorophenylalanine
PVG	:	periventricular grey
T	:	threshold
VT	:	ventral tegmentum

CHAPTER I - GENERAL INTRODUCTION

This thesis describes a series of studies undertaken to investigate several of those aspects of spinal inhibition which can be related to nociceptive processes in the central nervous system. The physiological model chosen to investigate such inhibition was the firing of neurones in the lumbar spinal cord of the cat. Neurones of the dorsal horn with cutaneous receptive fields to noxious stimuli were studied in many experiments since these cells are likely participants in spinal nociceptive processes.

These investigations have been divided into two sections. In the first, experiments examined the hypothesis that opioid peptides have a neurotransmitter role in the spinal cord, influencing the activity of dorsal horn neurones and motoneurones. In the second section, some of the supraspinal influences which can inhibit the firing of dorsal horn neurones were investigated. These experiments studied the tonic descending inhibition present on these neurones and that produced by electrical stimulation of supraspinal regions and descending pathways. In all experiments analogies have been drawn between the neurophysiological observations and nociception. The literature relevant to each section has been reviewed.

CHAPTER II - METHODS

A. ANIMAL PREPARATION

Cats of either sex weighing between 2.0 and 3.5 kg were anaesthetized with pentobarbitone sodium (35 mg/kg I.P. initially, supplemented with 2.5 mg/kg I.V. at approximately 2 hourly intervals). In all investigations the left cephalic vein, a common carotid artery and the trachea were cannulated. Body temperature of the animals was maintained between 36 and 38°C by a heating pad controlled by a probe placed between the rib cage and a scapula. Blood pressure was monitored continuously using a Statham pressure transducer (P23 Bd), and experiments were terminated if systolic pressure, in the absence of procedures such as block of spinal conduction or coagulation of brain stem regions, fell below 100 mm Hg.

To minimize movements of the spinal cord from respiratory excursions and to prevent limb movement from electrical stimulation of peripheral nerve and noxious heating of the footpad, animals were paralysed with gallamine triethiodide (4.0 mg/kg I.V. initially and maintained with 2.0 mg/kg every 30 minutes), and artificially ventilated (38 strokes/min). The stroke volume was adjusted to maintain the continuously monitored end tidal CO_2 level between 3.9 and 4.1%.

It was important to be certain of adequate anaesthesia during neuromuscular paralysis. In addition to regular supplements of pentobarbitone therefore, the blood pressure recording was continually observed during peripheral

nociceptive stimulation to ensure that no changes in blood pressure occurred.

With intracellular experiments, a bilateral pneumothorax was created to further reduce respiratory movements.

(a) Spinal Cord Preparation

Following a midline incision of the dorsal skin and retraction of overlying muscle, the laminae of the lumbar vertebrae were removed in a caudal-rostral sequence. The dural sac was opened dorsally and the exposed spinal cord was covered with a pool of paraffin oil (BP) formed by raising the dorsal skin flaps. The pool temperature was maintained at 37°C by constant heating. A coil of silver chloride-coated silver wire, covered with gauze impregnated with Ringer was sutured into muscle adjacent to the cord and served as a reference electrode. Fine forceps were used to make small holes in the pia mater overlying the L_6 and L_7 segments at sites of electrode entry.

In some experiments the spinal cord was divided at the thoraco-lumbar junction following infiltration with 2% lignocaine solution, while in other experiments spinal conduction was reversibly blocked by cooling. This was achieved by placing a metal chamber with a concave lower surface in close apposition to the dorsal surface of the first lumbar segment. The contact edges of the chamber were then sealed with a silicone grease and care was taken to prevent contact with the warm liquid paraffin bathing the rest of the spinal cord. Normally water at 38°C perfused this chamber; to block spinal

conduction, this was changed to a water-ethylene glycol mixture at -2°C. A thermistor incorporated in the base of the cooling chamber and in contact with the dorsal surface of the spinal cord usually showed a stable temperature of 2 to 4°C within 3 minutes of the start of cooling. Conduction in the spinal cord was restored by again circulating water at 38°C through the metal chamber. There is evidence that conduction in mammalian myelinated axons is blocked completely but reversibly at temperatures of 5 to 7°C, while at 4°C conduction block of unmyelinated C-fibres can be produced (Douglas & Malcolm, 1955; Franz & Iggo, 1968). The effectiveness of the present technique has been demonstrated by electrical stimulation in the cervical spinal cord (Duggan, Hall, Headley & Griersmith, 1977c).

In some experiments the L_6, L_7 and S_1 ventral roots were sectioned and mounted on platinum electrodes.

(b) Brain Preparation

The animal's head was positioned in a stereotaxic head frame. For access to the midbrain and diencephalon, the cranium was exposed by a midline incision. Bone was removed to create an opening approximately 15 x 15 mm within the range of Horsley-Clarke co-ordinates AP +16.0 to AP -4.0 and centred across the midline. The exposed dura was protected from drying by covering with cotton wool soaked in Ringer.

For access to the medulla oblongata, the head frame was rotated so that the head was ventroflexed. The dorsal surface of the brain stem and the caudal surface of the cerebellum

(which was not removed) were exposed by a midline incision followed by retraction of the overlying muscle and removal of the caudal cranium. After removal of the dorsal part of the dural sac, the exposed brain areas were sealed with agar (Bacto-Agar, "Difco", 4% in 165 mM NaCl) to prevent drying.

(c) Peripheral Nerve Preparation

In many experiments an excitatory input to spinal cord neurones was provided by electrical stimulation of peripheral nerves which, following exposure, were immersed in a pool of liquid paraffin and mounted on platinum stimulating electrodes. The tibial nerve (unsectioned) was used in most of these experiments, but in experiments with spinal reflexes and motoneurones the nerve to the biceps semitendinosus (BST), nerves to the gastrocnemii, and the sural nerve (all sectioned) were also stimulated. Nerves to the medial and lateral gastrocnemius were individually exposed, but stimulated together as one nerve (ML-G).

The electrical stimuli used to excite these nerves were square pulses of 0.2 to 0.5 msec duration, delivered via stimulus isolation units. The intensity of the stimuli was measured in multiples of the threshold for activation of the most excitable fibres, determined by recording the afferent volley with a platinum ball electrode placed on an appropriate dorsal rootlet close to its junction with the spinal cord.

(d) Measurement of Changes in Peripheral Circulation

In some experiments alterations in peripheral circulation were studied by recording changes in skin and muscle temperature. Changes in skin surface temperature were detected by placing a thermistor on either a hind limb digital pad, or on the inside of a pinna, with the contact points smeared with silicone grease. As ambient temperature affects the dilatation of cutaneous vessels, room temperature was controlled and maintained at 25 to 27°C in most of these experiments. Changes in muscle temperature were recorded by implanting a thermistor in the right gastrocnemius-soleus muscle. These thermistors formed part of conventional bridge circuits and after appropriate D.C. amplification the outputs were continuously displayed on a pen recorder. Records were calibrated as temperature changes rather than as absolute values : thus muscle and skin surface temperature *per se* were not measured. With the baseline variance present in these records, changes could be detected to the following limits: muscle temperature, 0.005°C; skin surface temperature, 0.01°C.

B. EXTRACELLULAR RECORDING TECHNIQUES

(a) Apparatus for Extracellular Recording

Electrical potential changes in the extracellular environment in the spinal cord were recorded using micropipettes containing 4 M NaCl. The pipettes were drawn out from 3 mm (outside diameter) Pyrex glass tubing in a vertical microelectrode puller and filled with the aid of fine glass fibres

which were inserted prior to pulling. Electrode tips were broken back under microscopic observation to the appropriate size (0.5 to 1.0 µm) with a fine glass rod mounted on a pneumatic micromanipulator (De Fonbrune, Paris). Electrical connection with the electrolyte was maintained by insertion of a silver chloride-coated silver wire embedded in saline (4 M NaCl) agar and encased in polythene tubing. In experiments employing multibarrel micropipettes, the centre barrel was used for extracellular recording.

The recording electrode was connected to a first stage emitter follower, an amplifier stage (time constant 3 msec) and then to an oscilloscope. The output of the emitter follower was also connected to a second amplifying system, the output of which was used to trigger a pulse generator. A window discriminator was used to select the size of action potentials which triggered this generator, and the derived standard pulses were counted by a ratemeter, with the frequency displayed simultaneously on two pen recorders. To ensure accurate counting, action potentials, the upper and lower levels of the discriminator window, and the derived standard pulses were all displayed on the same oscilloscope. The window was adjusted so as to exclude stimulus artefacts from records of cell firing rates.

In some experiments simultaneous extracellular recordings were obtained from two independent microelectrodes. The second recording electrode was connected to a first stage cathode follower, an amplifier stage (time constant 3 msec) and then to an oscilloscope.

The number of action potentials evoked by peripheral stimuli was determined by gating an electronic counter (Hewlett-Packard 5214L), with the counting period adjusted for each type of peripheral stimulus. With natural cutaneous stimuli, the period of stimulation and the gate duration were controlled by an automatic timer (Digitimer D4030). To determine the number of evoked action potentials during each counting period, it was necessary to subtract spontaneous action potentials for the duration of the counting gate. Spontaneous firing was measured from the ratemeter record between periods of cutaneous stimulation. With electrical stimulation of peripheral nerve, the gate was timed to include only those action potentials which, by their latency and stimulus threshold, were produced by impulses in particular classes of primary afferent fibres. In many experiments these gated counts were continuously displayed on a pen recorder, while in other experiments 16 such responses were used to compile peristimulus histograms, at appropriate intervals, by the use of an Ortec Time Histogram Analyzer. The means and standard errors of the means were determined for the 16 responses used to compile each histogram. In figures illustrating results, these means have sometimes been plotted instead of presenting histograms.

(b) Location and Identification of Neurones

A stepping motor microelectrode drive (Transvertex, Sweden) was used to advance the microelectrode through the

spinal cord. Lamina location of dorsal horn neurones was determined by the distance from the dorsal surface of the cord with electrode tracks approximately mid-way between the midline and the dorso-lateral sulcus. Lamina I was taken as lying between 0.9 to 1.0 mm; lamina II (substantia gelatinosa), 1.0 to 1.2 mm; lamina III-IV, 1.2 to 1.6 mm; lamina V, 1.6 to 2.0 mm; and lamina VI, 2.0 to 2.5 mm (Handwerker, Iggo & Zimmermann, 1975). In addition, neuronal location was confirmed in some experiments by electrophoretic ejection of pontamine sky blue from the recording electrode, with subsequent histological localization in 50 µm frozen sections.

Dorsal horn neurones were located by four methods:

(i) Spontaneous firing.

(ii) Firing produced by ejection of the excitant amino acid DL-homocysteate (DLH) from the advancing electrode. This method was only used to identify neurones of Rexed lamina I (Rexed, 1952) to determine its depth below the dorsal surface of the spinal cord.

(iii) Firing in response to electrical stimulation of the ipsilateral common tibial nerve.

(iv) Firing in response to noxious and innocuous cutaneous stimuli: radiant heat and deflection of hairs. Radiant heat was produced by a lamp focussed to a spot approximately 3 mm in diameter on one of the digital pads which had been previously blackened with ink. A thermocouple, placed in the

irradiated area, monitored the surface temperature attained and provided feedback to a circuit controlling the filament current of the heating lamp to accurately control this temperature.
The lower surface of the thermocouple was in direct contact with the skin surface, but the upper irradiated surface was coated with a layer of clear epoxy resin. Temperatures attained by 20 to 30 seconds of irradiation were between 48°C and 55°C and were displayed on a pen recorder together with cell firing (latency 5 to 15 seconds) produced by this stimulus. The innocuous stimulus, deflection of hairs adjacent to the heated area, was produced by a jet of air from a small polythene tube moved by the armature of an electrical relay activated by a Tektronics pulse generator. This arrangement allowed either continuous oscillation (2 to 4 Hz) for a selected period (commonly 12 to 15 seconds) or a single sweep of the air jet, repeated at 0.2 to 0.5 Hz, across part of a neurone's receptive field.

When studying nociception, it would seem appropriate to record from neurones activated by noxious cutaneous stimuli. Several workers have suggested that excitation of dorsal horn neurones by different sensory modalities depends to some extent on the spinal lamina location of the cell (see Price & Dubner, 1977). In the cat, it has been reported that neurones of Rexed lamina I are principally, if not exclusively, responsive to noxious cutaneous stimuli (Class 3 cells) (Christensen & Perl,

1970; Cervero, Iggo & Ogawa, 1976). On the other hand, neurones of lamina IV have been classed as non-nociceptive (Class 1), while those of lamina V are responsive to both noxious and non-noxious cutaneous stimuli (Class 2; multi-receptive; wide dynamic range) (Pomeranz, Wall & Weber, 1968; Heavner & De Jong, 1973; Guilbaud, Oliveras, Giesler & Besson, 1977). Several groups, however, have found that many neurones of laminae I, III, IV and V of the cat are excited by both noxious and non-noxious inputs (Handwerker et al., 1975; Price & Browe, 1975; Duggan, Hall & Headley, 1977a,b; Cervero, Iggo & Molony, 1977; Davies & Dray, 1978; Hall, 1979).

The preponderance of multireceptive neurones observed in the dorsal horn by conventional extracellular sampling techniques has important implications for the selection of neurones and their activating stimuli for the study of nociceptive processes. In particular, an induced decrease of nociceptive responses of neurones may be interpreted as being relevant to a reduced perception of pain, but this proposed relationship is more plausible if it can also be shown that non-nociceptive responses of the same neurones are concurrently unaffected. Conversely, a reduction of both types of response is difficult to interpret in terms of analgesia. Because of this consideration, most experiments described in this thesis examined excitation of dorsal horn neurones by more than one sensory modality.

(c) Some Considerations of the Stimuli Used

In experiments on nociception, it is often essential to produce a quantitative, reproducible noxious stimulus. Of those cutaneous stimuli which are painful, noxious radiant heat lends itself readily to this application. There is evidence that when skin temperature of the cat is raised above 45°C the input to the spinal cord from the heated area is almost exclusively from thermal nociceptors (Beck, Handwerker & Zimmermann, 1974). Although it has been reported that repeated noxious heat stimuli increase nociceptor firing (so-called sensitization; Perl, Kumazawa, Lynn & Kenins, 1976), heat stimuli lasting 25 to 30 seconds, repeated at 2-minute intervals, have produced constant responses in dorsal horn neurones for periods of up to 4 hours both in the present experiments and previous studies (Duggan & Griersmith, 1979a).

There are problems, however, with the use of radiant heating of the skin as a noxious stimulus. The temperature attained at subcutaneous thermal nociceptors is determined not only by the intensity of the noxious heat stimulus applied to the skin, but also by the state of the cutaneous circulation (Beecher, 1957). Thus with a constant stimulus (fixed intensity and duration of heating), procedures which alter blood pressure - and hence probably blood flow through the skin - can disturb the firing of nociceptors through changes in the rate of heat transfer from the skin surface to nociceptors (Duggan, Griersmith, Headley & Maher, 1978). When studying dorsal horn neurones of the cat, this has been observed as marked changes in cell firing to noxious cutaneous heat

following spinal cold block or intravenous administration of vasoactive substances (Duggan et al., 1978; Davies & Dray, 1980). The use of a thermocouple with a feedback circuit to accurately control the surface temperature has been shown to compensate to some extent for circulatory changes (Duggan et al., 1978). Under some conditions of changing circulation, however, even this regulated heating was shown to be inadequate to maintain reproducible nociceptor firing, and was thus unsuitable as a quantitative noxious stimulus (Duggan & Griersmith, 1979b).

In many of the experiments described in this thesis, experimental procedures such as spinal cold block and electrical stimulation and electrocoagulation in the brain stem altered blood pressure and thus probably affected cutaneous circulation also. It was often necessary to compare the effects of supraspinal stimulation and coagulation in different regions on the nociceptive responses of dorsal horn neurones. For quantitative purposes therefore, electrical stimulation of the ipsilateral tibial nerve at a strength adequate to excite unmyelinated (C) primary afferent fibres, many of which convey nociceptive information to the spinal cord (Bessou & Perl, 1969; Beck et al., 1974), was used in preference to noxious heat in many experiments. Stimulating C fibres electrically has the disadvantage that primary afferents from low threshold mechanoreceptors (LTMs) may be excited as well as nociceptive primary afferents. For example, a study with the posterior femoral cutaneous nerve of the cat found C-fibres from LTMs and nociceptors in approximately equal proportions (Bessou &

Perl, 1969). In the cat tibial nerve however, Beck et al. (1974) found three times as many primary afferent C-fibres exclusively from nociceptors than from LTMs, possibly due to the high proportion of glabrous skin (which lacks LTMs) in the innervated area. Thus it is probable that under conditions where changes in skin circulation can produce serious artefacts in responses to noxious heat that electrical stimulation of unmyelinated primary afferents of the tibial nerve is a satisfactory means of giving a quantitative nociceptive input to the spinal cord.

Graded electrical stimulation of the tibial nerve has shown that a stimulus strength of 20 to 100 times threshold for activation of a dorsal horn neurone produces action potentials with a latency indicating activation by impulses in unmyelinated afferents (Duggan, Griersmith & Johnson, 1981). In the present experiments such latency and threshold criteria were always applied when studying excitation of neurones by C-fibre impulses evoked electrically. With some neurones, action potentials of similar latency were produced by low stimulus strengths but these were not studied since such firing probably resulted from polysynaptic activation by myelinated primary afferents.

With hair deflection, the firing of dorsal horn neurones produced by one sweep of the air jet across part of the receptive field consisted of a group of action potentials often followed by a period of inhibition of spontaneous firing. When more than one sweep was used, the frequency of oscillation was selected so that the number of evoked action potentials was not

reduced by the inhibition following the preceding deflection. The time constant (1 second) of the counting system effectively integrated these bursts.

C. INTRACELLULAR RECORDING TECHNIQUES

(a) Apparatus for Intracellular Recording

Intracellular recordings were obtained from dorsal horn neurones and motoneurones with glass micropipettes filled with either 1.2 M potassium citrate or 2.0 M potassium chloride. For successful cell penetrations it was important for the micropipettes to have a very gradual taper to the tip. Those with very fine tips (resistance greater than 100 MΩ) were virtually unusable as resistance rose rapidly when introduced into the spinal cord and could rarely be lowered *in situ* by the passage of current. For dorsal horn neurones, most results were obtained with micropipettes broken or bevelled to a tip diameter of approximately 0.5 μm with resistances of 20 to 40 MΩ. For motoneurones 0.5 to 1.0 μm and 10 to 30 MΩ were appropriate parameters. Electrical connection with the electrolyte in the pipette was maintained as described for extracellular recording. Microelectrode resistance was continuously monitored during bevelling as an index of tip size, which was checked microscopically. It was found, however, that electrodes with tips broken back were equally suitable for intracellular recording, and thus for ease of preparation this method was employed in the majority of experiments.

The recording electrode was connected to the active bridge of a WPI model M701 microprobe unit. The output of the bridge was connected to a differential amplifier incorporated within the WPI unit. The single-ended "bridge" output from this amplifier was led to a further amplifier stage (time constant 1 second), then to an oscilloscope, a pen recorder, and an Ortec signal averager. Resting membrane potential was measured with a digital voltmeter connected to the WPI "x1 output", and continuously displayed on a Recti-riter rectilinear pen recorder. Intracellular stimulation was by means of a Grass S88 stimulator connected to the WPI unit through a Grass SIU5 Stimulus Isolation Unit.

In some experiments evoked changes in membrane potential were photographed directly from the oscilloscope screen with a Grass camera. With manipulations of membrane potential by current injection, the current pulse and resultant potential were always displayed and photographed together on the oscilloscope. With the depolarizing membrane potential changes (EPSPs) evoked by peripheral nerve stimulation, analysis was commonly performed with the signal averager, and the averaged potentials from 16 successive nerve stimuli were recorded with a pen recorder. For quantitative comparisons the areas of these averaged records were calculated. In some experiments samples of spontaneously occurring changes in membrane potential were photographed on moving film.

(b) Location and Identification of Neurones

For successful intracellular penetrations, it was found that high-speed steps of 10 to 20 µm by the electrode were required. Impalement of dorsal horn neurones was usually signified by the sudden appearance of a negative potential at the electrode and a burst of action potentials superimposed on a depolarization in response to electrical stimulation of the ipsilateral tibial nerve.

When locating motoneurones, the site of electrode entry into the cord was selected by using a ball electrode on the cord surface to determine the position of the largest field potential evoked by electrical stimulation of ventral roots. Motoneurones were identified by antidromic activation following ventral root stimulation. In addition, depolarizing changes in membrane potential were commonly produced by electrical stimulation of one or more of the ipsilateral BST, ML-G, sural and tibial nerves.

In motoneurone experiments, the stimuli used to excite these nerves were less than 10 times threshold for activation of the most excitable fibres, and thus it is improbable that unmyelinated fibres were excited. With dorsal horn neurones excited by impulses in tibial afferents, higher stimulus intensities were often used.

D. SPINAL REFLEX RECORDING TECHNIQUES

Spinal reflexes were recorded in L_6, L_7 and S_1 ventral roots following electrical stimulation of the ipsilateral BST, ML-G, sural and tibial nerves, with stimuli usually less than

10 times threshold. In a few experiments reflexes were recorded peripherally in the medial-gastrocnemius (M-G) nerve while stimulating large diameter afferents of the sural nerve. The platinum recording electrodes were connected to a differential amplifier (time constant 300 msec), which was connected to an oscilloscope and an Ortec signal averager. Reflexes were recorded as the averaged response to 16 or 32 successive stimuli, with the averaged records displayed with a pen recorder.

With the monosynaptic reflexes to stimulation of the BST and ML-G nerves, it was common to use two closely timed nerve stimuli, the first being submaximal for elicitation of a reflex. The number of averaged responses compiled as controls was determined by the variability of the individual reflexes. With some, the variance was small, but with many there was often significant variation in response amplitude and in these cases several control averages were prepared. As for the averaged records of EPSPs, the mean areas of these records were calculated for quantitative comparisons.

Reflexes to stimulation of primary afferent C fibres of the tibial nerve were also studied, and were usually recorded as relatively widely dispersed individual action potentials. In such cases a window discriminator was used to include all action potentials above the baseline and an electronic counter was appropriately gated to count only spikes which, by their latency and the nerve stimulus necessary to elicit them, were produced by C primary afferents. The nerve stimuli used were greater than 100 times threshold.

In all but two reflex experiments the spinal cord was divided at the thoraco-lumbar junction.

E. DRUG ADMINISTRATION

(a) Microelectrophoretic Methods

This technique was used to administer compounds as ions from glass micropipettes into the extracellular environment of single neurones in the dorsal horn of the spinal cord. This procedure has been described by Curtis (1964).

(i) Preparation of micropipettes

Five or seven barrel micropipettes were drawn out in a vertical microelectrode puller and the tips broken back to the desired size (5 to 7 μm) as described previously. Aqueous solutions of the compounds to be ejected electrophoretically were centrifuged (1600 g) for 10 minutes, and transferred to individual barrels of the micropipette. Centrifugation 1600 g, 10 minutes) of the micropipettes propelled the solutions to the barrel tips to complete the filling process. In some experiments the filling process was aided by fine glass fibres inserted in each barrel prior to pulling. The adequacy of filling was checked microscopically and the electrical resistance of each barrel measured.

(ii) Solutions

The solutions used in the microelectrophoretic study, with their concentrations (and pH if adjusted), are listed in Table I.

TABLE I AQUEOUS SOLUTIONS FOR ELECTROPHORETIC ADMINISTRATION

Compound	Concentration	pH (if adjusted)
γ-Aminobutyric acid (GABA)	500 mM	3 (HCl)
Met^5-enkephalinamide (M-ENKA)	20 mM	4 (HCl)
Morphine sulphate	70 mM	
Naloxone hydrochloride	100 mM	
Pontamine sky blue	2% w/v in 1.2 M sodium acetate	
Sodium DL-homocysteate (DLH)	200 mM	7.5 (NaOH)

(iii) Some considerations of the technique

Ionized compounds are ejected electrophoretically by the application of a potential gradient of suitable polarity within the solution. The amount ejected therefore depends on the current that flows between the solution in the micropipette and the external (extracellular) medium. This ejecting current was supplied and controlled by a polarizer circuit. The resistance of each barrel was determined with the microelectrode in the spinal cord by measuring the current required to maintain 0.5 V potential difference across the solution. The polarity of this voltage was selected for each compound to retard outward diffusion of the active ion by transporting it away from the tip of the micropipette. This retaining voltage was of opposite polarity to that applied for electrophoretic ejection of the ion.

The continuous application of a retaining voltage produces a relative decrease in the concentration of active ion in the micropipette tip. In addition, the concentrations attained in the extracellular environment of neurones cannot be accurately determined. Therefore, to compare the relative potencies of compounds at different sites in the dorsal horn, electrophoretic ejection was usually performed with fixed ejecting currents for fixed periods of time.

(iv) Micropipette assemblies

The microelectrophoretic experiments described in this thesis sought to make quantitative comparisons between the actions of morphine and met^5-enkephalinamide (M-ENKA) on the excitation of dorsal horn neurones by cutaneous noxious and

innocuous stimuli following administration near cell bodies, in the region of the substantia gelatinosa, and at intervening sites. This involved two independently-manipulated micropipette assemblies. With the aid of a stereoscopic microscope, a five and a seven barrel micropipette were aligned tip-to-tip, 50 to 200 µm above the dorsal surface of the spinal cord (the convergence point), and the appropriate micromanipulator readings obtained. The seven barrel pipette was then lowered vertically into the cord until neuronal activity from ejection of the excitant amino acid DLH was detected. Previous experiments (Duggan et al., 1977a) with dye deposits have confirmed that these superficial neurones lie within spinal lamina I. The micropipette was then advanced ventrally a further 50 to 150 µm so that the tip was positioned in Rexed lamina II or III, the substantia gelatinosa (Szentagothai, 1964; Scheibel & Scheibel, 1968). The distance from this point to the convergence point was then calculated. The five barrel pipette was introduced into the cord at 18° to the vertical so that its tip would pass 300 µm below the tip of the seven barrel pipette when both were in the same vertical line. Since it was exceptional to locate a neurone with the five barrel pipette at exactly this planned separation, it was decided to study neurones located from 40 µm superficial to 540 µm ventral to this point. Neurones were located by firing in response to noxious radiant heat to a digital pad, and selected for study if also activated by deflection of peridigital hairs, provided responses to both noxious and non-noxious stimuli were reproducible.

M-ENKA was administered from the seven barrel pipette, firstly in the region of the substantia gelatinosa and then at sites progressively closer to the cell body while observing the firing of the cell to cycled periods of noxious heat and hair deflection with the five barrel pipette. Infrequently, M-ENKA was also ejected near the cell body from this pipette. With many cells the amino acids DLH and γ-aminobutyric acid (GABA) were also ejected at the sites of M-ENKA administration with currents and times similar to those used with the enkephalin. With morphine, administration in the substantia gelatinosa depresses nociceptive activation of dorsal horn neurones for prolonged periods (Duggan et al., 1977a), making it difficult to subsequently study effects at sites close to cell bodies when using this dorso-ventral sequence. Since this prolonged action does not occur when morphine is ejected near cell bodies (Duggan et al., 1977a), this compound was administered first approximately 100 μm dorsal to cell bodies, and then at sites progressively more dorsal up to and including the substantia gelatinosa.

(b) Intravenous Administration

Solutions administered intravenously were:-

Naloxone hydrochloride 1.0 mg/ml

N-(3-furylmethyl)-α-normetazocine methanesulphonate (FMN) 1.0 mg/ml.

Both drugs were dissolved in 165 mM NaCl, and were administered into the left cephalic vein.

F. ELECTRICAL STIMULATION AND ELECTROCOAGULATION IN THE CENTRAL NERVOUS SYSTEM

(a) Preparation of Electrodes

Electrodes for electrical stimulation in the central nervous system were prepared from appropriate lengths of stainless steel, tungsten, or platinum-iridium (80:20) wire.

Straightened stainless steel wire of 250 µm diameter (Clark) was tapered to a point at one end by rotating the end against a rotating abrasive pad under microscopic observation. For tungsten wire (250 µm diameter, Clark), a tip was etched by repeated immersion in a tungsten etching solution (Table II). Both steel and tungsten electrodes were insulated by coating with Epoxylite insulating resin (Clark). The electrodes were dipped into the resin which was then cured by heat (100°C for 30 minutes followed by 180°C for 30 minutes). Five resin coats were usually required for effective electrical insulation. The desired tip exposure (usually 0.5 mm) was achieved by removing the appropriate amount of resin from the tip with a scalpel.

Platinum-iridium wire (210 µm diameter) was etched to a tip with the appropriate etching solution (Table II). The electrodes were insulated in glass, either by encasement in fine glass capillary tubing (450 µm outer diameter) with the wire anchored at both ends with cyanoacrylate adhesive ("Loctite"), or by immersion in molten glass. In both cases electrodes were insulated to within 0.3 mm of their tips.

TABLE II SOLUTIONS FOR ETCHING STIMULATING ELECTRODES

TUNGSTEN

Potassium hydroxide	34 g
Sodium Nitrite	71 g
Distilled water	100 ml

PLATINUM-IRIDIUM

Sodium hydroxide	30 g
Sodium cyanide	50 g
Distilled water	100 ml

Electrodes for electrocoagulation of regions of the brain stem were of stainless steel, prepared as for the stimulating electrodes, but insulated to within 1 to 2 mm of their tips.

(b) Stereotactic Techniques

Depending on the particular experiment, one, two, three, or four electrodes were fixed in a single plane to a perspex block with cold-curing dental acrylic (Neo-Simplex). This block was appropriately mounted in a Narashige manipulator.

For electrical stimulation and electrocoagulation in the medulla oblongata, steel electrodes separated by 1.0 to 1.5 mm were aligned in the rostro-caudal plane at 45° to the Horsley-Clarke horizontal plane. Because of this angular entry into the brain stem, the tips were staggered so that all would contact the basiocciput simultaneously. The manipulator readings were converted to Horsley-Clarke stereotactic co-ordinates by aligning the electrode tips against an artificial skull inscribed with these co-ordinates. The reading for the midline was obtained by placing the electrodes directly over the median fissure in the dorsal surface of the medulla, following removal of the dura.

With midbrain and more rostral regions, a pair of steel or tungsten electrodes 3 mm apart was used. The electrodes were aligned in the Horsley-Clarke frontal plane at 90° to the horizontal plane, and centred across the midline. In these experiments the position of a pointer was determined by reference to the inscribed artificial skull, and the electrodes were subsequently aligned tip-to-tip with the pointer.

In many experiments manipulator readings were obtained for the electrodes when just contacting the brain surface prior to entry and the bone of the base of the cranium. These readings were also used to determine electrode position.

The stereotactic co-ordinates of particular brain regions were based on the atlas of Berman (1968).

For electrical stimulation of the lateral spinal columns, the incised dura mater at L_1 to L_3 was retracted and a pair of steel or platinum-iridium electrodes 1.0 to 1.5 mm apart, aligned either rostro-caudally or medio-laterally, was introduced lateral to the dorsal roots entering the left L_2 to L_3 spinal segments. Occasionally a second electrode pair was positioned similarly on the right side of the cord. To facilitate electrode entry, small holes were created in the pia mater using fine forceps.

(c) Some Considerations of Stimulation Techniques

For electrical stimulation at sites in the midbrain and diencephalon, bipolar stimulation between an electrode pair aligned in the transverse plane and straddling the midline was used. This technique was chosen for the following reasons. Firstly, it was intended to investigate the neurophysiological basis of analgesia produced by electrical stimulation in the brain stem, and many of these behavioural experiments employed bipolar stimulation (Mayer, Wolfle, Akil, Carder & Liebeskind, 1971; Akil, Mayer & Liebeskind, 1976). Secondly, it was proposed to activate neurones and/or fibres of passage located in the region between the electrode tips while producing

minimal physical damage to this region by electrode placement. With this bipolar technique, preferential stimulation of neural elements between the tips is most likely (Wetzel, 1970). In self-stimulation experiments with rats, such an alignment of electrode tips in the transverse plane was more effective than rostro-caudal alignment (Szabo & Milner, 1972, 1973), and this arrangement has proved effective for activation of medullary raphé and dorsal raphé regions, producing inhibition in the lumbar dorsal horn (Duggan & Griersmith, 1979a; Griersmith, Duggan & North, 1981).

Bipolar stimulation between electrode pairs was also used to activate descending fibres in the lateral spinal columns.

This technique, however, presented problems when stimulating regions of the medulla. The object of these experiments was to excite the smallest number of brain stem neurones or fibres which would inhibit the responses of lumbar dorsal horn neurones. The bipolar technique was used initially, with the electrode pair aligned rostro-caudally and separated by 1.5 to 3.0 mm. This alignment was used on the assumption that the neurones to be stimulated, located ventro-laterally in the region of the lateral reticular nucleus, were also orientated in a rostro-caudal column. With this technique, however, inhibition of dorsal horn neurones was commonly produced from stimulation at widely-dispersed sites throughout the caudal brain stem. This may have resulted from excitation of relatively large volumes of neural tissue adjacent to both electrode tips (see Valenstein & Beer, 1961; Stark, Fazio & Boyd, 1962). Hence monopolar stimulation which, with currents

of less than 100 μA, is unlikely to excite neurones or fibres located more than 1mm from the electrode tip (Ranck, 1975), was subsequently used. Although a single electrode was sometimes employed for this purpose, it was more usual to use an array of three electrodes aligned rostro-caudally and separated by 1.0 or 1.5 mm. By stimulating from each electrode in turn, this arrangement allowed comparisons of the effect of stimulation at different rostro-caudal sites at any given vertical and lateral position.

In all experiments neural structures were stimulated using a tetanus of 0.2 to 0.5 msec pulses at 310 Hz. The duration of this tetanus and the stimulus current intensity (which was monitored continuously) varied among different groups of experiments, as follows:-

Diencephalon-midbrain (bipolar) : 90 to 120 msec,
20 to 300 μA

Medulla (bipolar and monopolar) : 100 to 200 msec,
5 to 100 μA

Lateral spinal funiculi (bipolar) : 20 to 40 msec,
100 to 900 μA

The effect of supraspinal stimulation was always tested against the gated response of a dorsal horn neurone to a peripheral stimulus. Thus the tetanic train and the peripheral stimulus were repeated at the same frequency so that each counting gate was preceded by a tetanus, which was timed to produce maximum inhibition of the gated response whilst remaining outside the gate. In most cases this occurred when the last pulse of the train was within 10 msec of the start of

the gate. To facilitate this timing, action potentials evoked by the peripheral stimulus, the stimulus artefacts of the tetanus, and the gating voltage were all displayed on the one oscilloscope.

With lateral funiculi stimulation during intracellular recordings from dorsal horn neurones, the counting gate was not used but rather depolarizing and hyperpolarizing potentials and action potentials were photographed from the oscilloscope screen.

(d) Electrocoagulation

To lesion particular brain stem regions, high frequency alternating current (20 to 75 mA at 500 kHz) from a Wyss coagulator was passed between adjacent pairs of an array of two, three or four electrodes aligned rostro-caudally and separated by 1.0 or 1.5 mm. The size of the brain stem structure to be destroyed determined the number of coagulating electrodes required. Thus with large plate-like structures such as the medullary raphé, four electrodes were used, but for discrete lesions in the region of the lateral reticular nuclei, two electrodes only were used. Adequacy of destruction was later determined histologically in 10 or 15 µm transverse paraffin sections.

G. HISTOLOGY

(a) Dye Marking Techniques

In many of the microelectrophoretic experiments, sites of

drug administration were confirmed by ejection of pontamine sky blue both from the five-barrel electrode recording at the cell body and from the seven barrel micropipette at one site of administration. The dye was ejected as an anion at 5 µA for 5 minutes at the conclusion of the experiment. A centimetre length of spinal cord containing the dye spots was then removed and fixed in 10% formal saline for three days. After mounting on a freezing microtome, 50 µm transverse sections were cut and examined microscopically. Sections containing dye spots were mounted in distilled water on a glass slide. A photographic enlargement of the sections was then made and onto it were marked the positions of the dye spots, as illustrated for one experiment in Figure 1.

(b) Electrode Marking Techniques.

In most experiments employing steel stimulating electrodes, one or more sites of stimulation were marked at the end of the experiment by passing direct current (10 to 20 µA for 60 seconds) between the stimulating electrode(s) and the indifferent electrode. The positive pole of the current source was connected to the stimulating electrode, thereby depositing ferric ions in the neural tissue. The entire brain, or the appropriate centimetre of spinal cord, was removed and trimmmed before fixing.

For visualization of iron, the tissue was fixed, trimmed, and sectioned in the transverse plane as described for dye marking techniques, in preparation for the Prussian blue test. Sections were immersed in freshly prepared 2% potassium

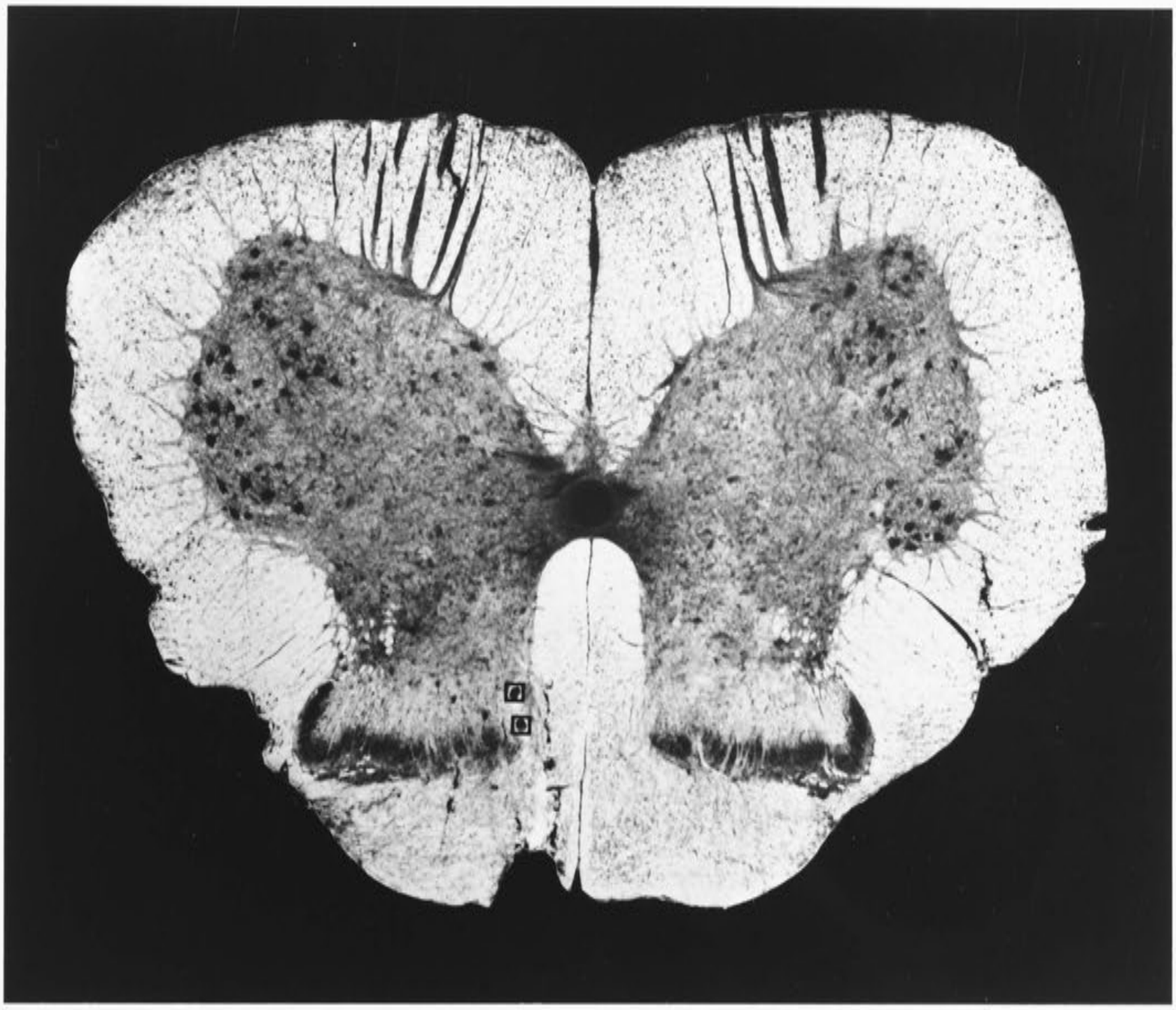


Fig. 1. The ejection of pontamine sky blue produced dye spots, marked by squares, in this 50 µm frozen section of the lumbar spinal cord. The sites are those of the five barrel micropipette recording at the cell body and the seven barrel micropipette at one more dorsal site of drug administration.

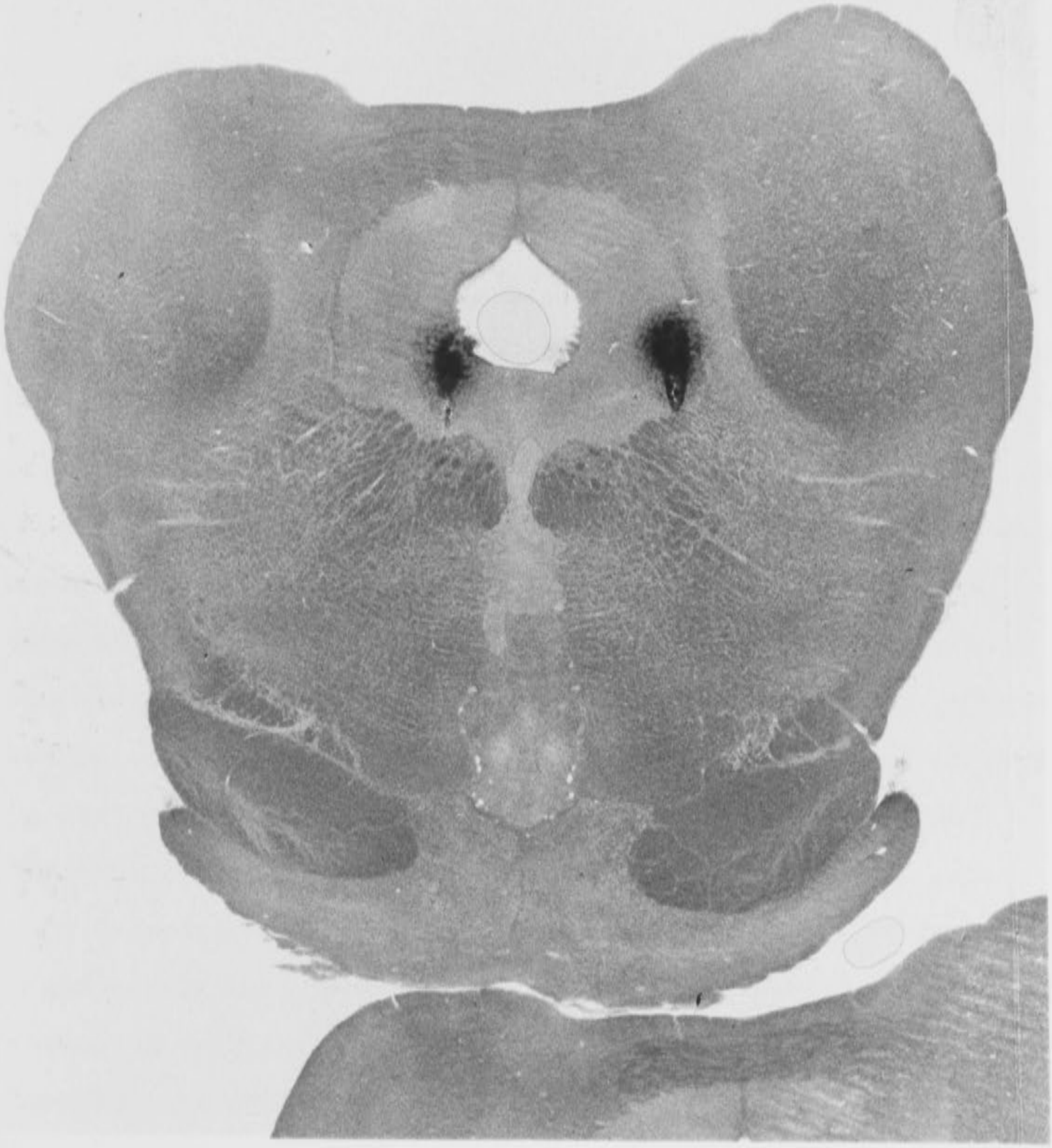
ferrocyanide for 5 minutes, to which was then added an equal volume of 0.2 N hydrochloric acid. After 20 minutes, the sections were examined for blue spots, denoting sites of reactive iron. Sections containing iron spots were washed in distilled water and mounted and photographed as described previously. Such a section, with iron spots in the ventral periaqueductal grey matter of the midbrain, is shown in Figure 2.

In some experiments both electrocoagulation and iron deposition were present in the brain stem, which was processed differently in order to visualize both the extent of lesions and the location of iron deposits. The trimmed brain stem was fixed in 10% formal saline for one to two weeks, then dehydrated in stages to absolute ethanol, transferred to chloroform and embedded in paraffin wax. Transverse sections 10 or 15 µm thick were cut, treated with xylene and absolute ethanol, defatted in a chloroform/alcohol/ether (6:1:1) mixture, and then re-hydrated in stages.

Sections to be examined for iron were then treated for the Prussian blue reaction. After 5 minutes in 10% potassium ferrocyanide solution, the sections were transferred to a mixture of 7% potassium ferrocyanide and 3% hydrochloric acid for 20 minutes. The sections were washed, counterstained with nuclear fast red, dehydrated and mounted. Iron deposits appeared as blue spots against the stained pink background.

In a small number of the experiments which employed tungsten stimulating electrodes, stimulation sites were marked similarly with direct current (100 µA for 60 seconds).

Fig. 2. A 50 μm transverse section through the midbrain at approximately AP 0. When treated with the Prussian blue technique, sites of iron deposition from the two steel stimulating electrodes were readily detected as blue spots (shown as black in the photograph) in the ventral periaqueductal gray matter.



Tungsten ions were then detected by the "tungsten blue" reaction (Pabst, 1973). The trimmed brain stem was fixed in a mixture of 70 ml 10% formaldehyde, 30 ml concentrated hydrochloric acid and 10 g stannous chloride. Transverse sections were then examined for blue spots.

(c) Lesions

In experiments involving electrocoagulation in the brain stem, the extent of destruction was determined by Nissl-staining the 10 or 15 µm transverse paraffin sections obtained as described above. The rehydrated sections were stained with 0.1% cresyl violet, rinsed and differentiated in ethanol, dehydrated in stages and mounted. Under microscopic observation, darkly-stained cell bodies were easily recognizable and areas of coagulation were distinguishable from the surrounding normal tissue. In regions of pronounced destruction, the damaged tissue was occasionally removed by the histological processing. Haemorrhage in the brain was observed as yellow-orange areas in the stained sections, and was commonly present in both coagulated regions and tissue of otherwise normal appearance.

In some experiments, the fixed brain stem block was transversely sectioned and the block faces thus exposed were photographed prior to further histological processing. Examples are shown in Figures 19 and 38.

CHAPTER III - OPIOID PEPTIDES IN THE SPINAL CORD

INTRODUCTION

The discovery of the endogenous opioid peptides was one of the more significant advances in neuroscience within the last decade, and has prompted much scientific inquiry. Since the detection (Hughes, 1975; Terenius & Wahlstrom, 1975) and characterization (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) of the enkephalins, many investigations have been aimed at testing the hypothesis (Kosterlitz & Hughes, 1975) that they act as neurotransmitters in the central nervous system. There are several well-known criteria for a substance to be accepted as a transmitter in the central nervous system, and it is proposed to review the evidence to date on the credibility of the enkephalins as transmitters, including their possible functions in this role. Most discussion will centre on the spinal cord since this was the region selected for study. The experiments described in this thesis were designed to investigate some aspects of enkephalin physiology, and do provide some evidence for a transmitter role.

Criteria for Transmitter Identification

Essentially the same criteria have been proposed by several authors (Paton, 1958; Curtis, 1961; Werman, 1966; Orrego, 1979). To qualify for acceptance as a central neurotransmitter, the proposed substance should be present in the central nervous system, located in synaptic vesicles in nerve

terminals, and the appropriate synthetic system should also be present presynaptically. In addition, physiological activation of these terminals should release the proposed substance, the membrane effects of the putative transmitter should in all ways resemble those of the natural transmitter (identity of action), and pharmacological antagonists should similarly modify the postsynaptic effects of the proposed transmitter and the natural transmitter. Of these criteria, the two most decisive are identity of action and release from nerve terminals.

In addition to a possible transmitter role, it has been suggested that enkephalins may function as neuromodulators, where they alter the responses of neurones to transmitters but do not <u>per se</u> directly affect cell excitability (Zieglgansberger & Fry, 1976; Zieglgansberger & Tulloch, 1979; Barker, Neale, Smith & Macdonald, 1978). This proposal will be discussed subsequently.

(a) <u>The Localization of Enkephalins within the Central Nervous System</u>

Several groups have investigated enkephalin distribution in the central nervous system, and immunohistochemical techniques have demonstrated a widespread, but uneven presence of enkephalin-like immunoreactivity. Initial studies performed on rats (Elde, Hokfelt, Johansson & Terenius, 1976; Simantov, Kuhar, Uhl & Snyder, 1977) found prominent networks of enkephalin-like immunoreactivity in axons and terminals in many parts of the nervous system. Subsequent studies localized immunoreactivity in cell bodies also (Hokfelt, Elde, Johansson,

Terenius & Stein, 1977a; Watson, Akil, Sullivan & Barchas, 1977a), and further reports confirmed these initial findings of immunoreactive axons, terminals and cell bodies in many regions from telencephalic to spinal cord levels (Sar, Stumpf, Miller, Chang & Cuatrecasas, 1978; Johansson, Hokfelt, Elde, Schultzberg & Terenius, 1978; Finley, Maderdrut & Petrusz, 1981). Other species investigated include cats (Moss, Glazer & Basbaum, 1981), monkeys (Aronin, di Figlia, Liotta & Martin, 1981; Haber & Elde, 1982) and man (La Motte & De Lanerolle, 1981).

The distribution studies relevant to this thesis are those concerning the spinal cord. The highest levels of enkephalin-like immunoreactivity have been found in the superficial laminae of the dorsal horn, especially the marginal layer (Lamina I) and the substantia gelatinosa (Lamina II), of rats (Hokfelt, Ljungdahl, Terenius, Elde & Nilsson, 1977b; Gibson, Polak, Bloom & Wall, 1981), cats (Glazer & Basbaum, 1981) and monkeys (Aronin et al., 1981). Although levels of immunoreactive enkephalin in the spinal cord appeared unaltered by dorsal rhizotomy and proximal cord transection, suggesting that intrinsic neurones were the source of the peptide (Elde, Hokfelt, Johansson, Ljungdahl, Nilsson & Jeffcoate, 1978; Seybold & Elde, 1980), subsequent studies have found brain stem neurones containing immunoreactive enkephalin and projecting to the spinal cord (Hokfelt, Terenius, Kuypers & Dann, 1979; Bowker, Steinbusch & Coulter, 1981). It is likely, however, that this descending contribution is rather small compared with that of spinal neurones.

Investigations on whether enkephalin occurs in axon terminals and thus might be released as a transmitter have been of two types: subcellular fractionation studies of brain homogenates, and ultrastructural studies of immunoreactive enkephalin.

In subcellular fractionation studies, transmitters should be found mainly in fractions containing synaptosomes, which are essentially nerve endings containing synaptic vesicles. Of the subcellular fractions obtained by differential centrifugation of rat brain homogenates, enkephalin activity was most concentrated in the crude synaptosomal (P_2) fraction (Pasternak, Goodman & Snyder, 1975; Simantov, Snowman & Snyder, 1976; Osborne, Hollt & Herz, 1978a). This synaptosomal localization of activity is consistent with a transmitter function for enkephalin.

Using immunohistochemical techniques at the electron microscopic level, enkephalin has been localized in axon terminals. In laminae I and II of rat spinal cord, met-enkephalin-labelled terminals were found forming axo-dendritic and axo-somatic but not axo-axonic synapses (Hunt, Kelly & Emson, 1980). A similar result was obtained for immunoreactive leu-enkephalin terminals in the superficial dorsal horn of the monkey (Aronin et al., 1981). Ruda (1982) observed many enkephalin-containing axonal endings on the somata and proximal dendrites of cat dorsal horn neurones projecting to the thalamus. In addition, a recent study of the caudalis portion of rat spinal trigeminal complex noted met^5-enkephalin immunoreactive terminals which formed predominantly axo-dendritic

synapses (Sumal, Pickel, Miller & Reis, 1982). In these studies, enkephalin-containing terminals were found to contain many small clear vesicles and fewer large granular vesicles, some of which contained immunoreactive enkephalin (Hunt et al., 1980; Aronin et al., 1981). These ultrastructural descriptions suggest that enkephalins act postsynaptically at dendritic and somatic sites in the dorsal horn.

(b) Synthesis of Enkephalins

Compared to the abundance of literature on enkephalin localization, there is a paucity of information concerning the biosynthesis of these peptides. When administered intracisternally or infused into the lateral ventricle of rats, the labelled amino acids [^{3}H]-tyrosine and [^{3}H]-glycine are incorporated into both met- and leu-enkephalin (Sosa, McKnight, Hughes & Kosterlitz, 1977; Yang, Hong, Fratta & Costa, 1978; Yang & Costa, 1979). Sosa et al. (1977) also demonstrated in vitro incorporation of labelled amino acids by isolated myenteric plexus-longitudinal muscle preparations, with labelled leucine and methionine giving the appropriate enkephalin. [^{35}S]-L-Methionine is similarly incorporated into met-enkephalin by cultured spinal cord cells, a process powerfully inhibited by antibiotic inhibitors of cytoribosomal protein synthesis (Neale, McKelvy & Barker, 1980).

The amino acid sequence of met-enkephalin is contained within the 31-amino acid sequence of the opioid peptide β-endorphin, which in turn constitutes amino acids 61-91 of the pituitary polypeptide β-lipotropin. Since it is possible that

the enkephalins are derived from large peptide precursors, it was generally thought that met-enkephalin was the end-product of a β-lipotropin-β-endorphin-met-enkephalin biosynthetic pathway. This hypothesis became less tenable, however, when it was found that the distribution of immunoreactive enkephalin among different brain regions did not parallel that of the putative precursors (Rossier, Vargo, Minick, Ling, Bloom & Guillemin, 1977; Watson, Barchas & Li, 1977b; Watson, Akil, Richard & Barchas, 1978). It now appears that there are three different precursors for the opioid peptides: pre-pro-opiomelanocortin for the endorphins (and lipotropins) (Nakanishi, Inoue, Kita, Nakamura, Chang, Cohen & Numa, 1979), pre-pro-enkephalin A for the enkephalins (Noda, Furutani, Takahashi, Toyosato, Hirose, Inayama, Nakanishi & Numa, 1982; Gubler, Seeburg, Hoffman, Gage & Udenfriend, 1982; Comb, Seeburg, Adelman, Eiden & Herbert, 1982), and pre-pro-enkephalin B (or pro-dynorphin) for the dynorphins, neo-endorphins and possibly leu-enkephalin (Kakidani, Furutani, Takahashi, Noda, Morimoto, Hirose, Asai, Inayama, Nakanishi & Numa, 1982) (reviewed by Rossier, 1982; Hollt, 1983). Pro-enkephalin A was first identified in extracts of bovine adrenal medulla and was thought to be the sole precursor for both met- and leu-enkephalin (Lewis, Stern, Kimura, Rossier, Stein & Udenfriend, 1980). Since pro-enkephalin B contains three leu-enkephalin sequences, however, this may not be so.

With the putative amino acid transmitter GABA, the synthesizing enzyme glutamate decarboxylase has been localized by immunoreactive techniques in terminals in the upper dorsal

horn (McLaughlin, Barber, Saito, Roberts & Wu, 1975). To date there are no comparable data for the enkephalins: details of the enzymatic processing of polypeptide precursors which eventually produces the enkephalins in neurones remain to be determined.

(c) Enkephalin Release

Transmitters are probably released from synaptic vesicles by a calcium-dependent process of exocytosis. Detection of transmitter release from presynaptic nerve terminals under physiological conditions, however, is a difficult procedure, particularly in the central nervous system. To date, enkephalin release from the central nervous system has only been demonstrated following clearly unphysiological stimuli. One approach has been to perfuse brain slices with a solution containing a high (50 mM) concentration of potassium to induce release from nerve endings. Using preparations of the rat corpus striatum (which contains high levels of enkephalins), several groups have shown a calcium-dependent release of enkephalin-like material, measured by radioimmunoassay, with this technique (Osborne, Hollt & Herz, 1978b; Richter, Wesche & Frederickson, 1979; Lindberg & Dahl, 1981). Similar results have been obtained with slices of rat globus pallidus (Iversen, Iversen, Bloom, Vargo & Guillemin, 1978; Bayon, Rossier, Mauss, Bloom, Iversen, Ling & Guillemin, 1978; Lindberg & Dahl, 1981), a rabbit striatal synaptosomal preparation (Henderson, Hughes & Kosterlitz, 1978), and cultured spinal cord cells (Neale et al., 1980). The relationship between this ionic stimulus for

release and that produced by the invasion of nerve terminals by nerve impulses, however, is uncertain.

Elevated levels of enkephalins have been detected in cerebrospinal fluid (CSF) following various electrical stimulation procedures. Akil, Richardson, Hughes and Barchas (1978b) reported a significant increase in enkephalin-like material in human ventricular CSF following stimulation of periventricular brain sites sufficient to produce analgesia. Similarly, tooth pulp stimulation in cats induced a prolonged increase in met-enkephalin-like material measured by radioimmunoassay, in the cisternal CSF, suggesting release from enkephalinergic neurones following nociceptive input to the central nervous system (Cesselin, Oliveras, Bourgoin, Sierralta, Michelot, Besson & Hamon, 1982).

Enkephalin release has also been studied by Yaksh and Elde with superfused spinal cord in situ. Firstly, they showed that the spinal cord perfusate of anaesthetized rats contained detectable levels of immunoreactive methionine-enkephalin, which underwent a calcium-dependent 3-fold increase when 40 mM potassium was added to the perfusing solution (Yaksh & Elde, 1980). In a subsequent study with anaesthetized cats, basal release of met-enkephalin-like immunoreactivity into both spinal and cerebral aqueduct perfusates was measured during bilateral stimulation of the sciatic nerves (Yaksh & Elde, 1981). Basal release was not altered by stimulation at low intensities (exciting only A-fibres), but was increased 2 to 3 fold by stimulation adequate to excite C-fibres. Intra-arterial administration of bradykinin also enhanced release.

Such in vivo release experiments, although utilizing unphysiological stimuli, are probably closer to physiological conditions than experiments using brain slices immersed in high potassium-containing solutions.

(d) The Actions of Enkephalins on Central Neurones

The criterion of identity of action prescribes that administration of a putative neurotransmitter near synapses should produce the same effects as the natural transmitter. Many investigations have sought to determine the effects of enkephalins when administered near neurones by a variety of techniques. Experiments have been of two main types. Either the enkephalins have been ejected microelectrophoretically near central neurones, or they have been added to the perfusate of slice or culture preparations of central neurones. In microelectrophoretic studies in rats and cats, brain regions examined include the cerebral cortex (Zieglgansberger, Fry, Herz, Moroder & Wunsch, 1976), caudate/putamen, hippocampus, cerebellum (Nicoll, Siggins, Ling, Bloom & Guillemin, 1977), nucleus accumbens (McCarthy, Walker & Woodruff, 1977), thalamus (Hill, Pepper & Mitchell, 1976), mesencephalic reticular formation (Hosford & Haigler, 1980), periaqueductal gray (PAG) (Frederickson & Norris, 1976), locus coeruleus (Scott-Young, Bird & Kuhar, 1977), brain stem reticular formation (Bradley, Briggs, Gayton & Lambert, 1976; Gent & Wolstencroft, 1976), trigeminal nucleus (Andersen, Lund & Puil, 1978b) and the dorsal horn of the spinal cord (Duggan et al., 1977b; Randic & Miletic, 1978; Zieglgansberger & Tulloch, 1979; Satoh,

Kawajiri, Ukai & Yamamoto, 1979). Although excitation has been observed with neurones of the rat hippocampus (Nicoll et al., 1977), cortex and brainstem (Davies & Dray, 1978) and Renshaw cells in the spinal cord (Davies & Dray, 1976, 1978), the most consistent feature of these investigations has been a depression of cell firing. Most experiments monitored the spontaneous activity of neurones, but sometimes excitant amino acids were ejected to excite otherwise silent neurones or to enhance slow spontaneous discharge. In most cases both types of activity were inhibited by enkephalin. The excitatory effects observed with Renshaw cells and pyramidal hippocampal cells may be due to inhibition of inhibitory neurones (Zieglgansberger, French, Siggins & Bloom, 1978). This generally depressant effect of enkephalins on both spontaneous and excitant-induced firing when administered near the cell bodies of neurones is consonant with a role for these peptides as inhibitory transmitters released at axo-somatic synapses. Furthermore, it is likely that this effect has greater functional significance when observed in regions of dense enkephalinergic innervation (such as the trigeminal nucleus caudalis) than in regions of no such innervation (such as the dorsal column nuclei) (Sar et al., 1978).

The enkephalins were discovered in a deliberate search for an endogenous ligand for the opiate receptor (Hughes, 1975; Terenius & Wahlstrom, 1975). The enkephalins thus appeared to provide an explanation for the presence of stereospecific opiate receptors in the brain, and it was assumed by many investigators that their function was to control nociceptive

processes. Indeed, the immunohistochemical distribution of enkephalin parallels that reported for opiate receptors (Elde et al., 1976; Sar et al., 1978) and particular attention has been drawn to its localization in regions probably associated with pain and analgesia: the PAG, medullary raphé nuclei, spinal trigeminal nucleus and the dorsal horn of the spinal cord (Hokfelt et al., 1977b).

If enkephalins do have an opiate-like function in the control of nociceptive processes, then one might expect to observe effects consistent with this role when these peptides are administered near neurones participating in these processes. This hypothesis has been tested by electrophoretic administration near neurones excited by noxious peripheral stimuli.

(i) Enkephalins and nociceptive responses: supraspinal neurones.

When supraspinal neurones have been studied in this way, met-enkephalin has been found to inhibit this type of excitation of thalamic (Hill et al., 1976) and trigeminal neurones (Andersen et al., 1978b), and neurones of the mesencephalic reticular formation (Hosford & Haigler, 1980) and medullary ventral reticular nucleus (Hill, 1980) and gigantocellular reticular nucleus (NGC) (Lovick & Wolstencroft, 1980a). In some of these investigations, the effect of enkephalin on excitation by non-noxious peripheral stimuli has also been examined. Andersen et al. (1978b) observed a selective effect of enkephalin with feline trigeminal neurones responding either to noxious and non-noxious stimuli or to only one of these

inputs. Met-enkephalin administered near the bodies of these neurones inhibited the nociceptive responses, with little effect on non-nociceptive responses. Also in the cat, met-enkephalin selectively depressed the responses of NGC neurones to Aδ input (tooth pulp stimulation) without affecting those to large myelinated fibre (Aβ) input (radial nerve stimulation) (Lovick & Wolstencroft, 1980a).

In other electrophoretic studies where enkephalin administration near cell bodies reduced nociceptive responses, non-nociceptive responses were not examined (Hill et al., 1976; Hill, 1980; Hosford & Haigler, 1980).

(ii) Enkephalins and nociceptive responses: spinal neurones

Some of the more definitive investigations of the function of enkephalins in nociception have been performed in the dorsal horn of the spinal cord, a region intensely investigated by both anatomical and physiological techniques. Enkephalin-containing neural structures are particularly abundant in the marginal layer and the substantia gelatinosa (Hokfelt et al., 1977b; Glazer & Basbaum, 1981), where the primary afferent nerve fibres conveying nociceptive information from the periphery terminate (Szentagothai, 1964; Light & Perl, 1977; Kumazawa & Perl, 1978). Opiate receptor binding experiments with the spinal gray matter have found the highest concentration of binding sites in the superficial laminae (I-III) of the dorsal horn (Lamotte, Pert & Snyder, 1976). Autoradiographic studies following intravenous administration of [^{3}H]-diprenorphine have also revealed a high density of binding

sites in the substantia gelatinosa (Pert, Kuhar & Snyder, 1975; Atweh & Kuhar, 1977). Thus the enkephalins appear ideally situated to influence the transmission of nociceptive information in the spinal cord.

With its high densities both of opiate binding sites and of immunoreactive enkephalin, the upper dorsal horn would seem the logical site to test the effects of enkephalins on neurones. Unfortunately it is difficult to obtain recordings from the small neurones of this region with the relatively large multibarrel microelectrodes used for microelectrophoresis. It is comparatively easier to record from lamina IV and V neurones, many of which (by extracellular sampling techniques in the cat) are multireceptive (Handwerker et al., 1975; Duggan et al., 1977a) and are likely to be involved in the spinal transmission of nociceptive information.

In discussing the effects of enkephalin in the dorsal horn, it is necessary to include some results obtained with morphine. When recording from multireceptive lamina IV and V neurones, activation of opiate receptors in the superficial dorsal horn inhibits their nociceptive responses. When morphine was administered electrophoretically into the substantia gelatinosa, the nociceptive responses of these neurones, situated up to 680 µm ventrally, were selectively reduced, but responses to non-noxious skin stimuli were little affected (Duggan et al., 1977a). Administered similarly under the same conditions, the effects of M-ENKA were very similar, although spontaneous firing was also often reduced and the duration of action was much shorter than that of morphine

(Duggan et al., 1977b). In these experiments, the inhibitory amino acids glycine and GABA, administered near the cell bodies, usually reduced spontaneous firing and that evoked by both noxious and non-noxious stimuli. Thus the selective reduction of nociceptive responses of these neurones by enkephalin administered in the substantia gelatinosa is unlikely to be due to the depression of spontaneous firing commonly observed with this peptide. Such results suggest an action of enkephalin on a structure integral to the nociceptive pathway to deeper neurones and located in the region of the substantia gelatinosa. These observations may provide an explanation for the analgesia produced in unanaesthetized animals by the administration of enkephalins directly into the spinal subarachnoid space (Yaksh, Huang & Rudy, 1977; Yaksh, Frederickson, Huang & Rudy, 1978).

When enkephalin has been administered electrophoretically near the cell bodies of dorsal horn neurones, however, its selectivity for nociceptive responses is more equivocal. When feline dorsal horn neurones responding exclusively to noxious stimuli were examined, met-enkephalin administered near cell bodies reduced nociceptive responses and spontaneous firing, but had little effect on the responses of cells activated only by non-noxious stimuli (Randic & Miletic, 1978). In the rabbit also, the nociceptive responses of multireceptive lamina V neurones were selectively inhibited by met- or leu-enkephalin administered near cell bodies, without alteration of non-noxious tactile responses (Satoh et al., 1979). In contrast, Duggan et al. (1977b) observed a non-selective inhibition of

both nociceptive and non-nociceptive responses of multireceptive dorsal horn neurones of the cat following administration of M-ENKA near cell bodies, similar to the effect of glycine and GABA. Such a reduction of both sensory modalities is difficult to interpret functionally, but does not appear appropriate for the regulation of nociceptive information alone.

The non-selective effect of enkephalin administered near the bodies of dorsal horn neurones (Duggan et al., 1977b) was not mimicked by morphine. When this opiate was similarly administered near the cell bodies of these neurones, it usually produced excitation and abnormalities in action potential configuration (Duggan et al., 1977a). When administered intravenously in analgesic doses, morphine selectively reduced the excitation of dorsal horn neurones by noxious skin stimuli, and by impulses evoked electrically in unmyelinated and small myelinated cutaneous afferent fibres, with little effect on excitation by impulses in large diameter fibres (Le Bars, Menetrey, Conseiller & Besson, 1975; Le Bars, Guilbaud, Jurna & Besson, 1976a). This resembles its effect when electrophoresed into the substantia gelatinosa (Duggan et al., 1977a). These comparisons lead to two conclusions. Firstly, in morphine analgesia the relevant receptors do not appear to be located on the cell bodies of dorsal horn neurones, but superficially in the substantia gelatinosa. Secondly, the cell body receptors appear to be activated by enkephalin, but not morphine. These results suggest that enkephalin may have other functions in the spinal cord besides regulation of nociceptive processes.

Investigations have been directed towards explaining how

these selective and non-selective effects of enkephalin occur. A selective reduction of nociceptive responses of dorsal horn neurones following administration of enkephalin in the substantia gelatinosa could be explained by an action on receptors located near the terminals of unmyelinated primary afferents. Enkephalin binding sites in dorsal roots and dorsal horn are significantly reduced following sciatic nerve section, suggesting a location on primary afferent fibres (Fields, Emson, Leigh, Gilbert & Iversen, 1980). Such receptors may be activated by endogenous enkephalin released from neurones of the substantia gelatinosa to presynaptically inhibit release of transmitter from nociceptive primary afferent fibres.

The anatomical substrates for such presynaptic inhibition are axo-axonic synapses, and enkephalin receptors near primary afferent terminals could be associated with these structures. There is some evidence that substance P is the transmitter released by nociceptive primary afferents (Nicoll, Schenker & Leeman, 1980), and the naloxone-reversible reduction of its potassium-evoked release from rat trigeminal slices by $[D\text{-ala}]^2$-Met-enkephalinamide and opiates was proposed to occur at such synapses on primary afferent terminals (Jessell & Iversen, 1977). A presynaptic action of met-enkephalin on nerve terminals was proposed to explain similar naloxone-reversible inhibition of the evoked release, from rat brain slices, of radiolabelled noradrenaline (Taube, Borowski, Endo & Starke, 1976), dopamine and acetylcholine (Subramanian, Mitznegg, Sprugel, Domschke, Domschke, Wunsch & Demling, 1977).

Evidence for a presynaptic action of enkephalin has also been obtained from in vitro studies using cultures of mouse spinal cord explants with attached dorsal root ganglia (DRG). In this preparation, focal DRG stimulation elicited a slow-wave negative potential in the dorsal cord, and this discharge was depressed, in a naloxone-preventable manner, by low concentrations of enkephalins in the bathing medium (Crain, Crain, Peterson & Simon, 1978).

Presynaptic inhibition of transmitter release from primary afferent fibres could result from a reduced probability of primary afferent terminal branches being invaded by afferent impulses, or by an alteration of the terminal membrane potential or conductance, factors which influence the amount of transmitter released by each incoming impulse. In the spinal cord, presynaptic inhibition of excitation by myelinated primary afferents is considered to result from depolarization of the terminals of these fibres (primary afferent depolarization, PAD), with less transmitter release per impulse (Eccles, Eccles & Magni, 1961; see Schmidt, 1971). The presence and mechanism of presynaptic inhibition is more equivocal for C fibres, where terminal hyperpolarization may be the relevant underlying event (see Hentall & Fields, 1979; Carstens, Klumpp, Randic & Zimmermann, 1981c; Calvillo, Madrid & Rudomin, 1982). Alterations in terminal polarization can be detected by changes in the threshold of fibre terminals to electrical stimulation with an intraspinal electrode. Using this technique in the cat, Sastry (1978, 1979) found an elevated threshold for both C and Aδ sural fibres following the

administration of met-enkephalin electrophoretically in the substantia gelatinosa near the intraspinal stimulation sites. This effect was antagonized by electrophoretic or intravenous naloxone. An increased threshold is consistent with a hyperpolarization of nociceptive afferent terminals by enkephalin, but as the mechanism of presynaptic inhibition of excitation by these afferents is not definitively known, the relationship between this effect and a selective reduction of nociceptive responses of dorsal horn neurones is difficult to determine. On the other hand, reduced terminal excitability is equally consistent with enkephalin acting to block the propagation of incoming impulses into the terminals (Duggan, Griersmith, Headley & Hall, 1979).

The concept of enkephalin acting as a transmitter at axo-axonic synapses in the dorsal horn, however, receives little or no support from the anatomical studies, since the incidence of such synapses formed by terminals containing immunoreactive enkephalin is relatively low. Ultrastructural studies have found the majority of these terminals forming axo-somatic and axo-dendritic connections, suggesting that enkephalins may act as postsynaptic inhibitory transmitters.

Activation of enkephalin receptors located at inhibitory synapses on the cell bodies of laminae IV and V neurones would probably reduce the responses of these cells to all excitatory inputs, whether dendritic or somatic, and this is consistent with the lack of selectivity produced by enkephalin administration at this site with both spinal (Duggan et al., 1977b; Zieglgansberger & Tulloch, 1979) and supraspinal (Andersen et

al., 1978b; Lovick & Wolstencroft, 1980a) neurones. When administered near the cell bodies of rat thalamic neurones (Hill et al., 1976) and cat dorsal horn neurones (Duggan et al., 1977b), the effects of met-enkephalin were similar to those of GABA. Since GABA is known to hyperpolarize spinal interneurones (Bruggencate & Engberg, 1968) and motoneurones (Curtis, Hosli, Johnston & Johnston, 1968), the cell body effects of M-ENKA may be due to hyperpolarization of the soma. Such an action of enkephalin has been noted for neurones in in vitro slice preparations of the guinea-pig locus coeruleus (Pepper & Henderson, 1980) and spinal cord dorsal horn of the rat (Murase, Nedeljkov & Randic, 1982) when enkephalin was added to the perfusing medium. With cat dorsal horn neurones and motoneurones in vivo, however, enkephalin did not alter membrane potential or conductance but reduced excitation by glutamate (Zieglgansberger & Fry, 1976; Zieglgansberger & Tulloch, 1979). In these experiments, enkephalin was ejected electrophoretically 100-180 µm from the presumed location of the cell body, a distance possibly too great to produce sufficient enkephalin at the soma to induce a detectable hyperpolarization at this site. This factor could have been allowed for had these authors employed glycine or GABA as a control. Nevertheless a similar result was obtained with cultured mouse spinal neurones (Barker et al., 1978), prompting both groups to propose a modulator role for enkephalin, where cell excitability is not directly affected but responses to transmitters are altered.

Thus, insofar as the criterion of identity of action is

concerned, there are many observations of the effects of enkephalins on neurones, but what is lacking are direct comparisons with synaptic events in the central nervous system. This task is made difficult by the fact that the physiological processes mediated by these peptides are not known. Although some evidence suggests a role in controlling nociceptive processes, enkephalin also has actions appropriate to an inhibitory transmitter released at axosomatic synapses. Collectively, the available data suggest a more extensive physiological function than merely regulation of nociception.

(e) Pharmacological Antagonism of Enkephalins

The final criterion to be considered is pharmacological antagonism: the postsynaptic effects of a proposed transmitter and the natural transmitter should be affected similarly by antagonist drugs. In studies of pharmacological antagonism, it is desirable to use a "pure" antagonist lacking any agonist activity at the appropriate receptors. By this contingency (Blumberg & Dayton, 1972), naloxone is considered the most suitable antagonist for investigating the actions of opioid peptides and their possible involvement in physiological processes.

(i) The adequacy of naloxone as an opioid antagonist

There is abundant information on the antagonism of opioid peptides by naloxone (reviewed by Sawynok, Pinsky & La Bella, 1979; Hill, 1981; Duggan & Johnson, 1983). In particular, naloxone has proved to be an adequate antagonist of the effects of exogenously-administered opioids on central neurones. Thus

the depressant effect of enkephalin on spontaneous neuronal activity has been reversed or blocked by prior or concurrent electrophoretic or systemic administration of naloxone (Bradley et al., 1976; Frederickson & Norris, 1976; Zieglgansberger et al., 1976; Duggan et al., 1977b; McCarthy et al., 1977; Nicoll et al., 1977; Scott-Young et al., 1977; Andersen et al., 1978b; Randic & Miletic, 1978; Zieglansberger & Tulloch, 1979; Hosford & Haigler, 1980). The excitatory actions of enkephalin observed with some neurones are also blocked by naloxone (Davies & Dray, 1976, 1978; Nicoll et al., 1977). Similarly, the reduction of evoked nociceptive responses by enkephalin is blocked by this antagonist (Duggan et al., 1977b; Randic & Miletic, 1978; Andersen et al., 1978b; Satoh et al., 1979; Hosford & Haigler, 1980; Hill, 1980). Some groups, however, have observed naloxone-resistant effects of opioids. The inhibitory action of electrophoretic enkephalin on cat brain stem neurones was not affected by similarly-administered naloxone (Gent & Wolstencroft, 1976), nor was a similar action on rat thalamic neurones affected by intravenous naloxone (Hill et al., 1976). With cat dorsal horn neurones, the reduction of spontaneous and evoked firing by enkephalin administered near cell bodies was antagonized by naloxone administered at the same site, but not intravenously (Duggan et al., 1977b). (The selective reduction in nociceptive responses of these neurones observed following enkephalin administration in the substantia gelatinosa, however, was reversed when naloxone was ejected in this region or administered intravenously (0.1 mg/kg)). Possible explanations for the ineffectiveness of naloxone under

some experimental conditions are that inadequate antagonist doses may have been used, or that the opioid effects may have been mediated by "non-opioid" receptors (Duggan & Johnson, 1983). The naloxone-resistant effects observed by Gent and Wolstencroft (1976) and Hill et al. (1976) were probably via such receptors, while in the study of Duggan et al. (1977b) an adequate concentration of naloxone at cell body receptors was apparently attained following electrophoretic, but not intravenous, administration.

(ii) The specificity of naloxone as an opioid antagonist

Many investigations have sought to implicate the participation of enkephalins in a physiological event by modification of that event with naloxone. A large proportion of these experiments have involved an alteration in behaviour of the whole animal and these will not be discussed. It is nevertheless worthy of comment that naloxone-induced enhancement of experimental and clinical pain has been reported, suggesting inhibition of nociception by a tonic release of opioid peptides (Grevert & Goldstein, 1977; Walker, Berntson, Sandman, Coy, Schally & Kastin, 1977;; Frederickson, Burgis & Edwards, 1977; Buchsbaum, Davis & Bunney, 1977; Levine, Gordon, Jones & Fields, 1978). This is not a consistent finding, however, since several studies have failed to observe hyperalgesia following naloxone administration (El-Sobky, Dostrovsky & Wall, 1976; Grevert & Goldstein, 1978; Lindblom & Tegner, 1979).

Since the interpretation of the effects of naloxone relies on its specificity for opioid peptides, the evidence for its interaction with other putative neurotransmitter systems should

be considered. There have been a number of behavioural experiments where the effects of naloxone have been attributed to interaction with dopaminergic systems (Sawynok et al., 1979). In these studies, however, it is still possible that the observed effects of naloxone were due to antagonism of endogenous opioids rather than direct interaction with non-opioid dopaminergic pathways. Considering single neurone studies, the depressant effects of electrophoretically-administered noradrenaline and 5-hydroxytryptamine (5-HT) on cat dorsal horn neurones were not reduced by naloxone administered electrophoretically or intravenously (0.3 to 3.6 mg/kg) (Headley, Duggan & Griersmith, 1978). With Renshaw cells of both cat (Duggan, Davies & Hall, 1976) and rat (Davies, 1976), however, naloxone reduced the excitatory effects not only of morphine but also of acetylcholine, although responses to excitatory amino acids were not blocked by the antagonist.

Of the reported effects of naloxone not related to opioid peptides, that which has received the most attention is antagonism of the action of GABA. Convulsions in rats have been reported following intracerebroventricular infusion of naloxone (100 µg) (Pinsky, Labella, Havlicek & Dua, 1978), and two groups found a silmilar effect in mice following intraperitoneal administration, but very high doses were required: 190 mg/kg (ED_{50}) (Dingledine, Iversen & Breuker, 1978) and 240 mg/kg (Gumulka, Dinnendahl & Schonhofer, 1979). At lower doses (90 mg/kg), naloxone potentiated the convulsant activity of the GABA antagonist bicuculline but not of the glycine antagonist strychnine (Dingledine et al., 1978).

Pretreatment with diazepam (5 mg/kg), which is considered to interact with GABA systems, protected mice against convulsions produced by bicuculline and naloxone, but not by strychnine. In binding experiments with homogenates of human and rat cerebellum and rat forebrain, naloxone was found to interfere with GABA binding to synaptic membrane fractions. For human cerebellum, however, the IC_{50} value for this effect (308 µm) was 40 times that of bicuculline, and similar activity was shown by morphine, levorphanol and its non-opiate isomer dextrorphan (Dingledine et al., 1978).

Since the interaction of ligands with opiate receptors is a stereospecific process, stereoisomers of opioid antagonists can provide evidence of opioid peptide involvement in physiological events since only the (-)-isomers have antagonist activity. (+)-Naloxone antagonized the normorphine-induced inhibition of evoked contractions of the guinea-pig ileum, and the morphine-induced activity of an adenylate cyclase assay, only at concentrations 1,000 to 5,000 times those effective for (-)-naloxone (Iijima, Minamikawa, Jacobson, Brossi & Rice, 1978). The (+)-isomer was also 10,000 times less potent than (-)-naloxone in opiate receptor binding assays of rat brain (Iijima et al., 1978), and unlike (-)-naloxone, did not antagonize the effects of morphine on myenteric neurones (Karras & North, 1981). The ability of naloxone to modify physiological events purported to involve endogenous opioids has also been confirmed with stereoisomers of antagonists. In the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum, naloxone increased the electrically-evoked

release of acetylcholine (Waterfield & Kosterlitz, 1975) and blocked the inhibition of evoked contractions produced by high frequency stimulation (Puig, Gascon, Craviso & Musacchio, 1977), and both effects were mimicked by the (-) but not the (+)-isomer of a benzomorphan antagonist, GPA 1843. Similarly, only the (-)-isomers of opiate antagonists enhanced the nociceptive responses of mice in the hot plate test (Jacob & Ramabadran, 1978) and altered the firing of rat dorsal horn neurones (Fitzgerald & Woolf, 1980). Insofar as an event is definitively produced by opioid peptides, the observation that the enkephalin-induced depression of the spontaneous activity of rat medullary neurones was consistently blocked by (-) but not (+)-naloxone is probably of greater significance (Gayton, Lambert & Bradley, 1978).

In electrophoretic experiments, (-)-naloxone reversibly antagonized the GABA-evoked depression of firing of olfactory tubercle neurones in the rat (Dingledine et al., 1978). A similar effect was observed during intracellular recordings from cultured mouse spinal neurones, where the responses elicited by electrophoretically administered leu-enkephalin and GABA were both antagonized by co-electrophoresis of (-)-naloxone (Gruol, Barker, Huang & Smith, 1979). With these cultured neurones, although the inhibitory response to GABA was more sensitive to naloxone antagonism than the excitatory and inhibitory responses to leu-enkephalin when compared on the same neurone, this group subsequently showed that (+)-naloxone equally depressed the GABA response (Gruol, Barker & Smith, 1980). In electrophoretic experiments, concentrations are

unknown but when applied to the cultured neurones by superfusion, high concentrations (0.1 to 1 mM) of naloxone were necessary to obtain the same result (Gruol et al., 1980).

The above observations have important implications for the proposed use of opioid antagonists in modifying physiological events. Firstly, when the antagonists are administered intravenously, the possibility of effects unrelated to opioid peptides is minimized by the use of low doses. With inhibition of GABA binding, the potency of naloxone was only 1/40 that of bicuculline (Dingledine et al., 1978), and the intravenous dose of bicuculline reducing GABA-mediated inhibition in the spinal cord and cerebellum of the cat was 0.2 to 0.4 mg/kg (Curtis, Duggan, Felix & Johnston, 1970). Thus it is extremely unlikely that in the cat, intravenous doses of naloxone in the order of 0.1 mg/kg, which were adequate to antagonize the effects on dorsal horn neurones of enkephalin administered in the substantia gelatinosa (Duggan et al., 1977b), would interfere with GABA transmission. Secondly, stereoisomers of antagonists should be employed, especially in electrophoretic experiments where concentrations are not known.

There is much evidence to suggest that the various opioid peptides and opiates act through different subclasses of opiate receptor, and this will be subsequently discussed at length. This complicates any consideration of the use of naloxone in revealing enkephalin-mediated events since an action of other opioid peptides such as ß-endorphin and dynorphin may be blocked simultaneously. Thus it is obviously ideal to use opioid antagonists which are selective for specific opiate

receptor subtypes (for example, the purported δ-antagonist of Shaw, Miller, Turnbull, Gormley and Morley, 1982), but to date such compounds have not been tested against opioid peptide-mediated events in the central nervous system.

(iii) Naloxone and spinal inhibition

Since the effects of enkephalin are most consistent with those of a central inhibitory transmitter, neurophysiological studies have sought to define naloxone-sensitive inhibition, especially in the spinal cord. Evidence for the presence of tonic inhibition involving opioid peptides and influencing the firing of spinal neurones is equivocal. In the spinal cat, intravenous naloxone has been reported to increase the spontaneous firing and the nociceptive responses of dorsal horn neurones responding to noxious cutaneous stimuli, but not the activity of cells responding to non-noxious stimuli (Henry, 1979). This effect was observed only when naloxone was administered during the daytime and early evening, but not during the early hours of the morning, prompting speculation that a circulating endogenous opioid, released in a diurnal cycle, produced inhibition in the dorsal horn (Henry, 1981). In studies with laminae IV and V neurones of the rat, spontaneous activity (Fitzgerald & Woolf, 1980) and responses to impulses in C fibres (Rivot, Chaouch & Besson, 1979; Fitzgerald & Woolf, 1980) were clearly increased by intravenous naloxone, but with substantia gelatinosa neurones, these activities were inhibited by naloxone (Fitzgerald & Woolf, 1980). Another form of naloxone-sensitive inhibition in the spinal cord of rats has been described as "diffuse noxious

inhibitory controls" (DNIC) by Besson's group. Noxious stimulation at widely dispersed sites on the body surface was shown to produce inhibition of multireceptive dorsal horn neurones (Le Bars, Dickenson & Besson, 1979a). This effect involved a supraspinal component (Le Bars, Dickenson & Besson, 1979b), was reduced by systemic naloxone (Le Bars, Chitour, Kraus, Dickenson & Besson, 1981), and was proposed to sharpen contrast in the ascending transmission of nociceptive information (Le Bars et al., 1979b). In contrast, several groups have failed to observe effects of naloxone in the dorsal horn. When multireceptive neurones of the cat dorsal horn were examined, systemic naloxone did not alter spontaneous activity nor nociceptive responses (Le Bars et al., 1975; Duggan et al., 1977c; Sinclair, Fox, Mokha & Iggo, 1980). With electrophoretic ejection of naloxone near the cell bodies of these neurones in amounts adequate to block inhibition by enkephalin, no significant effect was similarly observed (Duggan et al., 1977a; Belcher & Ryall, 1978). In the rat, the impulse transmission from afferent Aδ or C fibres to axons ascending in the spinal cord has also been reported to be unaffected by intravenous naloxone (Jurna, 1980). These discordant results do not provide clear evidence for inhibition involving opioid peptides in the dorsal horn.

There is also some controversy regarding the effects of naloxone on spinal reflexes. In these studies the activity of many motoneurones is usually observed by recording from their axons in ventral roots. Naloxone has been reported to increase ventral root spontaneous activity (Su, Gorodetzky &

Bell, 1980). In addition, there are several reports of increased spinal reflexes following naloxone administration, although different types of reflexes appear to be differentially affected. In the decerebrate spinal cat, naloxone (0.1-2.0 mg/kg) consistently increased monosynaptic reflexes of both extensor and flexor motoneurones and also polysynaptic reflexes to impulses in large myelinated cutaneous afferents (Goldfarb & Hu, 1976; Goldfarb, Kaplan & Jenkins, 1978). With this preparation, Bell and Martin (1977) found that reflexes to impulses evoked electrically in C fibres or to noxious radiant heat were also increased by naloxone (0.005-0.05 mg/kg), but Krivoy, Kroeger and Zimmermann (1973) failed to observe changes in either monosynaptic or polysynaptic reflexes to dorsal root stimulation following naloxone (1.0 mg/kg). In the unanaesthetized chronic spinal dog, a flexor reflex elicited by toe clamping was modestly increased by high (20 mg/kg) but not low (0.2 mg/kg) doses of naloxone (McClane & Martin, 1967a,b). In conscious man, however, intravenous naloxone (0.8 mg total dose) produced a modest (16%) increase in a monosynaptic extensor reflex (H reflex, recorded from the soleus muscle following stimulation of the tibial nerve transcutaneously) without affecting polysynaptic tactile and nociceptive flexor reflexes (Boureau, Willer & Dauthier, 1978). A further study with chronic spinal patients found that this monosynaptic reflex and the nociceptive flexion reflex were not altered by naloxone (0.01-0.03 mg/kg) (Willer & Bussel, 1980).

Thus there is clearly a lack of consistency in the attempts to define a naloxone-sensitive inhibition tonically

influencing spinal neurone activity. The effect of this antagonist on inhibitions of supraspinal origin will be discussed in Chapter IV.

The investigations described in the first section of this thesis are an endeavour to elucidate some aspects of the physiology of opioid peptides in the cat spinal cord. In the first part of this inquiry, multireceptive dorsal horn neurones of laminae III-IV, V and VI of spinalized cats were chosen for study. Previous investigations noted that although the actions of morphine and met-enkephalin on these neurones were similar when administered electrophoretically in the substantia gelatinosa, marked differences were observed when administered near the cell bodies. The cell body receptors appeared to selectively recognize enkephalin but not morphine, suggesting a wider role for spinal enkephalin than merely regulation in nociception. Differences between the actions of morphine and the enkephalins have also been noted when these compounds have been administered near other central neurones. The electrophoretic application of enkephalins to neurones of the rat medulla reduced the spontaneous activity of a high percentage of cells (Bradley, Gayton & Lambert, 1978), but with morphine applied similarly, excitation was the most common effect (Bradley & Dray, 1974; Bramwell & Bradley, 1974). With neurones of the mesencephalic reticular formation in the rat, Hosford and Haigler (1980) found that the nociceptive responses of many cells were inhibited by electrophoretic administration of either met-enkephalin or morphine, although both compounds were effective with some neurones. Collectively these findings

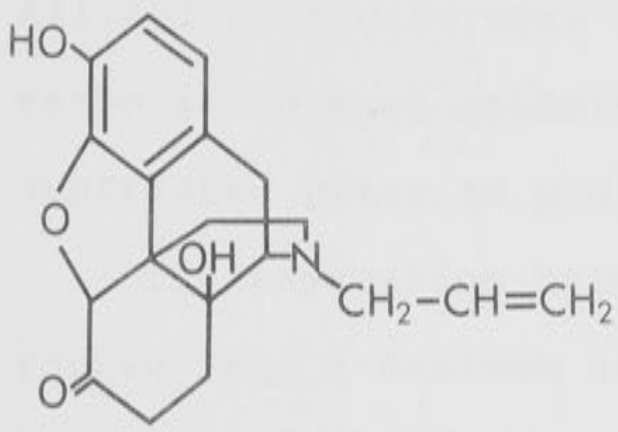
suggest a difference between receptors for opiates and enkephalins.

Since multiple forms of centrally-occurring opioid receptors have now been described, it was of interest to determine the extent to which the effects of morphine and enkephalin differed when administered at sites between the substantia gelatinosa and the more ventral cell body. Met-enkephalinamide rather than met-enkephalin was used in these experiments, as the amide has similar but more potent effects in a number of systems (see Duggan et al., 1977b).

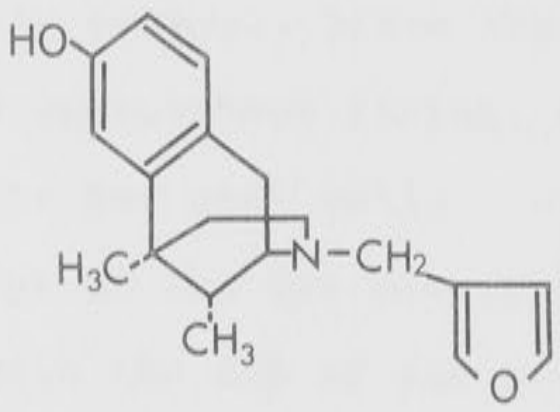
The second part of this study deals with spinal inhibition involving endogenous opioid peptides. In the cat, there is insufficient consistent evidence to implicate opioids in a tonic inhibitory process influencing the firing of dorsal horn neurones. The clear enhancement by naloxone of cat spinal reflexes reported by some groups, however, implies the existence of such a process somewhere in the spinal cord and ultimately affecting motoneurones. Because of the potential importance of this implication, it was considered necessary firstly, to confirm that systemic naloxone did alter a wide range of spinal reflexes, and secondly, to provide evidence for opioid peptide involvement by showing stereospecificity in antagonist action. Reflexes examined were monosynaptic reflexes to impulses in large diameter afferents in both flexor and extensor muscles, reflexes to stimulation of myelinated afferents of a cutaneous nerve and a mixed muscle-cutaneous nerve and to impulses in unmyelinated primary afferents of the mixed nerve. In some experiments the responses of a dorsal

horn neurone to impulses in unmyelinated primary afferents were observed concurrently with ventral root recorded reflexes to these impulses before and after naloxone administration. In addition, the finding of Catley and Pascoe (1978) that naloxone reduced the inhibition produced by conditioning stimulation to small diameter afferents of the reflex evoked in the medial gastrocnemius nerve by sural nerve stimulation was also investigated. Since the inactive (+)-isomer of naloxone is not readily available, stereoisomers of another opioid antagonist, N-(3-furylmethyl)-α-normetazocine (FMN) (Merz, Langbein, Stockhaus, Walther & Wick, 1973), were studied for effects on spinal reflexes. In a number of biological tests, this antagonist was found to have a similar profile of action to naloxone, although only about one-third as potent (Kuhn & Stockhaus, 1976). As with naloxone the (-)-isomer is an opioid antagonist but the (+) form is not. The chemical formulae of both naloxone and FMN are illustrated in Figure 3.

When it became clear that naloxone-sensitive inhibition of reflexes was tonically present under the experimental conditions used, investigations were then aimed at determining the mechanism of this inhibition. Reflexes record multiple motoneurone firing, so an initial step towards understanding the role of opioids in controlling this firing was to examine the properties and responses of single cells both before and after naloxone administration. This was done with intracellular recording.



NALOXONE



N-(3-FURYLMETHYL)- α -NORMETAZOCINE
(FMN)

Fig. 3. The structural formulae of naloxone and N-(3-furylmethyl)-α-normetazocine (FMN).

RESULTS

A. THE DISTRIBUTION OF OPIATE AND OPIOID RECEPTORS IN THE DORSAL HORN

Drugs were ejected electrophoretically at various sites in the dorsal horn while recording from multireceptive neurones responding to noxious heating of the skin and hair deflection. All cells studied were spontaneously active. Since the gated response to each stimulus included spontaneous firing, this was subtracted prior to plotting results for each cell.

The separation between the tips of the two micropipettes ranged from a maximum of 840 μm (with the tip of the seven-barrel pipette in the substantia gelatinosa) to a relatively close apposition when the tips of both pipettes were adjacent to the cell body. In some experiments the firing of the neurone could be recorded simultaneously from the five and the seven-barrel electrode when both were near the cell body.

(a) Effects of Met^5-enkephalinamide (M-ENKA) on Spontaneous Activity and Evoked Responses of Dorsal Horn Neurones

M-ENKA was ejected while studying fifteen neurones. Nine were located in Rexed lamina III-IV, five in lamina V, and one in lamina VI. To make quantitative comparisons between the effects observed at different sites, M-ENKA was administered with a fixed ejecting current for a fixed time (typically 100 nA for 4 to 6 minutes, corresponding to two or three cycles of noxious heat and hair deflection).

With thirteen of the fifteen neurones, M-ENKA reduced nociceptive responses and spontaneous firing not only when administered in the substantia gelatinosa but at all of the sites of administration closer to cell bodies. With these thirteen neurones, responses to hair deflection were either unaffected or only slightly reduced when compared to the reduction in nociceptive responses. Both nociceptive and non-nociceptive responses of two neurones were approximately equally reduced at all sites of administration.

With all fifteen neurones, following ejection in the substantia gelatinosa, there was no reduction in the effectiveness of a given dose of M-ENKA when ejected in the intervening region between the substantia gelatinosa and a site 100 µm dorsal to the calculated position of the cell body. With eight of these neurones, the effectiveness of M-ENKA was greater at the more ventral sites than in the substantia gelatinosa, with seven it was equi-effective at all sites. When ejected within 100 µm of the cell body with thirteen neurones, the effectiveness of M-ENKA then decreased with four, but with the remaining nine was either unchanged or increased.

Figures 4 and 6 illustrate this consistent effect of M-ENKA in reducing nociceptive responses over the whole distance between cell bodies and the substantia gelatinosa. In Figure 4 the effects of M-ENKA at 280 and 580 µm (substantia gelatinosa) dorsal to the cell body were similar in almost abolishing nociceptive responses. Adjacent to the cell body this action was increased as shown by prolonged depression beyond the period of ejection. The recording site near the cell body and

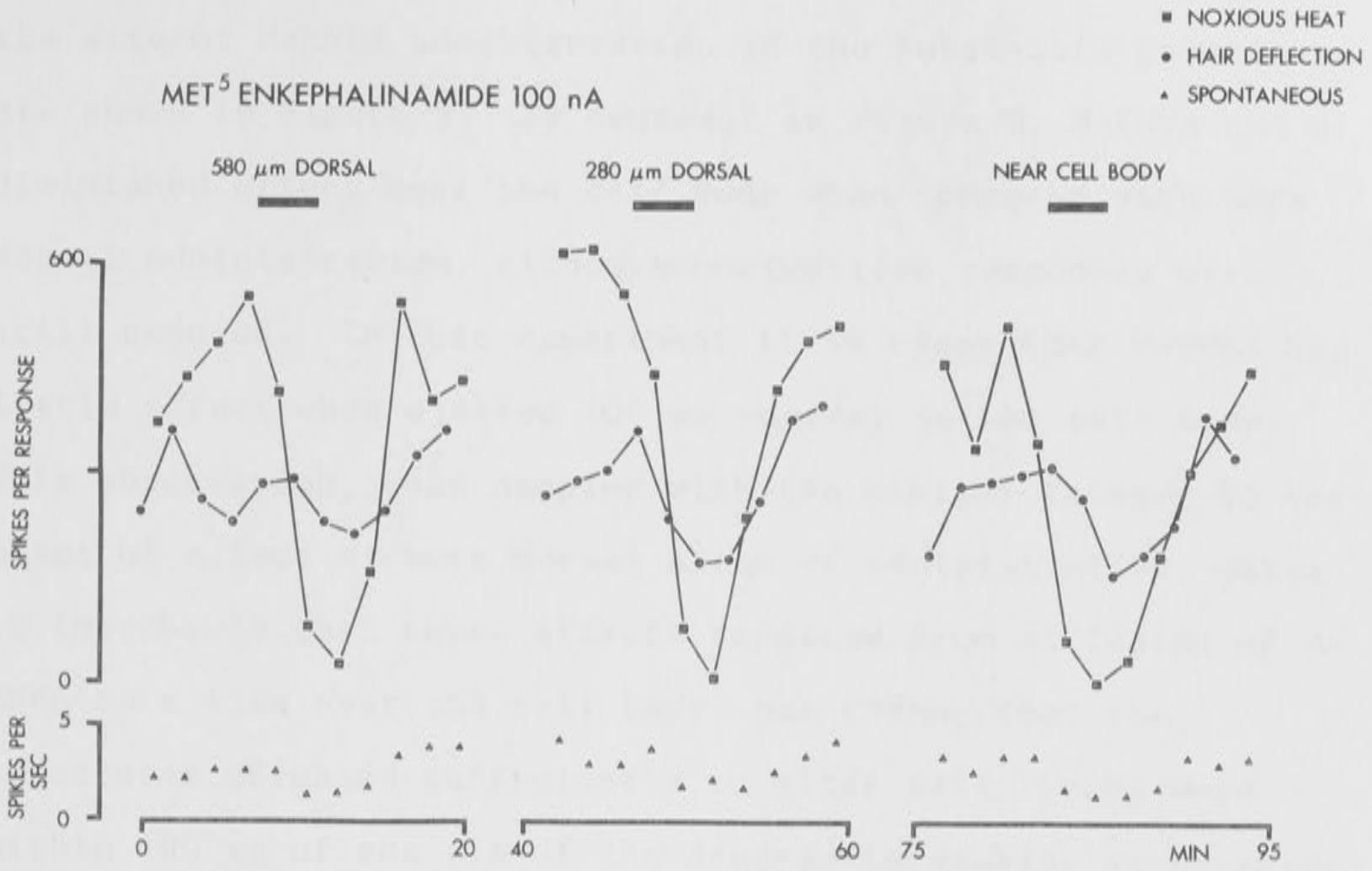


Fig. 4. The effect of Met^5-enkephalinamide administered near the body of a lamina IV dorsal horn neurone, in the substantia gelatinosa and at an intervening site. This multireceptive neurone was excited by alternately heating the skin of the fourth digital pad of the left hind limb to 55°C for 30 seconds (filled squares) and by continually deflecting adjacent hairs with an oscillating air jet for 12 seconds (filled circles). The upper graphs plot the number of action potentials evoked during each period of cutaneous stimulation, corrected for spontaneous firing, with respect to time. The lower graphs plot the spontaneous activity of the cell, measured between periods of cutaneous stimulation. Met^5-enkephalinamide was ejected electrophoretically (100 nA) from a seven barrel micropipette for the times indicated by the solid bars at three sites: in the substantia gelatinosa (580 μm dorsal to the cell body), at 280 μm dorsal, and adjacent to the cell body. Responses remained depressed for a longer period following ejection near the cell body. The recording site and the substantia gelatinosa site of administration are shown in Figure 5.

the site of M-ENKA administration in the substantia gelatinosa are shown in Figure 5. By contrast in Figure 6, M-ENKA had a diminished effect near the cell body when compared with more dorsal administration, although nociceptive responses were still reduced. In this experiment it is clear that M-ENKA had little effect when ejected 100 µm ventral to the cell body. This observation, when coupled with the similar latency to the onset of effect at more dorsal sites of administration, makes it improbable that these effects resulted from diffusion of M-ENKA to a site near the cell body, but rather that the structures affected sufficiently to alter cell firing were within 100 µm of the tip of the drug-administering seven-barrel micropipette.

M-ENKA was ejected near cell bodies from the five-barrel micropipette with only three neurones. In all cases lack of selectivity was observed: both nociceptive and non-nociceptive responses were reduced together with spontaneous firing.

The time course of the onset of reduction of nociceptive responses by M-ENKA was not determined accurately since the noxious heat stimulus (lasting 25 to 30 seconds) was applied at approximately two minute intervals. Nevertheless, a clear effect was often obvious within one cycle time (see Figures 4 and 6). The reduction of spontaneous firing had a similar time of onset with ejection of M-ENKA at all sites of administration. After cessation of M-ENKA ejection, nociceptive responses gradually returned to control levels over a period of 3 to 10 minutes.

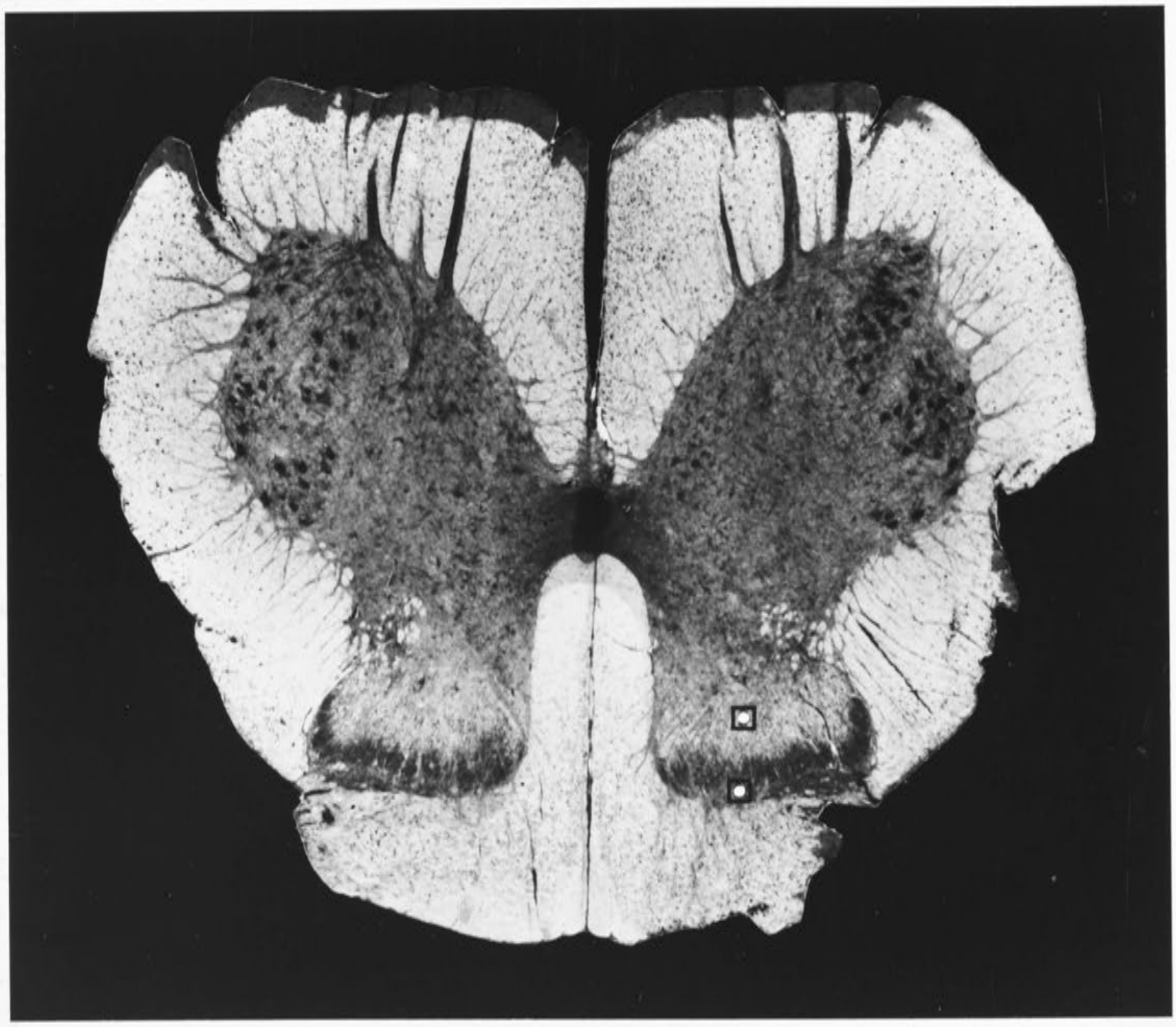


Fig. 5. Pontamine sky blue deposits (outlined with squares) marked the recording site near the lamina IV neurone and the site of electrophoretic ejection of M-ENKA in the substantia gelatinosa, 580 µm dorsal to the cell body, in this 50 µm frozen section. Results from this experiment are illustrated in Figure 4.

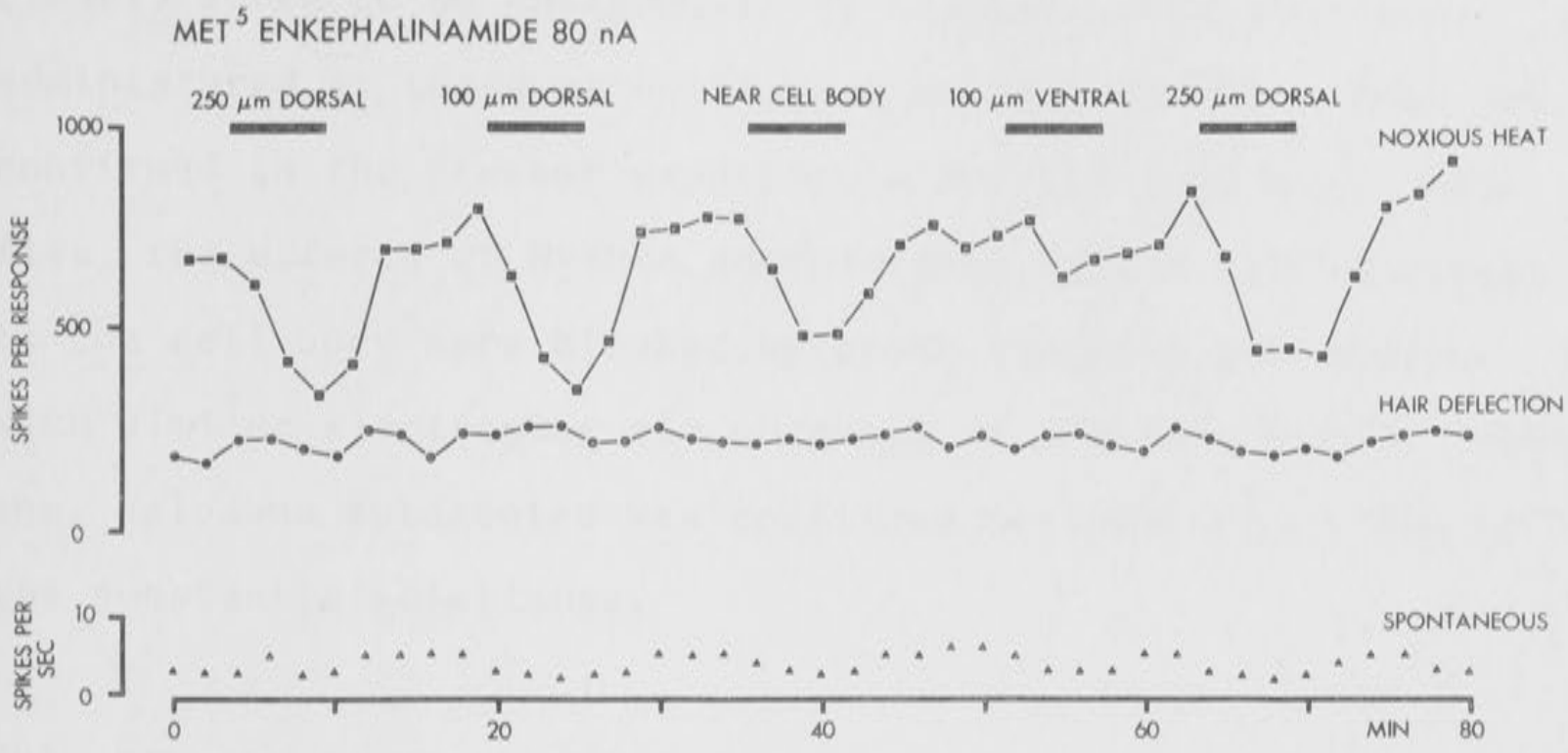


Fig. 6. Lack of effect of Met^5-enkephalinamide ejected ventral to the cell body of a dorsal horn neurone when compared with more dorsal sites of administration. This multireceptive lamina IV neurone was excited by alternately heating the skin of the third digital pad of the left hind limb to 55°C for 30 seconds (filled squares) and by continual deflection of adjacent hairs with an oscillating air jet for 12 seconds (filled circles). The upper graphs plot the number of action potentials evoked during each period of cutaneous stimulation, corrected for spontaneous firing, with respect to time. The lower graph plots the spontaneous activity of the neurone, measured between cutaneous stimuli. Met^5-enkephalinamide (80 nA) was ejected in the substantia gelatinosa (250 μm dorsal to the cell body), 100 μm dorsal to the body, adjacent to the cell body and 100 μm ventral to the cell body. Administration at the ventral site produced relatively little effect.

The effects of M-ENKA administered electrophoretically in the substantia gelatinosa and near cell bodies have been clearly shown to be antagonized by naloxone when similarly administered at these sites (Duggan et al., 1977b). This was confirmed in the present experiments for six neurones. With five, the effects of M-ENKA administered within 100 µm dorsal to the cell body were blocked by prior ejection of naloxone with similar electrophoretic currents at the same sites; with one, naloxone antagonism was confirmed for administration in the substantia gelatinosa.

(b) Effects of Morphine

Morphine was ejected electrophoretically in the dorsal horn while recording the spontaneous activity and the evoked responses of twelve neurones. Six were in Rexed lamina IV and six in lamina V. To compare effects at different sites, it was intended to administer morphine with a fixed ejecting current (80 to 100 nA) for a fixed time (6 to 10 minutes) at each site, but when near cell bodies, the onset of bursts of firing with abnormalities in action potentials sometimes prevented this. Of the twelve neurones examined, only in two was a depression of nociceptive responses produced by morphine administration near the cell body. These sites were 30 µm dorsal from one cell and 90 µm dorsal for the other. Both of these neurones were excited during morphine administration but this effect recovered quickly with the termination of ejection and was followed by a naloxone-reversible reduction in nociceptive responses.

With seven neurones morphine either had no effect or produced firing in the form of bursts of action potentials when administered at any site ventral to the substantia gelatinosa and only reduced nociceptive responses when administered in the latter region. In the substantia gelatinosa, morphine reduced nociceptive responses with minimal effect on spontaneous firing and excitation by deflection of hairs. In no instance was this selective action accompanied by the bursts of firing or abnormalities in spike configuration produced by administration near cell bodies. With three neurones morphine produced bursts of firing with increased cell responses when administered within 100 µm of the cell body but failed to modify cell responses at any of the more dorsal sites of administration.

The selective reduction of nociceptive responses by morphine ejected in the substantia gelatinosa has been shown to be reversed by naloxone administered similarly (Duggan et al., 1977a). In the present experiments, this reversal of the effects of morphine was observed for the seven neurones with electrophoresis of naloxone in the subtantia gelatinosa with an equal ejecting current to that of morphine.

An example of this site-selective action of morphine is illustrated in Figure 7. With this neurone, the ejection of morphine at sites 50 µm and 250 µm dorsal to the cell body produced bursts of firing and nociceptive responses were increased. This was particularly the case at the site closest to the cell body, where this irregular firing pattern also contributed to an apparently increased level of spontaneous

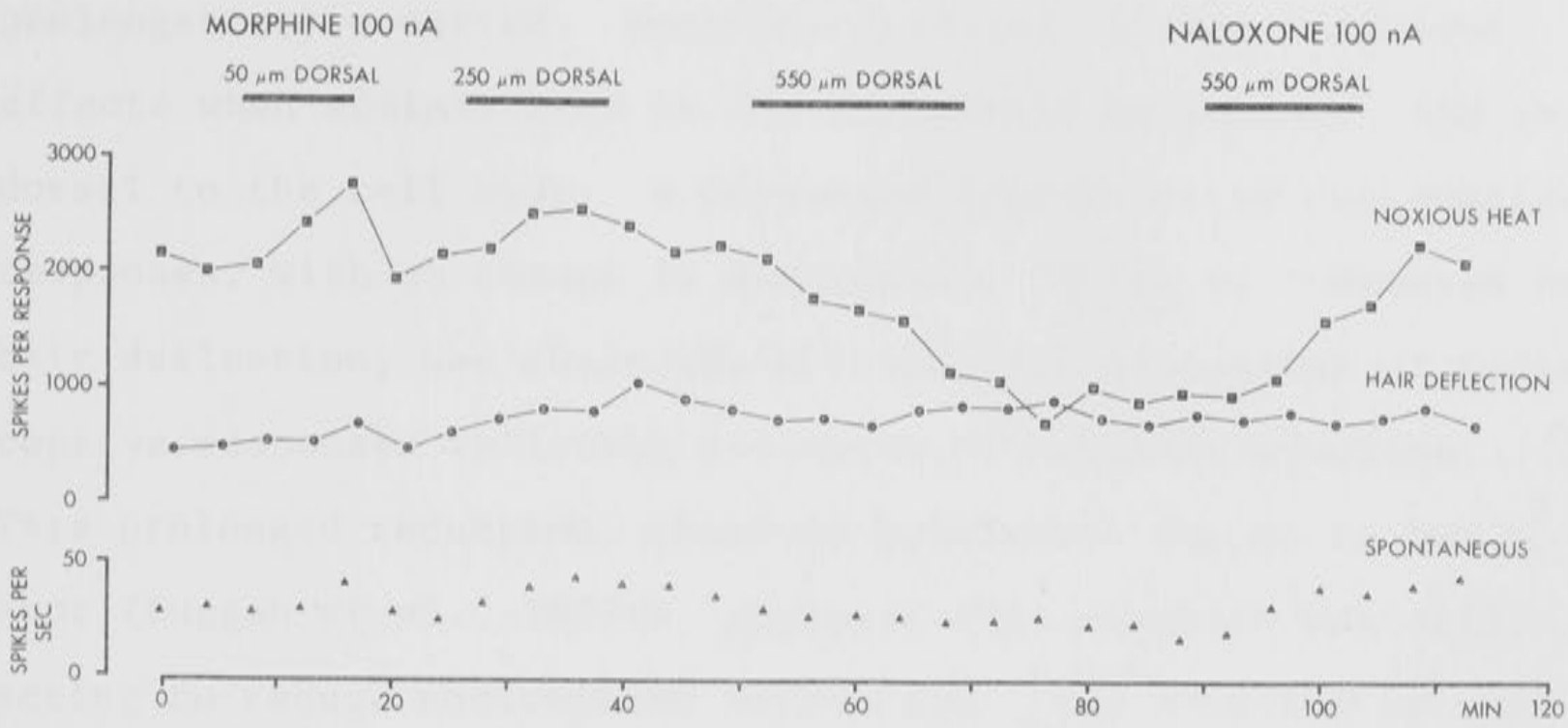


Fig. 7. Selective depression of nociceptive responses of a dorsal horn neurone by morphine ejected electrophoretically in the substantia gelatinosa but not at more ventral sites. This multireceptive lamina V neurone was excited alternately by heating the skin of the fourth digital pad of the left hind limb to 54°C for 30 seconds (filled squares) and by continual deflection of adjacent hairs with an oscillating air jet for 12 seconds (filled circles). The upper graphs plot the number of action potentials evoked during each period of cutaneous stimulation, corrected for spontaneous activity, against time. The lower graph shows the spontaneous firing of the cell, measured between cutaneous stimuli. Morphine (100 nA) was ejected 50 μm, 250 μm and 550 μm (substantia gelatinosa) dorsal to the cell body for the times indicated by the solid bars. Naloxone (100 nA) was also administered in the substantia gelatinosa.

activity. At both sites drug ejection was ceased when abnormalities in action potential configuration (such as spike prolongation) occurred. Morphine produced totally different effects when administered in the substantia gelatinosa, 550 µm dorsal to the cell body. A prolonged depression of nociceptive responses, with no change in spontaneous firing or responses to hair deflection, was observed, with further depression of nociceptive responses following cessation of morphine ejection. This prolonged reduction, observed previously for up to one hour (Duggan et al., 1977a), suggests that morphine was still acting to reduce nociceptive activation. The duration of this action was not determined, but instead naloxone was administered at the same site 20 minutes following cessation of morphine ejection. This returned nociceptive responses to control levels.

When ejected from the five-barrel micropipette, the effects of morphine were similar to those observed with administration within 100 µm of the cell body from the seven-barrel pipette, namely bursts of firing with a small increase in spontaneous firing.

(c) Effects of Amino Acids

Previous experiments had compared the effects of excitant and depressant amino acids with those of M-ENKA and morphine when ejected near cell bodies and in the substantia gelatinosa (Duggan et al., 1977a,b). In the present experiments therefore, GABA and DLH were ejected at these sites of administration but also in the intervening regions, to determine

whether the observed effects of M-ENKA and morphine at these sites were simply due to excitation or depression of neuronal firing.

Although nociceptive responses were clearly depressed by M-ENKA over a remarkable distance dorsal to the cell body, this constancy of effect was not observed with the amino acids. The inhibitory amino acid GABA non-selectively reduced all three types of cell firing, but this effect was mostly confined to sites of administration near cell bodies. Thus with seven of nine neurones tested, excitation by both noxious and non-noxious stimuli together with spontaneous firing was reduced by GABA (80 to 100 nA) ejected near cell bodies but not at dorsal sites of administration. With two neurones, ejection dorsal to cell bodies (210 µm for one and 240 µm for the other) depressed nociceptive responses.

The excitant amino acid DLH was studied with seven neurones. Although all were excited by administration near cell bodies, ejection at superficial sites produced inconsistent effects. Thus two neurones were unaffected by more dorsal administration, two were excited by administration at 150 µm dorsal but not more distally, and one was excited and two inhibited by DLH ejection in the substantia gelatinosa.

Thus the effects of the amino acids administered in the dorsal horn differed from those seen with M-ENKA or morphine at the same sites.

B. OPIOID INHIBITION AND THE VENTRAL HORN. I. OPIOID ANTAGONISTS AND SPINAL REFLEXES

(a) Naloxone and Reflexes Recorded in Ventral Roots

Reflex latency was measured as the time from the start of the stimulus artefact to the commencement of motoneurone firing, determined as the initial deflection recorded in the ventral root. When two closely timed nerve stimuli were used to elicit a single reflex, latency was measured from the second stimulus.

Intravenous naloxone increased all of the ventral root recorded reflexes in these experiments (see Figure 8). The percentage values quoted for reflex increases are based on increases produced by a naloxone dose of 0.05 mg/kg, although in some experiments the effects of other doses were also determined. Thus in two experiments an initial dose of 0.025 mg/kg was given but in only one was a clear effect observed. In five experiments two doses were given within 20 minutes of each other (in four the doses were 0.05 and 0.05 mg/kg, in one the doses were 0.05 and 0.025 mg/kg), with the second dose increasing reflex amplitudes still further in all five. In a few experiments higher doses were given subsequently (see Figure 8).

No systematic study was performed of dose-response relationships since it was considered more important to allow recovery from the effects of these low doses of naloxone so that the observations could be repeated in the same animal. Experiments on peripherally recorded reflexes (Section d)

showed that this recovery occurred in 2 to 2.5 hours. When recording from ventral roots, however, it is difficult to maintain constant recording conditions for that length of time due to the need to remove cerebrospinal fluid which accumulates adjacent to the roots. For this reason, attempts were made to repeat the effects of naloxone in the same animal allowing approximately 2 to 3 hours between doses. In the experiment illustrated in Figure 8, two hours elapsed between doses of naloxone and different stimulus parameters were used for the two control reflexes. Small increases were produced by a second dose of naloxone (0.05 mg/kg) (not illustrated), and the records shown were obtained following a larger dose (0.5 mg/kg).

(i) Monosynaptic reflex to stimulation of the BST nerve

The latencies of these reflexes were between 3.0 to 3.4 msec. Motoneurone firing was complete within 1.2 msec and the difference between the arrival of afferent volleys recorded approximately 5 mm from the dorsal root entry and the initial deflection recorded in the ventral root was within the range 1.2 to 1.9 msec. Examples are illustrated in Figures 8A and 9A. Intravenous naloxone (0.025 to 0.10 mg/kg) increased the amplitude of the BST monosynaptic reflex in all nine experiments. The mean increase was 80.9% ± 22.8 S.E.M. (n = 9). It should be noted that although the amplitude was increased, no new peaks of later firing were recorded (the period recorded extended well beyond that portion which is illustrated). Since the first peak of firing was complete within 1.2 msec, the increased amplitude must have resulted from recruitment of

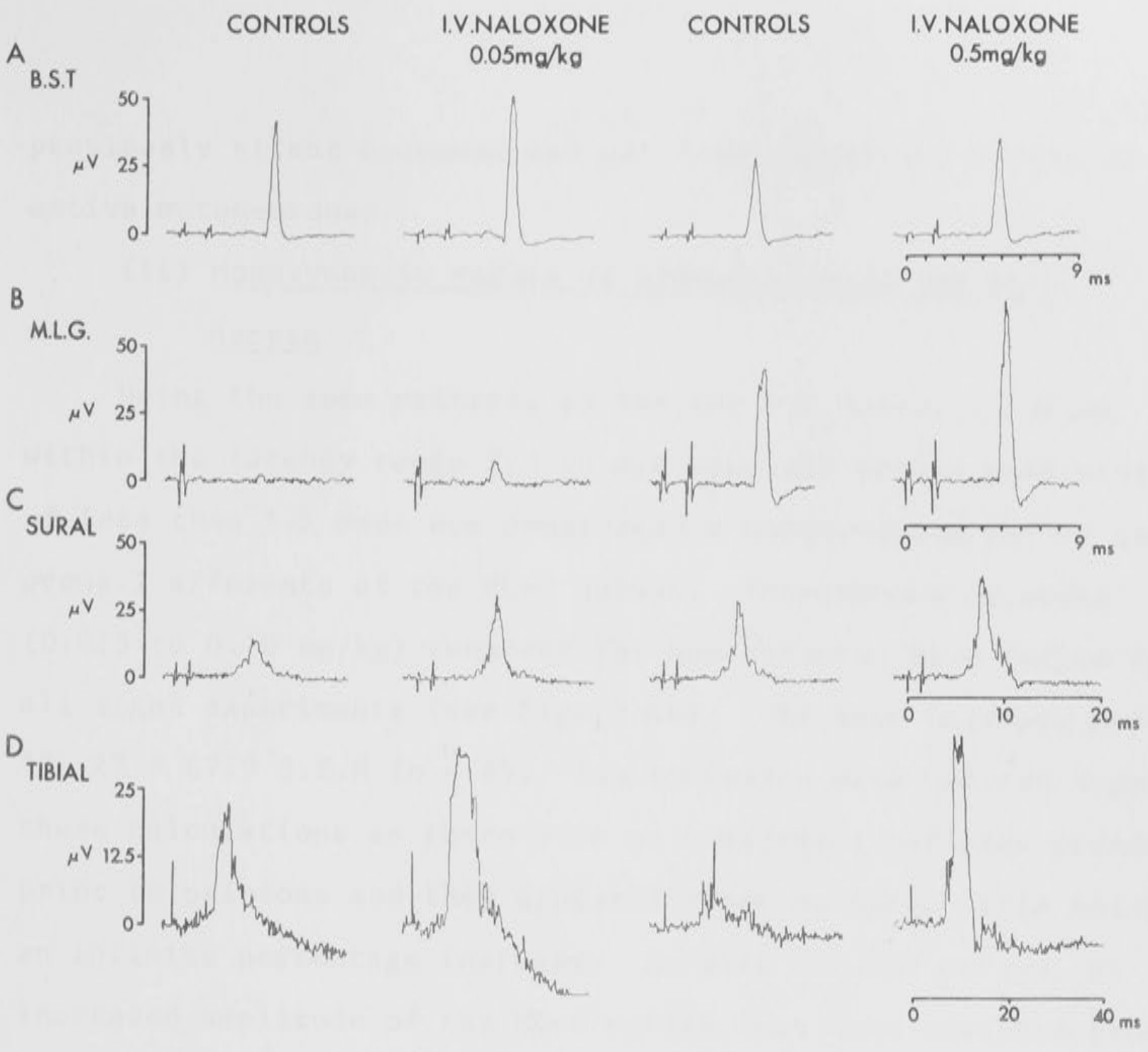


Fig. 8. Enhanced spinal reflexes following intravenous naloxone. Each pen recording is the average of 16 S_1 ventral root reflexes produced by electrical stimulation of a peripheral nerve. Following the first dose of naloxone (0.05 mg/kg), 3 hours were allowed for elimination of the drug and then new controls using different stimulus parameters were obtained. The increases produced by naloxone were in both cases recorded 9 to 12 minutes after the antagonist was given intravenously. In this and subsequent figures, the stimuli are expressed as multiples of the threshold (T) for excitation of the fastest conducting fibres in each nerve.

A. BST nerve stimulation: first control, 2 stimuli of 3T and 4T; second control, 2 stimuli of 2T and 5T.

B. ML-G nerve stimulation: first control, 1 stimulus of 20T; second control, 2 stimuli of 10T and 20T.

C. Sural nerve stimulation: first control, 2 stimuli of 7T and 10T; second control, 2 stimuli of 8T and 10T.

D. Tibial nerve stimulation: first control, 1 stimulus of 5T; second control, 1 stimulus of 10T.

previously silent neurones and not from repetitive firing of active motoneurones.

(ii) Monosynaptic reflex to stimulation of the ML-G nerves

Using the same criteria as for the BST nerve, a reflex within the latency range 3.5 to 4.0 msec and with a peak width of less than 1.2 msec was considered a monosynaptic reflex to group I afferents of the ML-G nerves. Intravenous naloxone (0.025 to 0.10 mg/kg) enhanced the monosynaptic ML-G reflex in all eight experiments (see Figure 8B). The mean increase was 138.2% ± 67.9 S.E.M (n = 6). Two increases were omitted from these calculations as there were no measurable reflexes present prior to naloxone and they appeared after naloxone, this being an infinite percentage increase. As with the BST reflex, an increased amplitude of the ML-G reflex must have resulted from recruitment of previously silent motoneurones.

(iii) Reflexes to stimulation of myelinated sural afferents

It was not possible to distinguish between reflexes to the larger (Aαβ) and smaller (Aδ) myelinated primary afferents of the sural nerve. In most experiments, the stimuli used were below 4 times threshold (4T) for the fastest conducting fibres and hence it is unlikely that Aδ fibres were stimulated. In a few instances (Figure 8C), larger stimuli were required to obtain reproducible control reflexes and it is probable that both Aαβ and Aδ afferents were stimulated in these experiments. Reflexes were of 4.0 to 6.6 msec latency and broadly dispersed when compared with monosynaptic reflexes to group I muscle afferents (see Figures 8C and 9C).

Naloxone (0.025 to 0.10 mg/kg) enhanced reflexes to sural nerve stimulation in six of eight experiments (Figure 8C), there being no change in one and a decrease in one. The mean increase for all eight experiments was 26.6% ± 12.9 S.E.M. (n = 8). Unlike monosynaptic reflexes to muscle afferents it is not possible to state whether recruitment of motoneurones or repetitive firing of previously active cells produced the observed increases.

(iv) Reflexes to stimulation of myelinated tibial afferents

As with the sural nerve most stimuli were below 4T for the fastest conducting fibres. These reflexes sometimes contained a short latency low amplitude component (presumed to be in response to group I muscle afferents) but always had a later broader component probably produced by impulses in large diameter cutaneous afferents.

Intravenous naloxone increased this reflex in all eight experiments. The mean increase was 47.3% ± 8.3 S.E.M. (n = 8). One such result is illustrated in Figure 8D.

(v) Reflexes to impulses in unmyelinated tibial afferents

Naloxone (0.025 to 0.05 mg/kg) increased reflexes to stimulation of C fibre afferents of the tibial nerve in all nine experiments. The mean increase was 138.6% ± 49.2 S.E.M. (n = 9). Results from one experiment are shown in Figure 10A.

(b) Stereoisomers of N-(3-furylmethyl)-α-normetazocine (FMN) and Reflexes Recorded in Ventral Roots

In commonly used tests of the effects of opiates, the

potency of FMN as a morphine antagonist was about one-third that of naloxone (Kuhn & Stockhaus, 1976). The effectiveness of FMN in blocking opioid peptide-mediated events, however, has not been determined. These relative potencies as antagonists of morphine were therefore used as a guide and low doses of FMN were administered initially, supplemented within a short time in some experiments.

The (+)-isomer of FMN in doses of 0.03 to 0.20 mg/kg had slight but variable effects on the reflexes studied. A decrease in two of the four reflexes occurred in one experiment (illustrated in Figure 9), and in two other experiments, two of the four reflexes were slightly increased. Overall (+)-FMN did not alter these reflexes significantly. By contrast all reflexes were clearly increased by (-)-FMN (0.03 to 0.20 mg/kg). The calculated increases were:

BST reflex increased by 27.0% ± 8.8 S.E.M. (n = 4)

ML-G reflex increased by 66.0% ± 39.7 S.E.M. (n = 3)

Sural reflex increased by 32.8% ± 10.1 S.E.M. (n = 4)

Tibial A reflex increased by 48.5% ± 13.9 S.E.M. (n = 4)

These calculations are based on the effects of doses of (-)-FMN equal to the total amount of (+)-FMN given previously to the same animal. In two experiments, higher doses of (-)-FMN were administered subsequently, producing a larger increase in reflexes.

The results illustrated in Figure 9 show all four reflexes increased by (-) but not (+)-FMN. With all reflexes, the amplitude was increased, but for the complex cutaneous reflexes, new later peaks of firing were also produced.

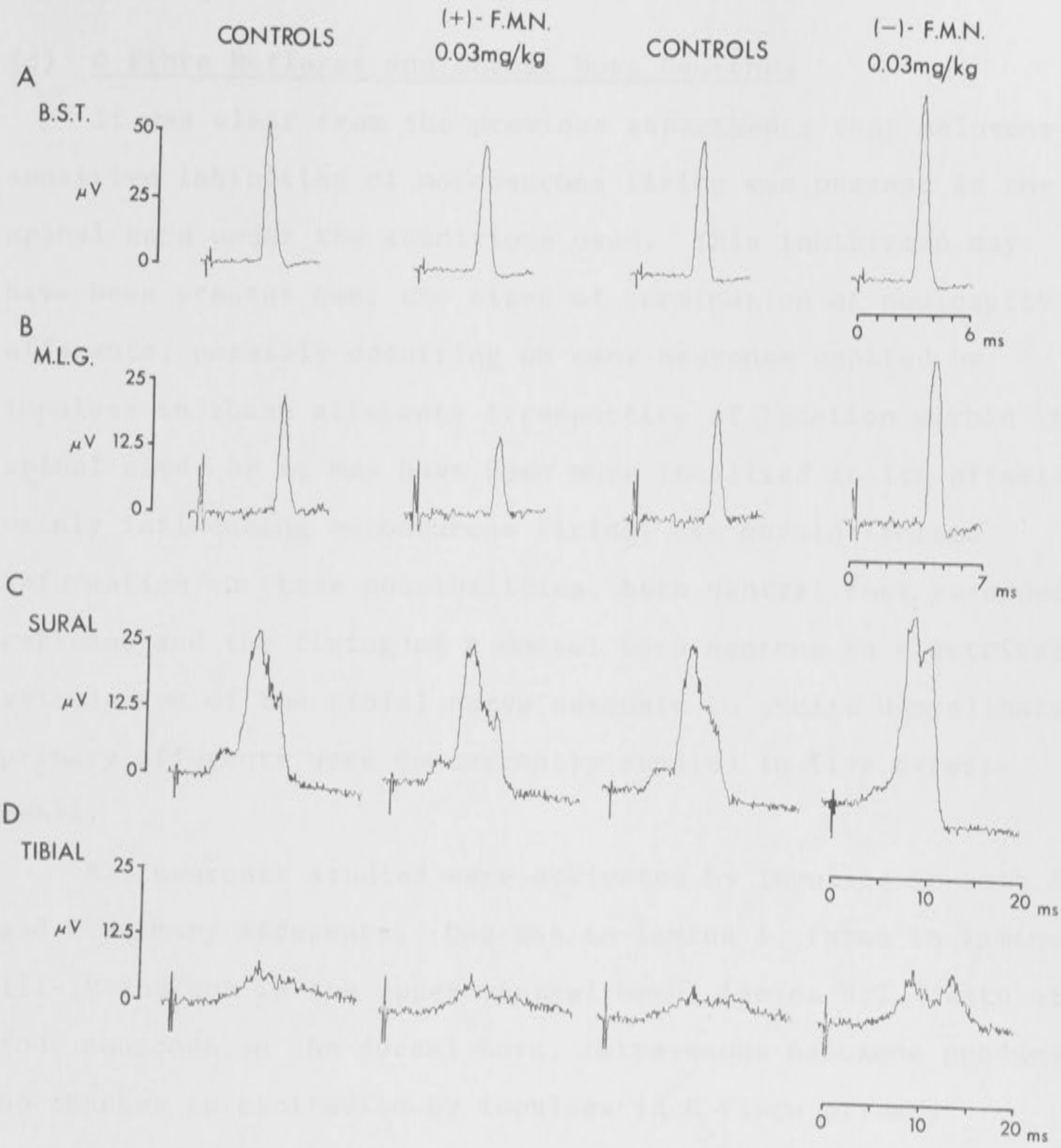


Fig. 9. Enhanced spinal reflexes following intravenous (-) but not (+)-FMN. As in Figure 8, each pen recording is the average of 16 reflexes. Unlike Figure 8 the same nerve stimuli were used throughout and the two sets of controls are comparable. The records illustrated for (-) and (+)-FMN (0.03 mg/kg) were obtained within 5 to 8 minutes of administering each drug.

A. BST nerve stimulation: 1 stimulus of 6T
B. ML-G nerve stimulation: 1 stimulus of 8T
C. Sural nerve stimulation: 1 stimulus of 3T
D. Tibial nerve stimulation: 1 stimulus of 3T

(c) C Fibre Reflexes and Dorsal Horn Neurones

It was clear from the previous experiments that naloxone-sensitive inhibition of motoneurone firing was present in the spinal cord under the conditions used. This inhibition may have been present near the sites of termination of nociceptive afferents, possibly occurring on many neurones excited by impulses in these afferents irrespective of location within the spinal cord, or it may have been more localized in its effects, mainly influencing motoneurone firing. To obtain limited information on these possibilities, both ventral root recorded reflexes and the firing of a dorsal horn neurone to electrical stimulation of the tibial nerve adequate to excite unmyelinated primary afferents were concurrently studied in five experiments.

All neurones studied were activated by impulses in both A and C primary afferents. One was in lamina I, three in lamina III-IV and one in the upper ventral horn, lamina VII. With all four neurones in the dorsal horn, intravenous naloxone produced no changes in excitation by impulses in C fibre primary afferents despite easily measured increases in reflexes to impulses in these fibres. Such a result is shown in Figure 10. In this experiment an 8 to 10-fold increase in impulses counted in the ventral root was observed but no change occurred in the responses of the lamina IV neurone to the same stimulus. In the case of the lamina VII neurone (2.77 mm from the dorsal surface), however, a increased C response was observed concurrently with the enhanced C reflex. In this experiment the ventral root response doubled following two doses of

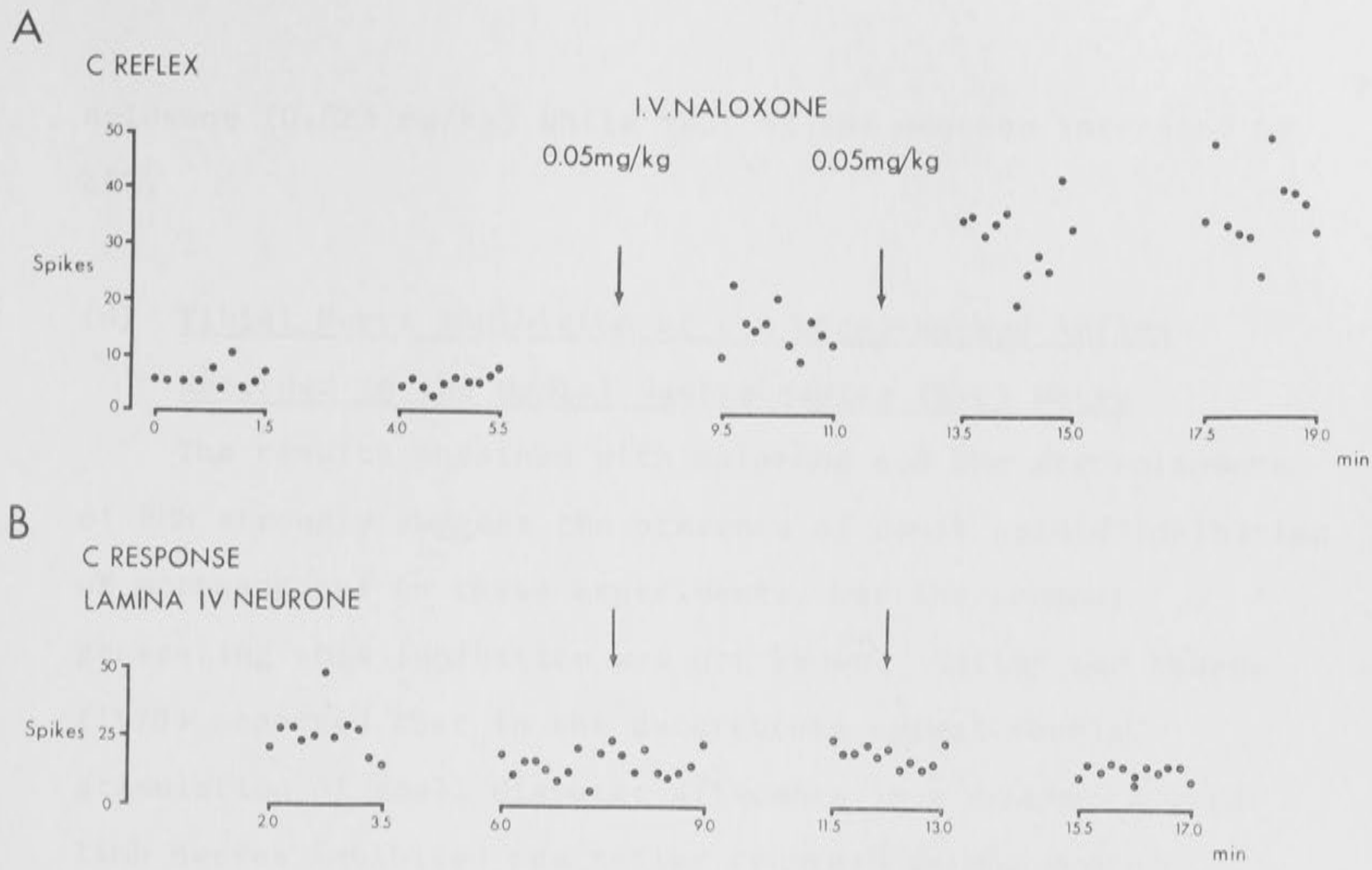


Fig. 10. Differing effects of naloxone on reflex firing in the S_1 ventral root to impulses in unmyelinated primary afferents of the tibial nerve (A) and the excitation of a lamina IV neurone by the same stimulus (B). The one gated electronic counter was used to count alternately, firing in the ventral root and of a lamina IV neurone. The times at which this was done are shown on the abscissae. The ordinates measure the total number of action potentials for the duration of the counting gate. This was timed to include only those spikes which, by their latency and the nerve stimulus necessary to produce them, were due to impulses in unmyelinated primary afferents. Two doses of naloxone (0.05 mg/kg) were given intravenously (indicated by arrows) producing a large increase in the reflex but no increase in the responses of the superficial neurone.

naloxone (0.025 mg/kg) while that of the neurone increased by 20%.

(d) Tibial Nerve Inhibition of the Sural-evoked Reflex Recorded in the Medial Gastrocnemius (M-G) Nerve

The results obtained with naloxone and the stereoisomers of FMN strongly suggest the presence of tonic opioid inhibition of motoneurones in these experiments, but the process generating this inhibition was not known. Catley and Pascoe (1978) reported that in the decerebrate spinal rabbit, stimulation of small diameter afferents in a number of hind limb nerves inhibited the reflex recorded in the medial gastrocnemius nerve following stimulation of large diameter afferents of the sural nerve and that naloxone (0.05 mg/kg) abolished this inhibition. This result suggested release of opioid peptides by impulses related to pain, and in the present experiments intense nociceptive input was certainly provided by the extensive surgery required for animal preparation.

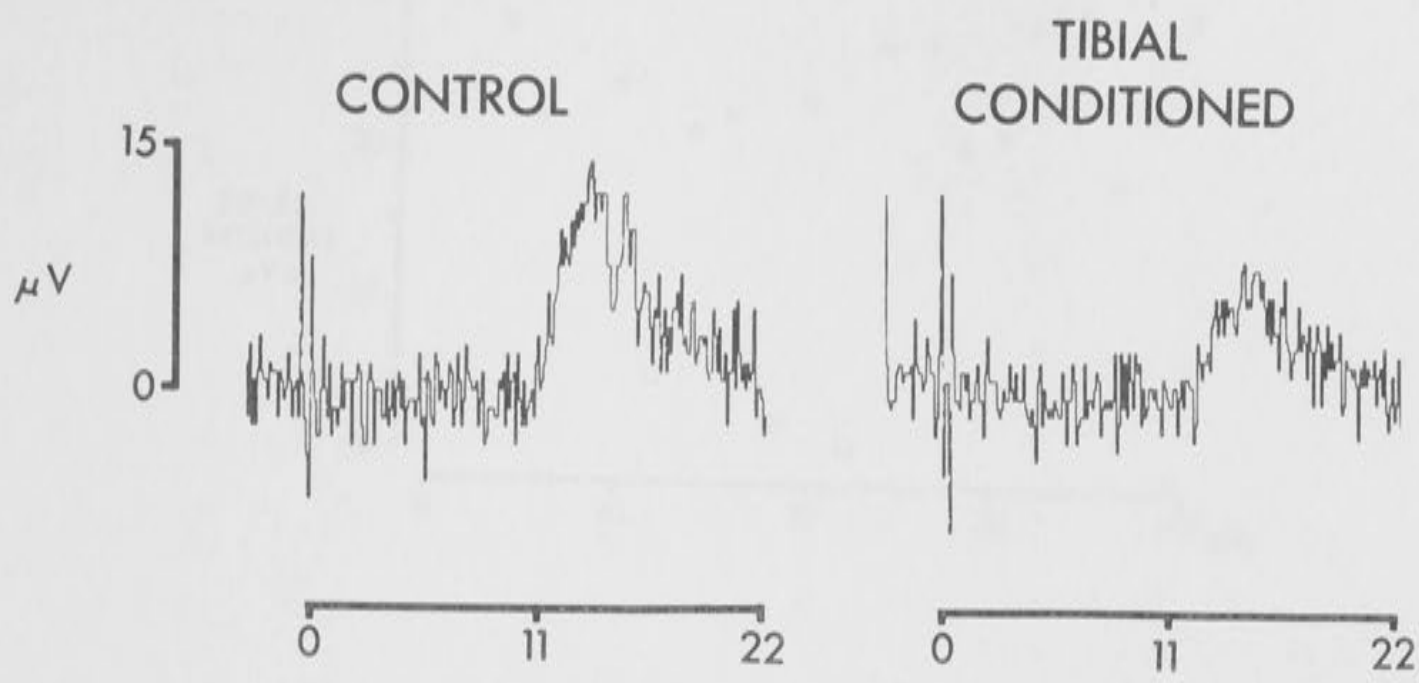
To investigate this finding of Catley and Pascoe, reflexes were recorded peripherally in the M-G nerve while stimulating low threshold sural afferents in five experiments. In four of these the effects of prior tibial nerve stimulation on this reflex were examined. The spinal cord was divided at the thoraco-lumbar junction in three experiments and left intact in the remaining two. No difference in results between spinal and intact animals was observed.

A single stimulus to the sural nerve (3 to 6T) produced a reflex discharge in the M-G nerve which, in three experiments,

was of such small amplitude that signal averaging was necessary to record it. In three, the latency of the single peak of activity was in the range 8.4 to 13.8 msec, in one there were two peaks (11.6 and 13.8 msec) and in the remaining experiment, three peaks of latency 4.2, 8.4 and 11.0 msec. A reflex of latency 11 msec is illustrated in Figure 11.

Tetanic stimulation of the tibial nerve (0.3 msec pulses at 50 Hz for 1 minute) inhibited the sural to M-G reflex but only when small diameter afferents were excited (see Figures 11 and 12). The graph in Figure 12A shows that stimuli of 2T, which excited only large (Aαβ) myelinated afferents, did not reduce the reflex. Stimuli of 10T produced significant inhibition (not illustrated), which was not greatly increased when C fibres were also stimulated (250T). This inhibition was of relatively short duration. Since 16 or 32 reflex responses (elicited at 1 Hz) were required for each averaged record, the time course of recovery could not be closely followed. In all instances, however, recovery was complete within 2.5 minutes of the cessation of tibial nerve stimulation.

Intravenous naloxone (0.05 mg/kg) increased the unconditioned reflex in only one of five experiments. In this case naloxone caused the appearance of a shorter latency discharge (6.2 msec, the later wave being of 8.4 msec latency) in the reflex response without altering the amplitude of the later response. Following recovery from naloxone (2 hours), the (-) but not (+)-isomer of FMN (0.05 mg/kg) also caused this peak to appear.



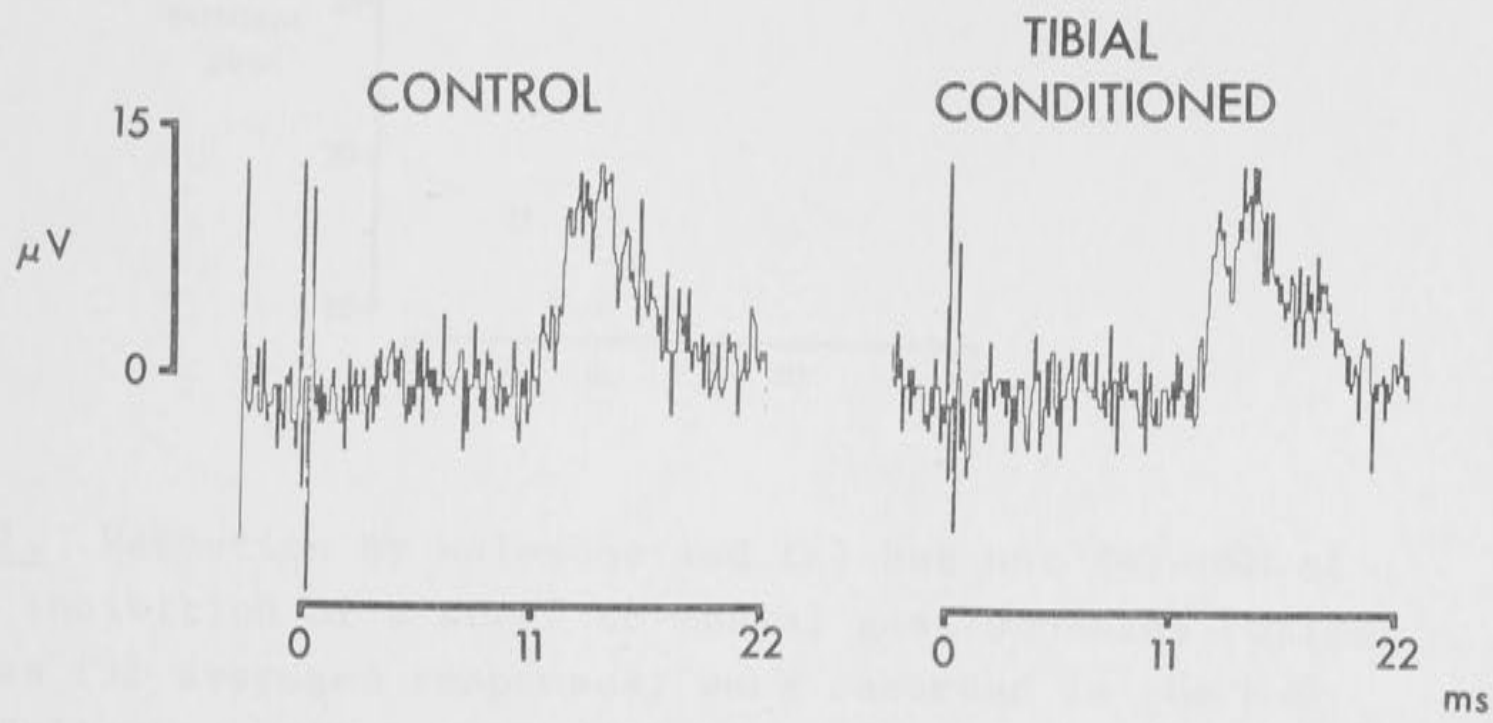
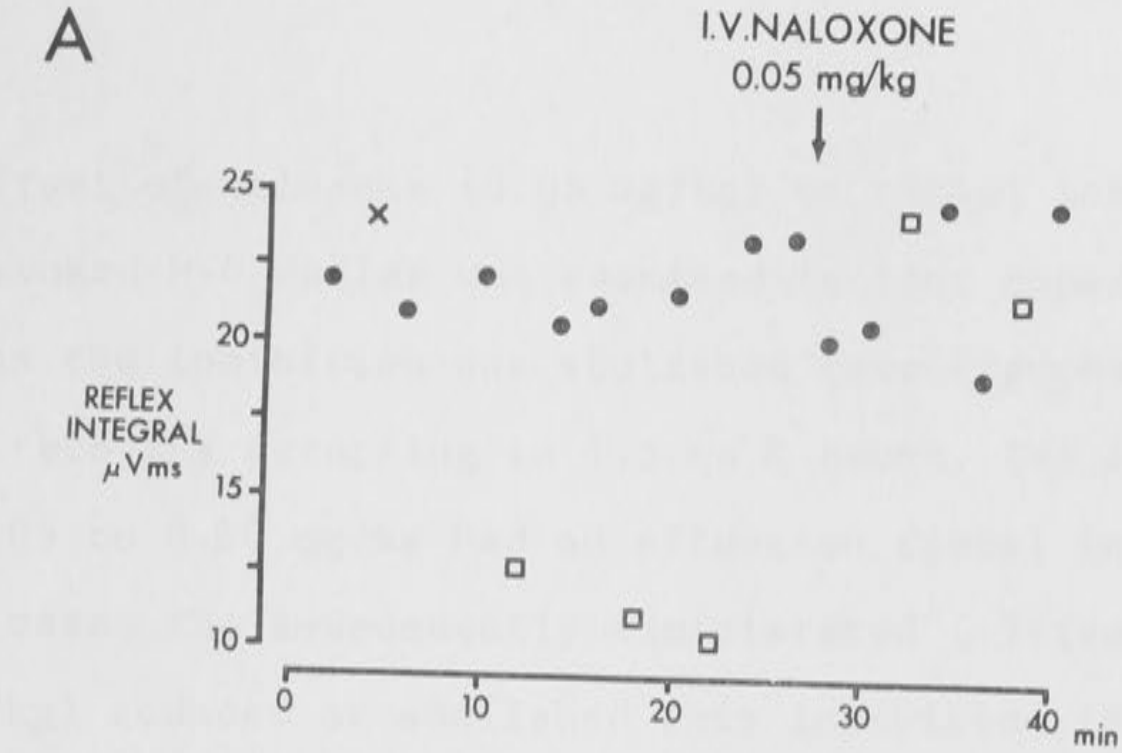


Fig. 11. Reduction by naloxone of tibial inhibiton of a sural to medial gastrocnemius reflex. The pen recordings are averaged records of 32 successive reflexes recorded in the M-G nerve following stimulation of the sural nerve with a 6T stimulus at time 0. The areas of such records are plotted in Figure 12. Tibial conditioning consisted of a continuous tetanus at 50 Hz, 250T intensity (adequate to excite C fibres) for 1 minute just prior to eliciting the reflexes. This tetanic stimulation reduced the amplitude of the reflex (upper record), and naloxone (0.05 mg/kg) prevented this inhibition (lower record).



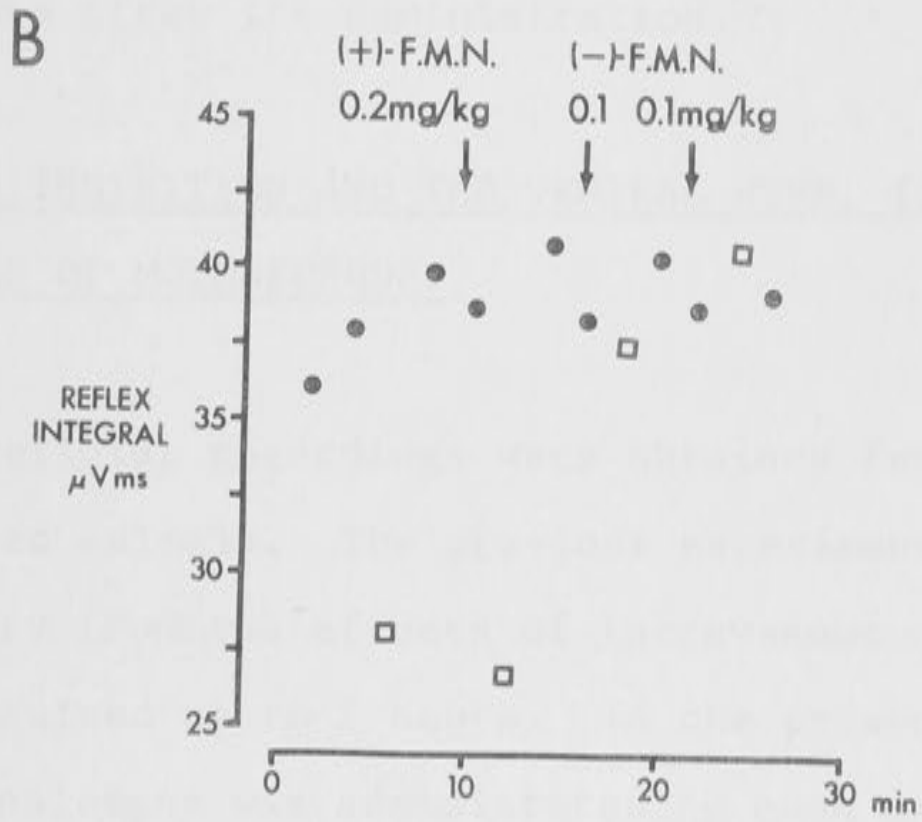


Fig. 12. Reduction by naloxone and (-) but not (+)-FMN of tibial inhibition of a sural to medial gastrocnemius reflex. Reflexes (32 averaged responses) were recorded in the M-G nerve following stimulation (6T) of the sural nerve at 1 Hz. The plotted symbols are areas calculated from averaged records. Stimulation of the tibial nerve with a continuous tetanus of 50 Hz, 2T intensity for 1 minute just prior to eliciting the reflex did not reduce its amplitude (cross). With a stimulus intensity of 250T (suprathreshold for C fibres) the reflex was inhibited (open squares).

A. The reduction by intravenous naloxone of tibial inhibition of the sural to M-G reflex.

B. The reduction by (-) but not (+)-FMN of tibial inhibition of the sural to M-G reflex.

The effect of naloxone (0.05 mg/kg) on tibial inhibition of the sural-evoked M-G reflex was examined in four experiments. In all cases the inhibition was abolished (see Figures 11 and 12A), with recovery occurring in 1.5 to 2 hours. (+)-FMN in doses of 0.05 to 0.20 mg/kg had no effect on tibial inhibition but in all cases the subsequently administered (-)-isomer (0.05 to 0.20 mg/kg) reduced or abolished this inhibition (Figure 12B). Recovery from the effects of the (-)-isomer was not seen up to 3 hours after its administration.

C. OPIOID INHIBITION AND THE VENTRAL HORN. II. INTRACELLULAR STUDIES OF MOTONEURONES

Intracellular recordings were obtained from motoneurones of spinalized animals. The previous experiments had indicated that recovery from the effects of intravenous naloxone on spinal reflexes required up to 2 hours. In the present experiments therefore, naloxone was administered to each animal up to three times while studying different motoneurones, allowing 2 to 3 hours between doses. To detect possible naloxone-induced changes in motoneurone properties and responses, it was important to ensure that reflexes were increased by the doses of naloxone used, and thus reflexes were concurrently studied. When a successful motoneurone penetration was made, a typical recording sequence consisted of averaging EPSPs evoked by stimulation of peripheral nerves, averaging ventral root reflexes from the same stimuli, sampling spontaneous potential changes on film, photographing action potentials evoked

antidromically by ventral root stimulation, determining voltage/current relationships for hyperpolarizing and depolarizing intracellular current pulses, repeating the EPSP and reflex observations and then, provided that these were reproducible and membrane potential was stable, naloxone was administered intravenously.

(a) Effect of Naloxone on Resting Membrane Potential

The resting membrane potentials of seventeen motoneurones were observed during naloxone administration. The significance of small changes in this potential was determined by the stability in the record. For two neurones the record was so stable that a change of 1 mV could be detected, with eight neurones a change of 2 mV was detectable but greater than 2 mV was necessary with seven. On this basis, a single intravenous dose of naloxone which increased spinal reflexes (0.05 mg/kg) produced no change in the resting membrane potential of nine of twelve motoneurones. With the remaining three, depolarizations of 1.5, 3 and 6 mV were recorded within 4 minutes of naloxone administration.

Two doses of naloxone (0.05 mg/kg) were given within 15 minutes while recording from another five motoneurones since the first dose did not increase spinal reflexes. No change in resting membrane potential was observed with two but a depolarization of 3 mV was measured with two and 1 mV in one. Figure 13B illustrates a depolarization of 3 mV observed within 3 minutes of a second dose of naloxone (0.05 mg/kg). As typified in this record, depolarizations usually occurred rapidly,

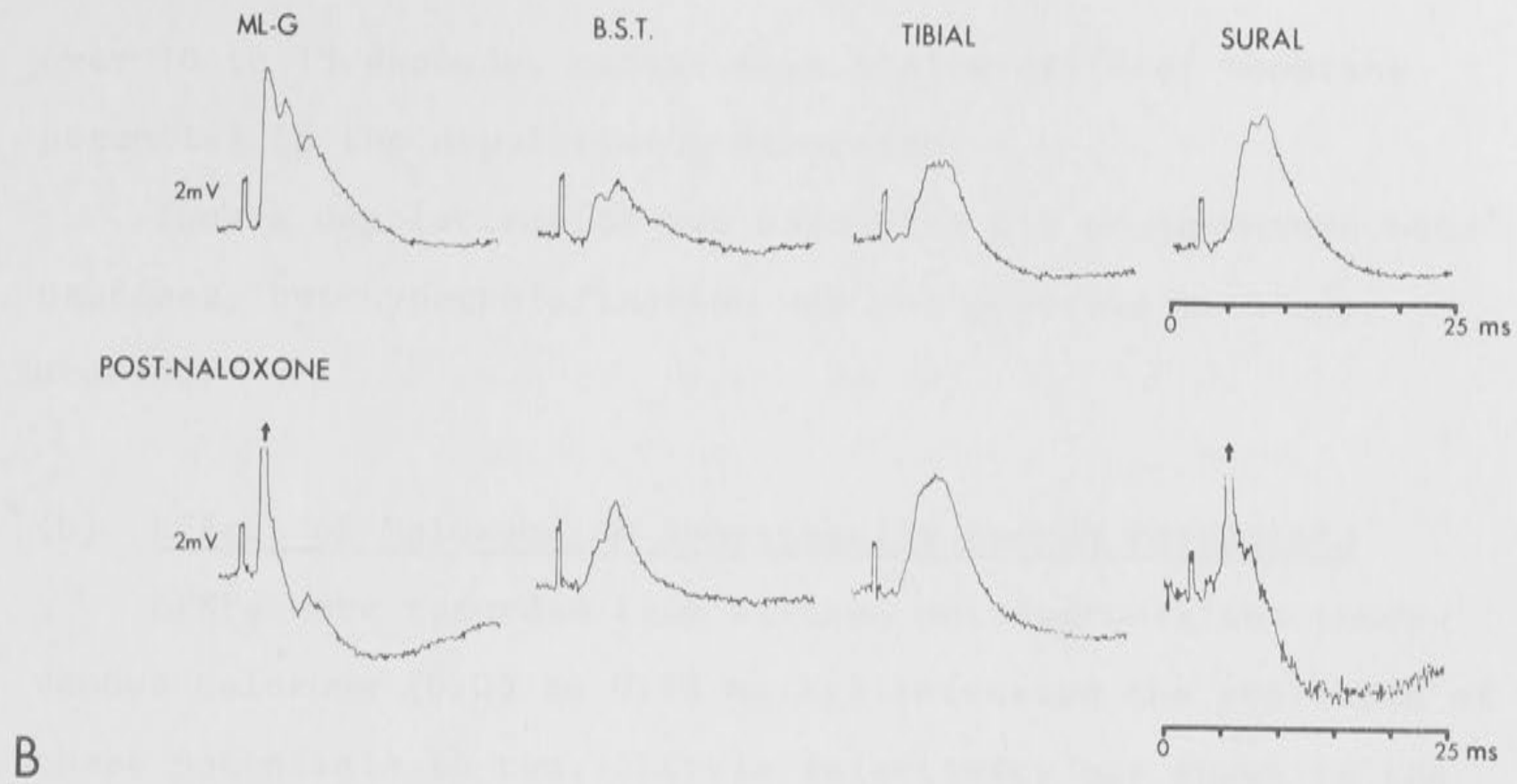


Fig. 13. Increased motoneurone EPSPs and a depolarizing change in resting membrane potential following intravenous naloxone.

A. The EPSPs were evoked by stimulation of the ML-G nerve (1.6T), BST nerve (1.8T), tibial nerve (1.5T) and sural nerve (1.5T). Each record is the average of 16 EPSPs evoked at 1 Hz. A 2 mV calibration pulse is present on each record and the trailing edge of the stimulus pulse coincides with the trailing edge of the calibration. Amplifier time constant, 1 second. After naloxone (0.10 mg/kg) an action potential was evoked constantly by the ML-G stimulus and irregularly by the sural stimulus. Since averaging the latter gave a misleading record, 1 sweep only is illustrated in the post-naloxone record. The post-naloxone EPSP records were obtained at the times shown by the arrows in B.

B. A pen recording of the resting membrane potential of the motoneurone. A small depolarization (3 mV) was recorded approximately 3 minutes after the second dose of naloxone (0.05 mg/kg).

over 10 to 15 seconds, rather than a slow drift of membrane potential in the depolarizing direction.

Thus a depolarization was seen with six of seventeen motoneurones, but hyperpolarization was not observed with any neurone.

(b) Effect of Naloxone on Synaptically Evoked Potentials

EPSPs were recorded from sixteen motoneurones and intravenous naloxone (0.05 to 0.10 mg/kg) increased the amplitude of these potentials in ten. Little selectivity was shown in the increases in EPSPs with responses to stimulation of large diameter muscle and cutaneous afferents being similarly increased. Thus of five neurones activated from two nerves, evoked potentials from both were increased by naloxone in all five. Of two neurones activated by four nerves, naloxone increased potentials from all four in one neurone and in three of four in the other. Of the remaining six motoneurones, EPSPs were unchanged by naloxone in four and decreased in amplitude with two.

This non-selective action of naloxone is illustrated in Figure 13A. With this neurone the latency of the EPSP from the ML-G nerve (2.0 msec from the peripheral nerve stimulus) suggests monosynaptic activation whereas the longer latency for the BST nerve indicates a polysynaptic process. Prior to naloxone the neurone did not fire to any nerve stimulus but after a total dose of 0.10 mg/kg it fired constantly to the ML-G stimulus, irregularly to the sural stimulus (sural EPSPs which did not evoke firing were increased), and EPSPs from BST

and tibial stimulation were increased. The higher dose of naloxone was used in this experiment since the reflexes concurrently measured were not increased by the first dose of 0.05 mg/kg.

Changes in synaptically evoked potentials were large and readily detected compared with the effects on membrane potential. In interpreting the mechanisms of the changes in evoked potentials two points require comment. Firstly, it was clear that synaptically evoked potentials could be increased in the absence of any change in resting membrane potential. This occurred with six of ten neurones and results from one are illustrated in Figure 14. With this neurone, the area of the sural-evoked EPSP was increased by 116% at 11.5 minutes, and by 104% at 15.5 minutes, after naloxone administration. These increases occurred with no change in resting membrane potential. Secondly, with three of the four neurones in which the largest membrane potential changes occurred, the changes in evoked potentials were also relatively large. Figure 15 shows results from the neurone depolarized by 6 mV following naloxone. Initially noted was a 30% increase in the area of the sural-evoked EPSP recorded one minute after naloxone administration (Figure 15B). By two minutes post-naloxone, spontaneous firing had commenced at 5 Hz, the neurone previously being silent (Figure 15C), and the sural stimuli constantly evoked action potentials whereas, before naloxone, they did not. Similarly, the neurone shown in Figure 13 was depolarized by 3 mV following naloxone and the changes in evoked potentials were relatively large.

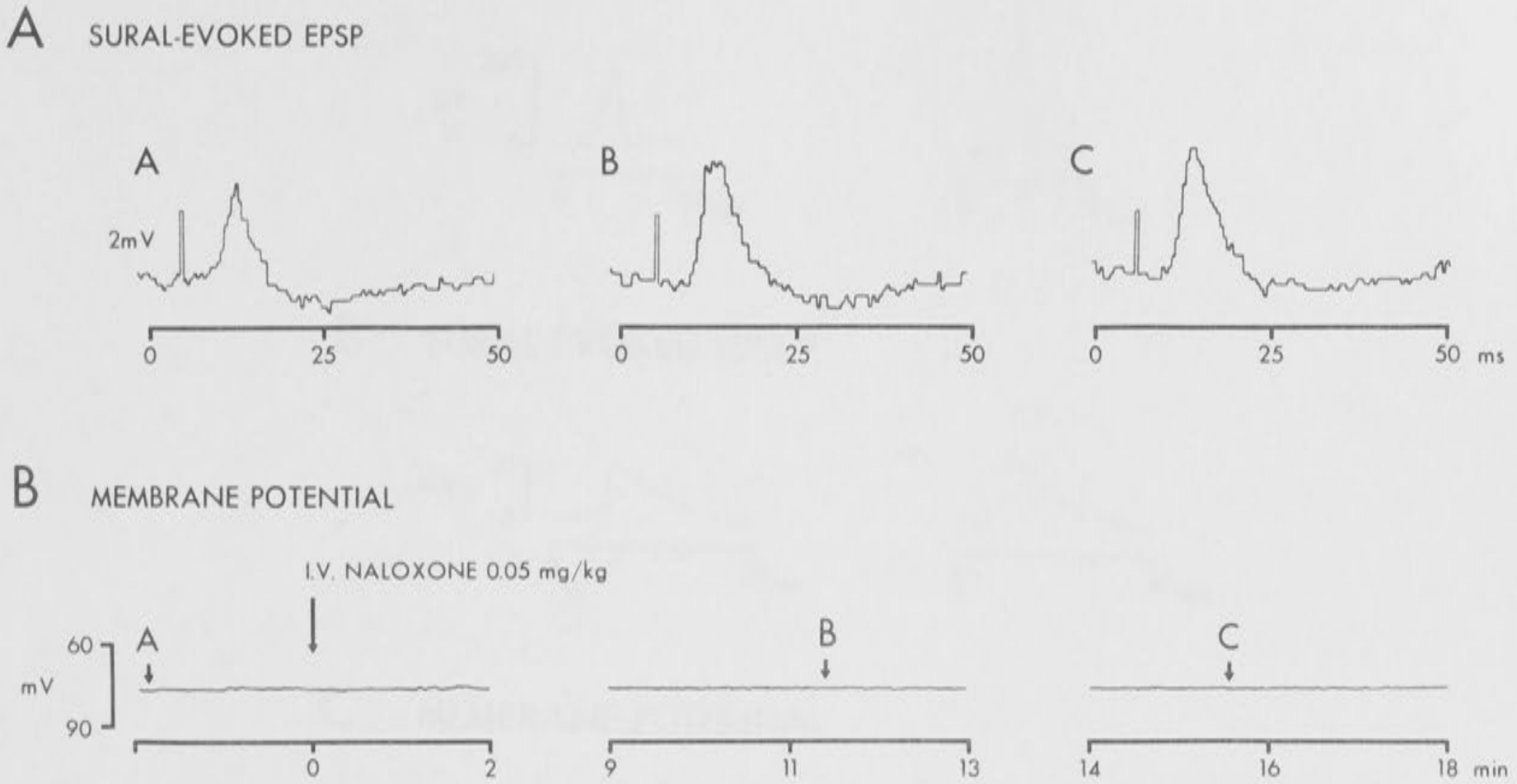


Fig. 14. Increased sural nerve-evoked EPSPs without a change in resting membrane potential in a motoneurone following intravenous naloxone.

A. The EPSPs are averages of 16 successive records. The sural nerve stimulus (3.5T, 1 Hz) coincides with the trailing edge of the 2 mV calibration pulse. Amplifier time constant, 1 second. The times of recording the EPSPs are shown by the arrows in B. The areas of the EPSPs recorded 11.5 and 15.5 minutes after naloxone (0.05 mg/kg) are 216% and 204% respectively of the area of the pre-naloxone record.

B. A pen recording of the resting membrane potential of the motoneurone, showing no change following intravenous naloxone (0.05 mg/kg).

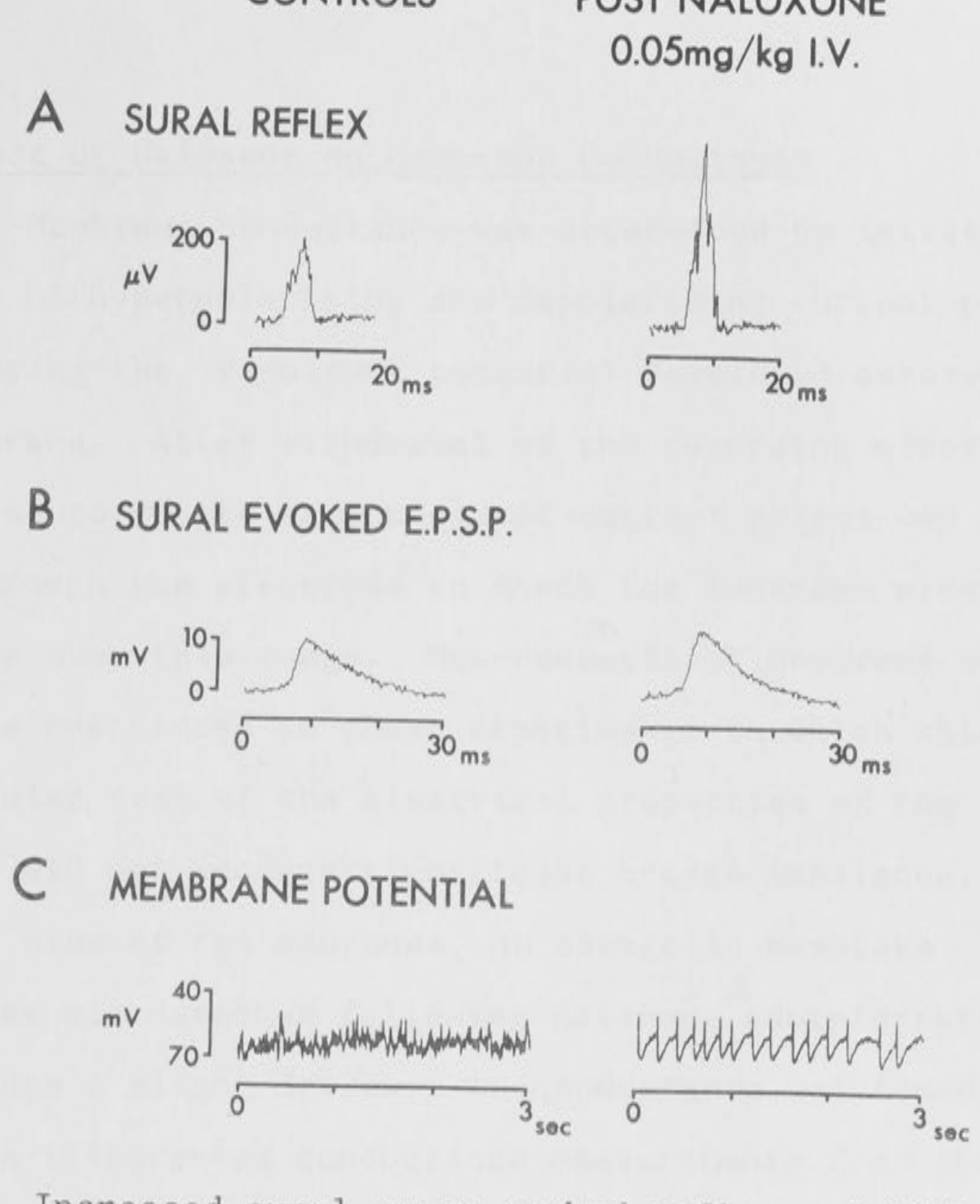


Fig. 15. Increased sural nerve-evoked reflex and EPSP and spontaneous firing of a motoneurone following intravenous naloxone (0.05 mg/kg).

A. The pen recordings are averages of 16 S_1 ventral root reflexes evoked by sural nerve stimulation (4T) at 1 Hz, delivered at time 0. The area of the reflex, recorded 2 minutes after naloxone administration, is 184% of the area of the pre-naloxone record.

B. EPSPs (average of 16) evoked by sural nerve stimulation as in A. The area of the EPSP, recorded 1 minute after naloxone administration, is 130% of the area of the pre-naloxone record.

C. Pen recordings of the spontaneously-occurring changes in resting membrane potential. Prior to naloxone, the neurone was silent and spontaneous fluctuations in membrane potential were recorded. The recording obtained 4 minutes post-naloxone shows spontaneous firing of the neurone at 5 Hz. Action potential amplitudes were not reproduced accurately by the pen recorder and have been truncated.

(c) Effect of Naloxone on Membrane Conductance

Membrane conductance was determined by intracellular injection of hyperpolarizing and depolarizing current pulses, and measuring the resultant potential developed across the cell membrane. After withdrawal of the recording electrode from the neurone, the same range of current pulses was again passed through the electrode to check for constant electrode resistance over this range. Measurements of membrane conductance were restricted to those experiments in which this extracellular test of the electrical properties of the electrode did not produce significant bridge imbalance.

With nine of ten neurones, no change in membrane conductance was detected following naloxone administration. In one instance a slight decrease in conductance was found. Figure 16A illustrates conductance measurements from the motoneurone described in Figure 13. Despite large increases in EPSPs (Figure 13A), the current/voltage relationships for 20 msec pulses obtained 15 and 27 minutes after naloxone (0.10 mg/kg) were not different from controls. The height of the action potential evoked antidromically by ventral root stimulation was also measured before and after naloxone. No significant alteration in its amplitude was found (see Figure 16B), supporting the conclusion that somatic membrane conductance was unchanged by the opioid antagonist.

Filmed records of spontaneously occurring fluctuations in membrane potential showed no consistent changes following intravenous naloxone.

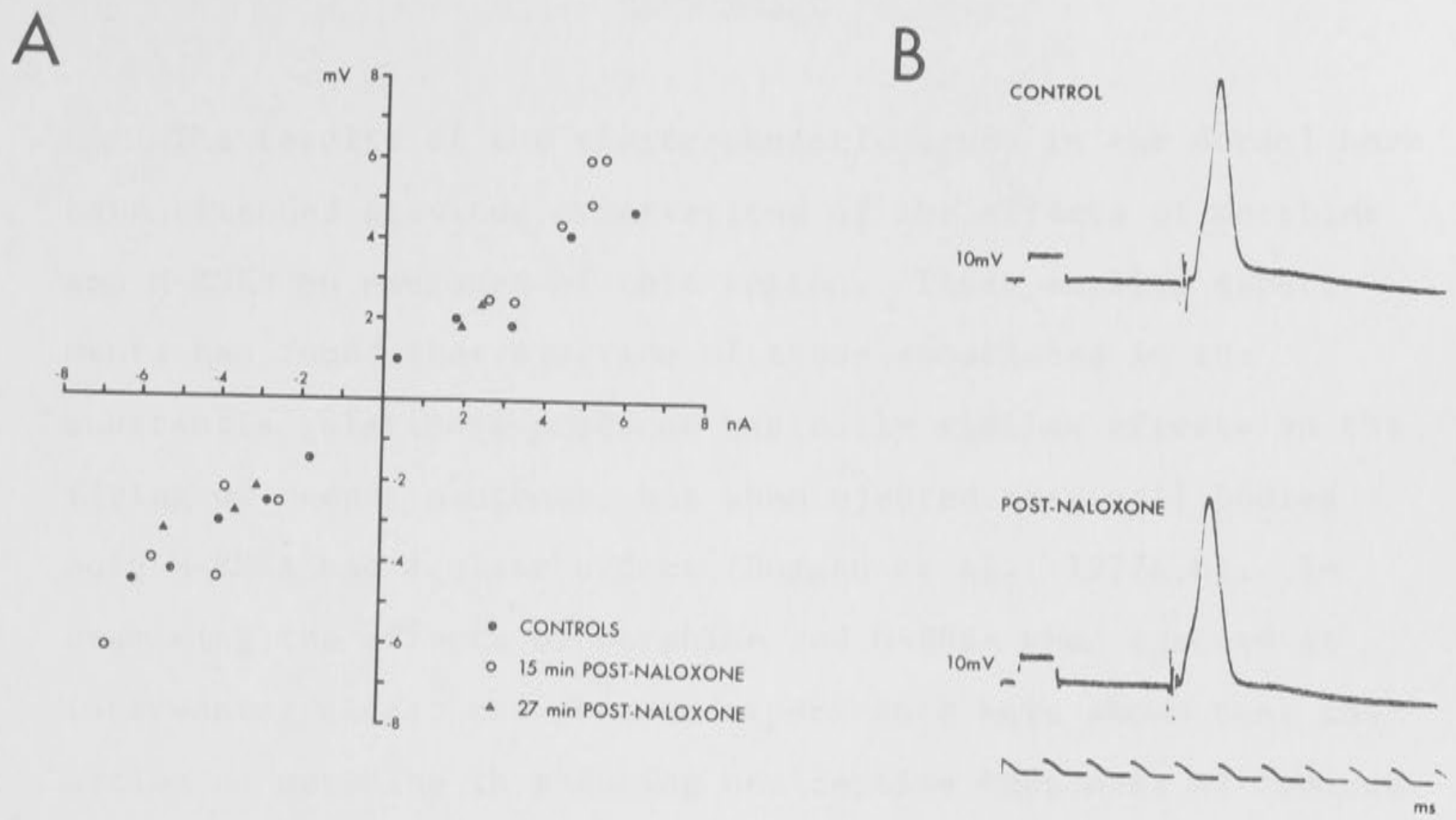


Fig. 16 Lack of effect of intravenous naloxone on the somatic membrane conductance of the motoneurone described in Figure 13.

A. Voltage/current relationships were measured prior to, and 15 and 27 minutes subsequent to, naloxone administration (0.10 mg/kg). The depolarizing and hyperpolarizing current pulses were 20 msec in duration. The slope of the plot (membrane resistance) was not altered by naloxone.

B. Photographs of the antidromic action potential evoked by ventral root stimulation. The amplitude of this potential was not affected by naloxone administration (0.10 mg/kg).

DISCUSSION

The results of the electrophoretic study in the dorsal horn have extended previous observations of the effects of morphine and M-ENKA on neurones of this region. These earlier experiments had found that ejection of these substances in the substantia gelatinosa produced basically similar effects on the firing of deeper neurones, but when ejected near cell bodies only M-ENKA had a clear effect (Duggan et al., 1977a,b). In examining the effects of morphine and M-ENKA when ejected at intervening sites, the present experiments have shown that the action of morphine in reducing nociceptive responses of laminae IV and V neurones was restricted to the region of the substantia gelatinosa. With ejection at more ventral sites down to and including near the cell body, morphine had no effect or produced firing in bursts with abnormalities in action potential configuration. It is likely that these effects of morphine near cell bodies were not produced by an action on opiate receptors, but resulted from high concentrations of the opiate close to the soma membrane. This is supported by the previous observation that such effects were not antagonized by electrophoretic naloxone (Duggan et al., 1977a). Although similar ejecting currents were used in the substantia gelatinosa, these effects were not seen but rather a naloxone-reversible reduction of nociceptive responses. This suggests that the predominant process in these circumstances was an action on opiate receptors.

In contrast to morphine, M-ENKA depressed nociceptive

responses when administered over a wide area adjacent to and dorsal to the somata of these cells, up to and including the substantia gelatinosa. Since these effects of M-ENKA were susceptible to naloxone, they were most likely due to an action on opiate receptors.

This different distribution of effects observed in these experiments is most easily explained by the presence of differing receptors for morphine and enkephalins in the dorsal horn of the cat, and a probable location on different neural structures.

Multiple Opiate Receptors

There is now a wealth of data supporting the existence of multiple opiate receptors (recently reviewed by Chang, Hazum & Cuatrecasas, 1980; Chang & Cuatrecasas, 1981; Simon, 1981; Zukin & Zukin, 1981; Wood, 1982). The first proposal along these lines suggested that agonists such as morphine and mixed agonists/antagonists such as nalorphine acted on different receptors to produce analgesia (Martin, 1967). On the basis of behavioural and neurophysiological observations in the chronic spinal dog, Martin and his colleagues have postulated the existence of at least three different opiate receptors in the canine central nervous system. They proposed the μ-receptor, preferentially recognized by morphine-like compounds; the κ-receptor, recognized by certain benzomorphans such as ketocyclazocine; and subsequently the σ-receptor, for which N-allylnorcyclazocine (SKF-10,047) was the prototype agonist (Martin, Eades, Thompson, Huppler & Gilbert, 1976; Gilbert & Martin, 1976).

These observations on the heterogeneity of opiate receptors have been matched by investigations of the interaction of the opioid peptides with opioid receptors. Evidence against the original expectation that the enkephalins were endogenous ligands for central morphine receptors (Hughes, 1975) was provided by Lord, Waterfield, Hughes and Kosterlitz (1976, 1977), who suggested that the opioid peptides and the opiate analgesics interact with different receptors. On the basis of differing agonist potencies in three in vitro assay systems, this group described a class of receptors (named δ-receptors) in the mouse vas deferens with which opioid peptides preferentially interacted (Lord et al, 1976). This proposal was reinforced by the use of antagonists. In the guinea-pig ileum, naloxone equally antagonized the enkephalins and normorphine, but in the mouse vas deferens, about ten times more naloxone was required to antagonize the action of the enkephalins than to block that of normorphine (Lord et al., 1977). These findings provided good evidence for the existence of receptors preferring morphine and its surrogates (μ-receptors) and those preferring the enkephalins (δ-receptors) in peripheral tissues.

In the brain, much of the evidence for separate μ- and δ-receptor populations comes from binding experiments. Investigations based on the inhibition of binding of labelled ligands in guinea-pig brain homogenates suggested the presence of both μ- and δ-receptors, corresponding to the peripheral subtypes (Lord et al., 1977; Kosterlitz, Lord, Paterson & Waterfield, 1980). Further studies using labelled enkephalins and opiate agonists and antagonists found two distinct binding sites in a

rat brain membrane preparation (Chang & Cuatrecasas, 1979), with variations in binding activity in different brain regions (Chang, Cooper, Hazum & Cuatrecasas, 1979). More definitive evidence was provided by selective protection experiments in which pre-exposure of brain homogenates with μ- or δ-ligands protected the respective binding site from irreversible inactivation by phenoxybenzamine (Robson & Kosterlitz, 1979) or N-ethylmaleimide (Smith & Simon, 1980). Regional variations in the density of μ- and δ-binding sites in rat brain have also been proposed from autoradiographic studies using relatively selective ligands (Goodman, Snyder, Kuhar & Young, 1980; Duka, Schubert, Wuster, Stoiber & Herz, 1981).

Binding studies with brain homogenates have also revealed a putative κ-binding site, which binds [^{3}H]-ethylketazocine but only minimally recognizes agonists for the other opioid receptor types (Kosterlitz & Paterson, 1980; Wood, Charleson, Lane & Hudgin, 1981; but see Hiller & Simon, 1980). In experiments on isolated peripheral tissues such as the guinea-pig ileum myenteric plexus and mouse and rabbit vas deferens, evidence has been obtained that the dynorphins may be endogenous ligands for the κ-opioid receptor (Chavkin, James & Goldstein, 1982; Corbett, Paterson, McKnight, Magnan & Kosterlitz, 1982; Yoshimura, Huidobro-Toro, Lee, Loh & Way, 1982; Oka, Negishi, Suda, Sawa, Fujino & Wakimasu, 1982). In the central nervous system, however, this proposal is more equivocal (Quirion & Pert, 1981; James, Chavkin & Goldstein, 1982). In addition, high-affinity stereospecific binding sites

for the 31-amino acid polypeptide β-endorphin have been found in rat brain membrane preparations (Ferrara, Houghten & Li, 1979; Hazum, Chang & Cuatrecasas, 1979; Akil, Hewlett, Barchas & Li, 1980), with a differential distribution of binding in different brain regions (Law, Loh & Li, 1979). In the rabbit spinal cord, β-endorphin binding sites are concentrated in the dorsal half of the cord (Ferrara & Li, 1980), and such sites which appear to preferentially recognize this peptide have been designated as ε-opioid receptors. Finally, specific binding sites for phencyclidine have been proposed as the σ-opiate receptor (reviewed by Zukin & Zukin, 1981), originally proposed by Martin and co-workers.

The Sites of Action of Morphine and M-ENKA

In interpreting the results of the present electrophoretic experiments, it is pertinent to consider the evidence for morphine-preferring (μ) and enkephalin-preferring (δ) receptors in the spinal cord. In the rat, binding studies using the selective radioligands [^{3}H]-morphine (μ sites) and [^{3}H]-D-ala^2, D-leu^5-enkephalin (δ sites) have revealed both types of receptor in dorsal roots as well as the dorsal horn, suggesting a location, at least in part, on primary afferent fibres (Fields, et al., 1980). A significant reduction of both μ- and δ-binding sites on dorsal roots followed sciatic nerve section suggesting a location of both types on primary afferents. These findings strengthen previous evidence for the location of a significant proportion of the opiate receptors of the upper dorsal horn on primary afferent fibres. Following dorsal

rhizotomy, Lamotte et al. (1976) observed a 40% reduction of [^{3}H]-naloxone binding in the upper dorsal horn of the monkey (although the possible contribution of transsynaptic degeneration was not discounted). A similar result has been obtained for [^{3}H]-diprenorphine binding in the rat dorsal horn following dorsal rhizotomy (Jessell, Tsunoo, Kanazawa & Otsuka, 1979) and after neonatal capsaicin treatment (Gamse, Holzer & Lembeck, 1979), which probably selectively destroys a population of nociceptive primary afferents (Jancso, Kiraly & Jancso-Gabor, 1977).

(a) Morphine

The results of the present experiments are readily explained by an action of morphine on morphine-preferring (μ) receptors located near the terminals of unmyelinated primary afferents in the substantia gelatinosa. This is consistent with the selective effects of morphine on the nociceptive modality observed both in these and previous (Duggan et al., 1977a) experiments. This selective effect of morphine might equally be observed by an action on substantia gelatinosa interneurones which received only nociceptive afferent information and projected to the deeper neurones from which recordings were obtained. If this were the case, however, then other cell excitants or depressants should have produced clear effects when administered in this region, including a similar action to that of morphine. The lack of consistent effects of DLH and GABA so administered in these experiments does not favour this explanation, but rather that of an action

of morphine on the unmyelinated fibres, which terminate predominantly in the substantia gelatinosa (Light & Perl, 1977).

Morphine acting near primary afferent terminals could presynaptically inhibit release of transmitter from these terminals. In the study of Jessell and Iversen (1977), morphine, normorphine and levorphanol as well as [D-ala]^2-Met-enkephalinamide suppressed the K^+-evoked release of substance P from rat trigeminal slices, an effect postulated to occur by an action at opiate receptors on primary afferent terminals. This opiate effect, which was stereospecific and blocked by naloxone, has been confirmed <u>in vivo</u> using the superfused spinal cord of cats and rats (Yaksh, Jessell, Gamse, Mudge & Leeman, 1980). Addition of morphine to the superfusing solution reduced both spontaneous substance P release from the cord and that evoked by electrical stimulation of nociceptive afferents of the sciatic nerve.

The mouse spinal cord-DRG explant preparation of the Crain group has also provided evidence for a presynaptic action of morphine. Low concentrations of the opiate in the bathing medium had similar effects to a number of enkephalins in depressing the DRG-evoked discharge in the dorsal cord, but not that in the ventral cord, in a naloxone-reversible manner (Crain, Peterson, Crain & Simon, 1977). A subsequent study found that the binding of [^{3}H]-diprenorphine was much higher in the outgrowing neurites of DRG cells than in the spinal cord, suggesting a location of opiate receptors on potential primary afferent fibres (Hiller, Simon, Crain & Peterson, 1978). Intracellular recordings from cultured mouse spinal

cord cells have shown that monosynaptic EPSPs evoked by DRG stimulation were reversibly depressed by etorphine administered electrophoretically between the DRG and the spinal cord cell (Macdonald & Nelson, 1978). This depression, which was dose (current)-dependent and reversible by electrophoretic naloxone, was attributed to a reduction in quantal content rather than in quantal amplitude, suggesting a presynaptic locus of action of the opiate. No change in membrane potential or conductance concurrently with the reduced EPSPs would have provided further evidence for this proposal, but the authors did not describe these postsynaptic parameters.

In terminal excitability experiments, two groups have reported an elevation in the threshold of nociceptive afferent terminals by morphine. Carstens, Tulloch, Zieglgansberger and Zimmermann (1979b) noted an increased threshold for the antidromic activation of sural C fibres by intraspinal stimulation following intravenous morphine, but no consistent effect was observed for A fibres. Intravenous naloxone, however, reversed this effect in only one-third of cases. In the experiments of Sastry (1978, 1979), both electrophoretic and intravenous administration of morphine in low doses produced a naloxone-reversible increase in the intraspinal threshold of C (and Aδ) sural primary afferents, similar to the effect of electrophoretic met-enkephalin. These low doses of morphine and met-enkephalin also potentiated PAD of these afferents produced by superficial peroneal nerve stimulation, an effect suggested to explain, at least in part, the analgesic actions of these compounds (Sastry, 1979, 1980). In experiments of this type,

results obtained with intravenous administration of vasoactive substances require cautious interpretation since haemodynamic changes can alter the distance relationship between the terminal and the intraspinal electrode, and blood pressure changes may not accurately reflect alterations in spinal cord circulation. The similar findings by two groups, however, reinforced by the electrophoretic results suggests a real effect. Although the observed changes in threshold were opposite in direction to that produced with group Ia afferent terminals by GABA, a putative transmitter at axo-axonic synapses (Curtis, Lodge & Brand, 1977), the relationship between terminal excitability and presynaptic inhibition may be different for C fibres. Another possible explanation is that reduced excitability of nociceptive afferents may block impulse propagation into the terminals, thereby decreasing transmitter release.

(b) M-ENKA

From the present experiments, limited conclusions can be reached regarding the structures affected by electrophoretically administered enkephalin. Since in binding assays with rat spinal cord neither morphine nor an enkephalin were completely selective ligands for μ and δ sites respectively (Fields et al, 1980), it is possible that the effects of M-ENKA administered in the substantia gelatinosa may have been partly due to activation of morphine-preferring receptors on unmyelinated fibres. The specificity of ligands is concentration dependent and since concentrations are not known in electrophoretic

experiments, the possibility of cross-activation of receptors is even greater in experiments of this type. At ventral sites however, the lack of effect of morphine with any dose used indicates that cross-activation of enkephalin-preferring receptors by the opiate did not occur.

The study of Fields et al. (1980) provided evidence for both enkephalin-preferring and morphine-preferring receptors on dorsal root fibres, with a relatively higher proportion of the latter. The evidence for actions of both enkephalin and morphine on primary afferent terminals has been discussed previously. Thus the possibility has to be considered that both morphine and M-ENKA may have acted predominantly on their respective receptors to presynaptically inhibit the release of nociceptive afferent transmitter.

Although the ejection of both morphine and M-ENKA in the substantia gelatinosa selectively reduced the nociceptive responses of deeper neurones with little effect on excitation by hair deflection, their effects were not identical since only M-ENKA consistently reduced spontaneous firing also. This suggests that the enkephalin acted at sites other than or in addition to those for morphine.

Ventral to the substantia gelatinosa, however, it is more difficult to explain the effects of M-ENKA by activity at receptors on the terminals of primary afferents. Morphine was inactive at these ventral sites and this hypothesis therefore requires a location of morphine-preferring receptors on unmyelinated fibres terminating in the substantia gelatinosa, but those penetrating this area and terminating more ventrally

would possess only enkephalin-preferring receptors. As discussed previously, ultrastructural studies of the distribution of enkephalins by immunohistochemical techniques do not support the proposals for a presynaptic inhibitory function at axo-axonic synapses in the dorsal horn.

The non-selective effects of M-ENKA ejected near cell bodies are readily explained by an action at inhibitory synapses on the somata, producing a hyperpolarization. The evidence for such an effect was reviewed earlier. In this case, the anatomical data are in agreement since terminals containing immunoreactive enkephalin and forming axo-somatic synapses in the dorsal horn are relatively common. The ultrastructural studies also reported many enkephalin-containing terminals forming axo-dendritic synapses in the dorsal horn, so similar inhibitory receptors for enkephalin may also be situated more distally on the dendrites of laminae IV and V neurones. The dendritic trees of many of these neurones extend dorsally up to the substantia gelatinosa (see Brown, 1982), and M-ENKA, ejected at these intervening sites, selectively reduced nociceptive responses. This selectivity requires that the receptors be preferentially located adjacent to synapses which receive nociceptive afferents. This implies a segregation of afferent inputs to different areas of the dendritic tree of these neurones, as illustrated in Figure 17. If the present results are explained in terms of activity by enkephalin at dendritic receptors, then it is perhaps surprising that DLH did not consistently excite neurones when administered at the same sites as the enkephalin. The variable effects of DLH, however,

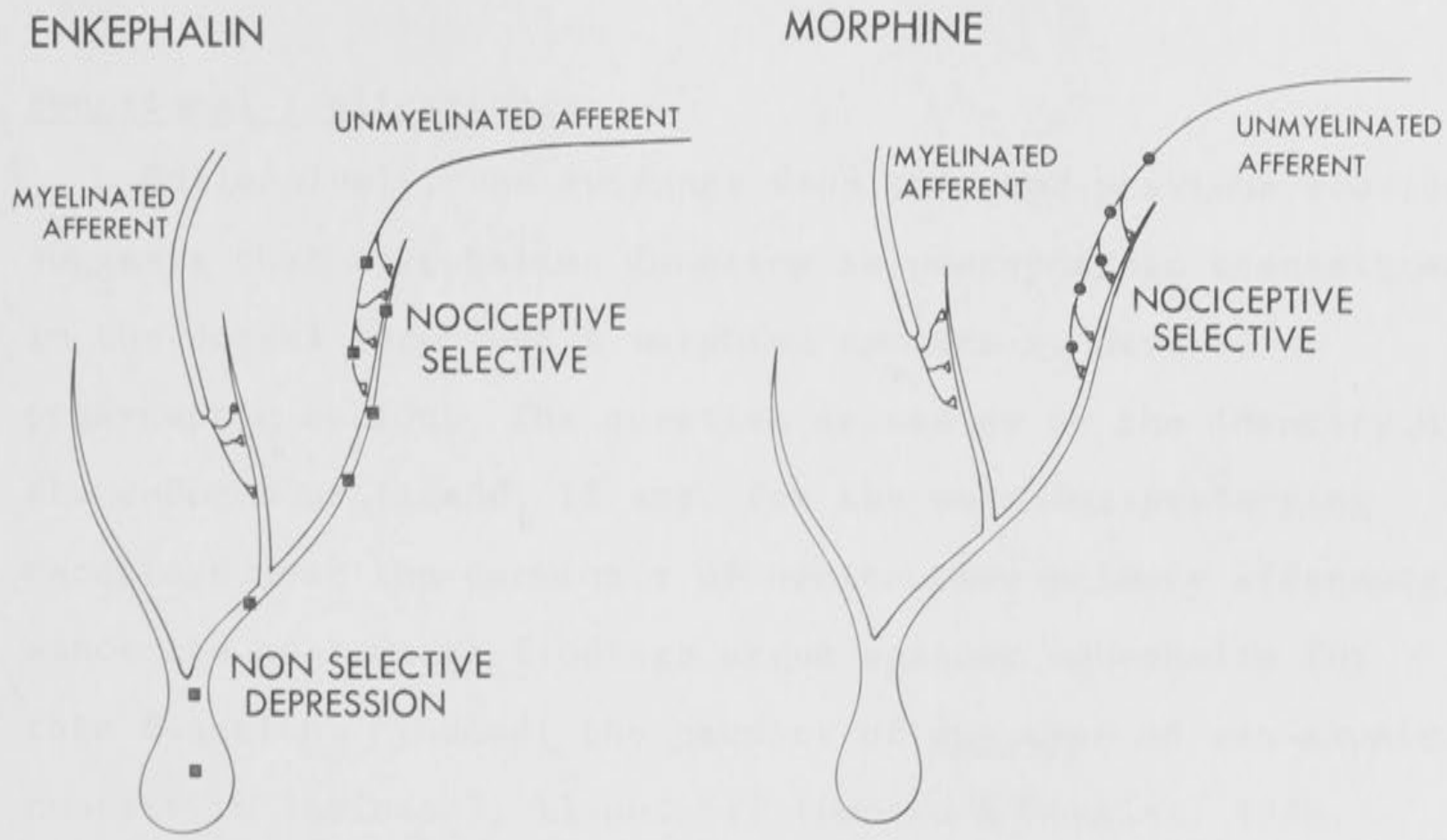


Fig. 17. A proposed distribution of receptors for enkephalin and morphine in the dorsal horn of the cat spinal cord. Two drawings of the one hypothetical neurone have been used for clarity. The neurone is in lamina IV or V with its dendrites projecting dorsally, and myelinated and unmyelinated primary afferents are shown terminating on different dendritic regions. Receptors for enkephalin are indicated by the filled squares on the unmyelinated primary afferent and the cell body and a dendrite of the neurone. Receptors for morphine are indicated by the filled circles on the unmyelinated primary afferent.

may have resulted from complex effects on local interneurones both excitatory and inhibitory as well as directly on the cells studied.

Functional Implications

Collectively, the evidence from this and previous studies suggests that enkephalins function as postsynaptic transmitters in the dorsal horn, while morphine appears to have a presynaptic action. The question arises as to the identity of the endogenous ligand, if any, for the morphine-preferring receptors near the terminals of nociceptive primary afferents, since the anatomical findings argue against enkephalin for this function. Indeed, the paucity of any type of axo-axonic contact in laminae I, II and III (Duncan & Morales, 1978; Ralston, 1979; Zhu, Sandri & Akert, 1981) raises the possibility that such opiate receptors may not be acted upon in the conventional sense by synaptically-released transmitter. There is evidence that these receptors are not restricted to the terminal regions of nociceptive primary afferents in the substantia gelatinosa, but are also prevalent for some distance along the incoming fibres in the dorsal roots (Wamsley, Zarbin, Young & Kuhar, 1982). If these receptors have a physiological role, they may be activated by a circulating ligand and are ideally located to block the propagation of incoming impulses into nociceptive afferent terminals, thus reducing transmitter release.

Thus it may have been a premature assumption that the pharmacology of the opiates would be an accurate predictor of

the physiology of the opioid peptides. The evidence suggests a much wider role for these peptides in the spinal cord than merely regulation in nociception, and this concept is supported by the reflex and motoneurone experiments as discussed below.

Reflexes

The results obtained in the study of spinal reflexes have confirmed and extended the findings of some other groups (Goldfarb & Hu, 1976); Bell & Martin, 1977) on the effects of naloxone on these reflexes in the cat. Naloxone increased all of the ventral root recorded reflexes examined in the present experiments, indicating that naloxone-sensitive inhibition of many motoneurones was present under the experimental conditions used. Moreover, low doses (0.025 to 0.10 mg/kg) of the antagonist produced these effects and similar activity was shown by the (-) but not (+)-isomer of another opiate antagonist, FMN. These two observations favour interpretation of the effects of the antagonists as resulting from block of the action of opioid peptides.

As noted by Goldfarb and Hu (1976), the inhibition present on reflexes showed no functional specialization, as monosynaptic reflexes to impulses in large diameter afferents in both flexor and extensor muscles, and polysynaptic reflexes to impulses in myelinated afferents of a cutaneous nerve and in myelinated and unmyelinated afferents of a mixed muscle-cutaneous nerve, were all enhanced by naloxone. Also in agreement with these authors, there was a large variation in the magnitude of the reflex increases, both among different

types of reflex in the one animal and for the one reflex type in different experiments. Although reflexes recorded in ventral roots were consistently enhanced by naloxone, the reflex recorded in the M-G nerve following stimulation of large diameter afferents of the sural nerve was increased by naloxone (0.05 mg/kg) in only one of five experiments. In contrast, Catley and Pascoe (1978) found that naloxone (0.005 mg/kg) enhanced this reflex in the decerebrate spinal rabbit although the incidence of this effect and numbers of experiments performed were not described by the authors. If this effect was consistently produced, then there is no apparent reason for the discrepancy between their results and the present experiments.

Catley and Pascoe (1978) also found that conditioning stimulation of small diameter afferents of the ipsilateral sural, lateral or medial popliteal or medial or lateral gastrocnemius nerves at 50 Hz for 10 seconds potently inhibited the sural to M-G reflex for up to 30 minutes, and that naloxone (0.05 mg/kg) abolished this inhibition. Block of such inhibition by naloxone was confirmed by the present results in anaesthetized cats using tibial conditioning and (-)-FMN in addition to naloxone, but the opioid antagonist-sensitive inhibition lasted for less than 3 minutes.

When an antagonist is used to block the action of a putative neurotransmitter, knowledge of its specificity for the transmitter is important. Both <u>in vitro</u> and <u>in vivo</u> experiments have indicated that naloxone is a relatively specific antagonist of opiates and opioids (see Duggan &

Johnson, 1983). The evidence discussed previously indicates that antagonism of the action of GABA required high concentrations of naloxone and the effect was not stereospecific. It is unlikely that such concentrations were attained near spinal cord receptors in the present experiments by the intravenous administration of 0.05 to 0.10 mg/kg of naloxone, doses which have proved adequate to antagonize the effects of enkephalin administered in the substantia gelatinosa (Duggan et al., 1977b). Thus it is highly improbable that enhanced spinal reflexes by 0.05 mg/kg of naloxone were due to antagonism of a GABA-mediated inhibition. The similar results obtained with (-) but not (+) FMN are further evidence against involvement of GABA.

It is not possible to completely exclude an excitant action by naloxone, unrelated to antagonism of the action of opioid peptides, as being responsible for the enhancement of spinal reflexes. Such an effect of naloxone is extremely rare, however, when it is administered electrophoretically near cat spinal neurones (Duggan et al., 1976, 1977b; Zieglgansberger & Bayerl, 1976; Zieglgansberger & Tulloch, 1979) and depression of firing has been observed more often (Duggan et al., 1976). On the dual considerations of dose and stereospecificity of antagonist action, it is much more likely that the enhancement of reflexes observed in the present experiments resulted from antagonism of inhibition by opioid peptides.

Since transmission of a monosynaptic reflex occurs before any inhibitory interneurones also activated by the peripheral nerve stimulus can act upon motoneurones, enhancement of these

reflexes by the opioid antagonists must have resulted from either an enhanced release of transmitter from primary afferent terminals or a raised level of excitability of motoneurones. In either case this indicates the presence of tonic inhibition exerted on motoneurone firing and involving opioid peptides. If this is also correct for the motoneurones participating in the other (polysynaptic) reflexes studied, it indicates that, under the conditions of these experiments, many motoneurones were under tonic inhibition which involved opioid peptides at some stage.

Motoneurones

Intracellular recordings from motoneurones during naloxone administration confirmed the results of the reflex experiments by showing that opioid peptidergic inhibition not only affected many motoneurones, but that the responses of each neurone to a variety of afferent inputs of both muscle and cutaneous origin were inhibited. With some neurones, the release from inhibition was accompanied by the commencement of spontaneous firing and the production of action potentials from nerve stimuli which previously evoked only subthreshold EPSPs. Since recruitment of previously silent neurones rather than repetitive firing of active motoneurones increased the amplitude of the monosynaptic reflexes, such intracellular results were probably direct observations of this process.

Opioids could produce tonic inhibition of motoneurones by directly mediating either presynaptic inhibition of transmitter release from primary afferent terminals or postsynaptic

inhibition of motoneurones. Alternatively such inhibition could be effected by inhibition of interneurones tonically exciting motoneurones, or by inhibition exerted on the first of a chain of inhibitory interneurones which ultimately inhibits the motoneurone. The experiments on reflexes could not decide which of these possibilities was the likely underlying process, but the intracellular results did provide some information relevant to elucidating the mechanism involved.

In these experiments, it was clear that EPSPs could be increased in the absence of changes in membrane potential and conductance. In these instances it is reasonable to conclude that naloxone did not enhance EPSPs by block of a postsynaptic inhibition on the somata of the recorded motoneurones. The significance of the depolarization observed with approximately one-third of cells is uncertain. It could indicate that there was a postsynaptic element in the inhibition reduced by naloxone but it could equally have resulted from an increased input of spontaneously occurring EPSPs although this was not detected in filmed records. An apparent depolarization from a deterioration in recording conditions cannot be excluded since the doses of naloxone used commonly produced a gradual elevation of 5 to 10 mmHg in blood pressure and thus could have altered circulation through the cord with its associated recording problems.

Inhibition which reduces EPSPs in the absence of changes in somatic membrane potential or conductance is the usual description of presynaptic inhibition (Frank & Fuortes, 1957; Eccles, Schmidt & Willis, 1962). As discussed previously, the evidence for the participation of enkephalins in such a process

in the spinal cord is incomplete. In particular, electron microscopic studies of the dorsal horn have described a relative paucity of axo-axonic synapses containing enkephalin, most of the peptide being localized within axo-somatic and axo-dendritic synapses (Hunt et al., 1980; Aronin et al., 1981). There are no comparable ultrastructural accounts of enkephalin-containing neural elements in the ventral horn, but by light microscopy enkephalin-positive axons and terminals have been found in this region, in close proximity to the somata and dendrites of presumed motoneurones (Aronin et al., 1981; Lamotte & De Lanerolle, 1981). Thus although enkephalin-containing terminals appear to be present near motoneurones, the available anatomical data give little support for enkephalin acting as a presynaptic transmitter released at axo-axonic synapses. Tonic opioid inhibition could effectively act presynaptically, however, through controlling the activity of GABA-releasing neurones mediating presynaptic inhibition, and thus, even with monosynaptic EPSPs, it is not necessary to postulate that the peptide acts directly on primary afferent terminals to ultimately inhibit motoneurones.

Exogenously administered enkephalin has hyperpolarized neurones in some (North, Katayama & Williams, 1979; Pepper & Henderson, 1980; Murase et al., 1982) but not all (Barker et al., 1978) in vitro preparations and this effect is consistent with the GABA-like non-selective depression of cat dorsal horn neurones by enkephalin in vivo (Duggan et al., 1977b; the present experiments). The failure by some workers to observe this effect could result from a different type of postsynaptic

action by this peptide on some neurones. When enkephalin was electrophoretically administered near cat motoneurones, Zieglgansberger and Tulloch (1979) did not observe changes in membrane potential or conductance despite a decrease in depolarization by glutamate. Electrophoretic naloxone partially blocked this effect, but per se did not alter membrane conductance. These results, which suggest a neuromodulatory role for enkephalin, are not inconsistent with the effect of intravenous naloxone in the present experiments.

Tonic opioid inhibition of motoneurones is an unexpected result since as previously described, several lines of evidence have associated opioid peptides such as enkephalin with the spinal transmission of nociceptive information, although a wider physiological role has been suggested, based on its effects when administered near dorsal horn neurones (Duggan et al., 1977b) and on its very wide distribution in the central nervous system (Hokfelt et al., 1977a). However, it needs to be pointed out that the present experiments in no way indicate the identity of the opioid peptide mediating this inhibition of motoneurones. Rather than an enkephalin therefore, the relevant opioid might equally be β-endorphin, or even dynorphin which has been found (by radioimmunoassay) in high levels in the spinal cord (Goldstein & Ghazarossian, 1980; Botticelli, Cox & Goldstein, 1981). The use of opioid antagonists which are selective for the receptor subtypes may offer a means of discrimination for future experiments.

The stimulus for the release of opioid peptides in the present experiments was not known. The cats had been subjected to extensive surgery some hours before naloxone administration, and it is possible that this surgery was the stimulus since there is evidence that afferent impulses related to pain are associated with enkephalin release. Cesselin et al. (1982) noted increased levels of immunoreactive enkephalin in cat cisternal CSF following nociceptive input (tooth pulp stimulation), and Yaksh and Elde (1981) also observed this in ventricular and spinal cord perfusates following stimulation of the sciatic nerve at C- but not A-fibre strength. Blocking ascending and descending spinal conduction by cooling prevented ventricular but not spinal enkephalin release, indicating that spinal neurones were responsible (Yaksh & Elde, 1981). In the experiments of Catley and Pascoe (1978) and the present series it was necessary to excite small diameter primary afferents of a hind limb nerve to produce naloxone-sensitive inhibition of the sural to gastrocnemius reflex.

When administered centrally, enkephalin has a relatively short duration of action compared with morphine. This has been noted both for the analgesia produced by intracranial injection (Belluzzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976; Buscher, Hill, Romer, Cardinaux, Closse, Hauser & Pless, 1976) and for its effects when ejected electrophoretically in the dorsal horn (Duggan et al., 1977b; the present experiments) suggesting rapid enzymatic degradation of these peptides. Thus, if impulses related to pain do release enkephalin to tonically inhibit motoneurones, then a continuous nociceptive afferent input, such as in the present experiments, may be required to

detect opioid inhibition some hours after surgery. It is likely that the tibial conditioning represented additional nociceptive input via Aδ and C fibres and the short duration of its effect is consistent with a further release of enkephalin.

It is unlikely that the tonic opioid inhibition revealed in these experiments has any relevance to analgesia. An inhibition of motoneurones of differing functional types would tend to restrict all movements. Immobility has been observed when opioids have been administered intrathecally to the lower spinal cord of rats, particularly with dynorphin (Herman, 1982). Restriction of movement is not an appropriate response during the inflicting of injury (analgesia permits an organized response to prevent further injury) but it is appropriate subsequently, as part of the repair process. Thus an important function of opioid peptides in the spinal cord may be to partly immobilize an injured area. Wall (1979) has described sequential behavioural responses to injury in a number of species and the later stages were marked by pain and reduced movement.

This hypothesis may also explain the inconsistent effects of naloxone on the responses of neurones in the dorsal horn in preparations similar to those of the present experiments. These and previous studies (Duggan et al., 1977c; Sinclair et al., 1980) have shown that changes in the responses of these cells in the cat are rarely seen with intravenous naloxone. This suggests that under the conditions of these experiments, many hours after surgery, opioids are not acting in the dorsal horn on the enkephalin receptors which, when activated,

readily reduce the firing of laminae IV and V neurones. Nevertheless, small consistent effects of intravenous naloxone on dorsal horn neurones have been reported for the cat (Henry, 1979), larger changes seen in the rat (Rivot et al., 1979; Fitzgerald & Woolf, 1980), and such changes have been related to analgesia. It is possible, however, that the anaesthetized cat subjected to extensive surgery is not a suitable preparation to study the possible role of opioid peptides in analgesia. Such analgesia may occur rapidly and transiently as an initial response to injury so as to permit an organised response where motor performance is not disrupted by reflexes to nociceptive afferents. Several hours after injury, however, the action of opioids most readily observed appears to be in the ventral horn rather than the dorsal horn. This hypothesis requires that opioid peptides be released to alter the activity of different neurones at different times after injury. In neurophysiological experiments of this type, however, the time course of the release of opioid peptides immediately following injury cannot be studied due to the time required for animal preparation.

CHAPTER IV - SUPRASPINAL INHIBITION OF DORSAL HORN NEURONES

INTRODUCTION

Analgesia from brain stem stimulation

One of the more remarkable observations in neurobiology is the production of analgesia from electrical stimulation of discrete regions in the central nervous system. The first reports of this phenomenon appeared in the mid-1950s. Using implanted electrodes, it was found that electrical stimulation of the septal area in man repeatedly produced immediate relief from intractable pain (Heath, 1954; Heath & Mickle, 1960). A similar result was also obtained by Pool and colleagues (Pool, 1954; Pool, Clark, Hudson & Lombardo, 1956) who observed analgesia in patients following electrical stimulation near the supra-optic nuclei. Subsequently, a series of studies commencing in the early 1960s showed that stimulation of the thalamic nucleus ventralis posterolateralis effectively suppressed pain (see Mazars, Merienne & Cioloca, 1979). This group maintained, however, that only pain due to sensory deafferentation, rather than "nociceptive" pain (cancer, posterior root compression), was susceptible to thalamic stimulation. In a study with a single patient with intractable pain, stimulation of the caudate nucleus produced pronounced pain relief (Ervin, Brown & Mark, 1966). Gol (1967), however, obtained disappointing results with septal stimulation, satisfactory pain relief being achieved in only one of six patients. In experimental animals, an early study found that

stimulation of the lateral hypothalamus of rats attenuated the effects of a painful peripheral stimulus (footshock) (Cox & Valenstein, 1965).

One of the most convincing demonstrations of this stimulation-produced analgesia was that of Reynolds (1969), who reported that stimulation at the dorsolateral perimeter of the midbrain PAG of rats induced sufficient analgesia to abolish aversive responses to noxious pinch and to permit laparotomy in the absence of anaesthesia. Since the animals were normalgesic before and shortly after stimulation, the effect was attributed to the stimulation rather than possible trauma from electrode placement. In confirming this observation, Mayer et al. (1971) also reported analgesia from stimulation of the ventral tegmentum and dorsal and medial thalamus. Subsequent studies in the rat clearly established that the midbrain PAG, especially the ventral aspect, is an effective region for the production of analgesia by electrical stimulation (Akil & Mayer, 1972; Mayer & Liebeskind, 1974; Akil & Liebeskind, 1975; Akil et al., 1976; Young, Yaksh & Rudy, 1977; Lewis & Gebhart, 1977; Oleson, Twombly & Liebeskind, 1978; Mohrland & Gebhart, 1980; Swajkoski, Mayer & Johnson, 1981). In most of these studies, analgesia was assessed by variations of the classic analgesic test, tail flick in a rodent in response to a noxious stimulus (D'Amour & Smith, 1941), using either radiant heat or strong pinch. In addition to pain of somatic origin, visceral pain (induced by intraperitoneal injection of hypertonic saline) was also found susceptible to PAG stimulation (Giesler & Liebeskind, 1976).

Investigations in the cat have yielded similar results. Electrical stimulation in the PAG in this species evoked comparable analgesia, tested by abolition of aversive reactions to noxious pinch (Liebeskind, Guilbaud, Besson & Oliveras, 1973; Oliveras, Besson, Guilbaud & Liebeskind, 1974a; Oliveras, Guilbaud & Besson, 1979), by inhibition of a nociceptive reflex (the jaw opening reflex to tooth pulp stimulation) (Oliveras, Woda, Guilbaud & Besson, 1974b; Oliveras et al., 1979), or by reduction of pain-evoked behaviour following either subcutaneous injection of formal saline (Melzack & Melinkoff, 1974) or radial nerve stimulation at high intensities (Gebhart & Toleikis, 1978).

In monkeys, reports of analgesia from PAG stimulation are less consistent. Some studies in this species have described an absence of antinociceptive effects from electrical stimulation of the periventricular-periaqueductal gray region (Pert & Yaksh, 1974; Oleson, Kirkpatrick & Goodman, 1980), or even a decreased pain threshold (Schmidek, Fohanno, Ervin & Sweet, 1971). In contrast, other investigations have noted clear attenuation of behavioural responses to noxious heat with PAG stimulation (Goodman & Holcombe, 1976; Hayes, Price, Ruda & Dubner, 1979).

The other brain stem regions extensively studied in this context are the raphé nuclei of the medulla. In the rat, analgesia produced by electrical stimulation of the nucleus raphé magnus (NRM) was reported by Proudfit and Anderson (1975). This observation was subsequently confirmed by other groups (Oleson et al., 1978; Satoh, Akaike, Nakazawa & Takagi,

1980; Zorman, Hentall, Adams & Fields, 1981; Zorman, Belcher, Adams & Fields, 1982). In the cat also, these midline structures have proved to be potent sites for the elicitation of stimulation-produced analgesia (Oliveras, Redjemi, Guilbaud & Besson, 1975; Oliveras, Hosobuchi, Redjemi, Guilbaud & Besson, 1977; Oliveras, Hosobuchi, Guilbaud & Besson, 1978; Oliveras et al., 1979). In this species, a comparison of the different raphé nuclei from the midbrain to the caudal medulla (raphé dorsalis, raphé centralis superior, and raphé centralis inferior including raphé pontis, raphé magnus, raphé obscurus and post-pyramidal raphé nucleus) found that the most potent effects were obtained by electrical stimulation in the raphé magnus (Oliveras et al., 1979). In the monkey, stimulation-produced analgesia from NRM has been reported, although this region was much less effective than diencephalic sites (Oleson et al., 1980).

In experimental animals, stimulation at a number of other supraspinal sites has produced analgesia. These seldom-investigated, but nevertheless effective, regions include the septum in rats (Breglio, Anderson & Merrill, 1970) and monkeys (Schmidek et al., 1971; Oleson et al., 1980), the caudate nucleus (Schmidek et al., 1971; Lineberry & Vierck, 1975), cerebellum (Siegel & Wepsic, 1974), medial geniculate body (Schmidek et al., 1971), anterior medial thalamus, mammillary nucleus and preoptic nucleus (Oleson et al., 1980) in monkeys, the lateral hypothalamus in the region of the medial forebrain bundle in rats (Balagura & Ralph, 1973; Yunger, Harvey & Lorens, 1973) and monkeys (Black, Cianci & Markowitz, 1972),

and the medial posterior thalamus (Rhodes & Liebeskind, 1978; Oleson et al., 1978), nucleus reticularis paragigantocellularis (NPGC) (Akaike, Shibata, Satoh & Takagi, 1978; Satoh et al., 1980) and locus coeruleus and substantia nigra (Segal & Sandberg, 1977; Sandberg & Segal, 1978) in rats.

The effectiveness of this technique for pain relief in man is more controversial (reviewed by Sweet, 1977; Sedan & Lazorthes, 1978; Gybels, 1979). In addition to the early studies cited previously, stimulation of the posterior ventralis medialis thalamic nucleus adequately controlled pain from facial anaesthesia dolorosa (Hosobuchi, Adams & Rutkin, 1973), and internal capsular stimulation was reported effective against pain secondary to central nervous system injury (Fields & Adams, 1974; Adams, Hosobuchi & Fields, 1974). Since the animal experiments had clearly demonstrated the effectiveness of the mesencephalic PAG, this region was also stimulated in human studies, but the analgesia produced was associated with undesirable side effects such as nystagmus, nausea, and vertigo (Richardson & Akil, 1977a). For this reason, stimulation in the rostral extension of the PAG, the periventricular gray (PVG), at approximately the level of the posterior commissure, has been preferred, with success reported by several groups (Adams, 1976; Hosobuchi, Adams & Linchitz, 1977; Richardson & Akil, 1977a,b; Hosobuchi, Rossier, Bloom & Guillemin, 1979a; Meyerson, Boethius & Carlsson, 1979). It was noted, however, that although chronic intractable pain was relieved by this procedure, acute pain thresholds to noxious heat or mechanical stimuli (such as are used in analogous animal experiments) were

elevated less consistently (Hosobuchi et al., 1977; Gybels, 1979). The effectiveness of medial thalamic sites was confirmed in a recent study by Boivie and Meyerson (1982), with all stimulation sites producing good pain relief being localized post-mortem to the PVG or the transitional zone between PVG and the medialis and parafascicular nuclei. In contrast, others have found little or no analgesia or pain relief to result from electrical stimulation of PAG, PVG and thalamic sites (Cosyns & Gybels, 1979; Mazars et al., 1979). Although several authors have emphasized that electrode tip location is critical, particularly in the medio-lateral direction, the differences between investigators have not been satisfactorily explained.

The latency and duration of analgesia produced by supraspinal stimulation vary with species. In man, latencies of 5 to 15 minutes following commencement of stimulation have been reported (Adams, 1976; Hosobuchi et al., 1977), with pain relief lasting many hours after cessation of stimulation (Hosobuchi et al., 1977; Richardson & Akil, 1977a), although short duration analgesia (minutes) has been observed (Boivie & Meyerson, 1982). In contrast, onset of analgesia is rapid (within seconds), and outlasts the period of stimulation by only seconds to minutes, in rats (Mayer et al., 1971; Satoh et al., 1980), cats (Oliveras et al., 1974a, 1975) and monkeys (Hayes et al., 1979). Human and animal studies are also at variance with respect to the peripheral field of analgesia induced by supraspinal stimulation. In the former, generalized pain relief unassociated to a dermatomal pattern has usually

been observed (Hosobuchi et al., 1977; Richardson & Akil, 1977a), while incomplete body coverage, commonly restricted to the extremities (tail and limbs), has been described for the latter (Mayer et al., 1971; Balagura & Ralph, 1973; Mayer & Liebeskind, 1974). A recent extensive study of the somatotopic organization of midbrain sites in the rat related dorsal sites to a rostral peripheral field of analgesia, and progressively ventral sites to more caudal fields (Soper & Melzack, 1982), but there is no comparable description for other species including man. These differing characteristics of stimulation-produced analgesia in humans and animals suggest different mechanisms of action, but are complicated by psychological aspects with humans. All investigators are agreed, however, that analgesia is usually not accompanied by other sensory deficits.

Neurophysiological Studies of Stimulation-produced Analgesia

The initial descriptions of analgesia from stimulation of PAG regions were impressive (Reynolds, 1969; Mayer et al., 1971), but the neural substrates responsible were not known. Since the direct application of morphine to these sites had previously been shown to produce potent analgesia (Herz, Albus, Metys, Schubert & Teschemacher, 1970), it was suggested that a common descending inhibitory pathway may mediate both types of analgesia (Mayer et al., 1971). (There is an extensive literature on the analgesic effects of microinjection of opiates into supraspinal structures, and the relationship between analgesia produced by supraspinal stimulation and that

produced by opiates. This has been reviewed by Yaksh and Rudy (1978) and Willis (1982), and it is not intended to discuss these studies in this account). Many previous studies had documented the existence of descending inhibitory systems exerting effects in the spinal cord. Magoun and Rhines (1946) had shown that stimulation of medial reticular areas inhibited stretch reflexes and movements from stimulation of the cerebral cortex. A more lateral area facilitated these responses. Tonic descending inhibition of activity in flexion reflex pathways had been described in both decerebrate and barbiturate-anaesthetized cats by Lundberg and his colleagues (Holmqvist & Lundberg, 1959; Eccles & Lundberg, 1959; Holmqvist, Lundberg & Oscarsson, 1960). Lesion experiments revealed that this inhibition originated in the medial part of the caudal brain stem (Holmqvist & Lundberg, 1961; Engberg, Lundberg & Ryall, 1968a). In terminal excitability experiments, electrical stimulation of this region produced PAD in the lumbar cord, suggesting the existence of presynaptic inhibitory mechanisms (Carpenter, Engberg & Lundberg, 1962, 1966). It was later established that such stimulation, when subthreshold for PAD production, could inhibit transmission in flexion reflex pathways, and intracellular results led this group to conclude that this inhibition was exerted on interneurones (Engberg, Lundberg & Ryall, 1968b,c). Partial cord transection indicated the descent of this pathway, named the dorsal reticulospinal system, via the dorsolateral funiculus (DLF) (Engberg et al., 1968b). Most of these experiments on reflexes have been primarily concerned with the control of movements and more

detailed studies of this are beyond the scope of this discussion. Studies of descending controls of neurones in the dorsal horn excited by noxious peripheral stimuli are relevant and these will be examined at length.

Inhibition of spinal neurones by supraspinal stimulation in the cat was described by Taub (1964). Electrical stimulation of cerebellar nuclei and a number of mesencephalic and medullary regions inhibited the spontaneous, nociceptive and non-nociceptive responses, and reduced the cutaneous receptive fields, of cells in the spinocervical tract. The effective supraspinal sites in the pontomedullary tegmentum included part of the midbrain PAG and ventral tegmentum, and the inhibition was clearly not modality-selective. Similar non-selective inhibition of the activities of laminae IV and V neurones of the cat lumbar cord was observed following electrical stimulation in the pyramidal tract (Fetz, 1968) and the orbital cortex (Wyon-Maillard, Conseiller & Besson, 1972).

A close parallel between the production of analgesia and inhibition of spinal neurones by supraspinal stimulation in the cat was drawn by Besson and his colleagues. Electrical stimulation at sites in the ventral PAG adjacent to the dorsal raphé nucleus (DRN) produced profound analgesia and, in the anaesthetized animal, inhibited the responses of lamina V neurones to noxious cutaneous stimuli (Liebeskind et al., 1973; Oliveras et al., 1974a). When both nociceptive and non-nociceptive responses were examined with multireceptive neurones, midbrain stimulation selectively inhibited nociceptive responses in

about half of these cells (Oliveras et al., 1974a). Similar results were subsequently obtained in the monkey. Electrical stimulation in the PAG reduced behavioural responses to noxious heat stimuli and selectively inhibited the nociceptive responses of multireceptive spinothalamic neurones (Hayes et al., 1979). Such preferential inhibition of excitation by painful stimuli seems appropriate to behavioural analgesia in the awake animal.

Since this definitive work, a large number of investigators have shown that electrical stimulation of the supraspinal regions eliciting analgesia in experimental animals inhibits the excitation of dorsal horn and trigeminal neurones by noxious cutaneous stimuli. In the cat, the effects of midbrain stimulation have been confirmed largely by Zimmermann's group. The responses of dorsal horn neurones to noxious heating of the skin were inhibited by electrical stimulation in both dorsal and ventral PAG (Carstens, Yokota & Zimmermann, 1979c), with the ventral PAG having a lower threshold for inhibition (Carstens, Klumpp & Zimmermann, 1980b). When nonnociceptive responses (hair deflection) of these neurones were also examined, both were similarly inhibited at low PAG stimulus intensities, although at higher currents heat responses were inhibited to a greater extent (Carstens et al., 1980b). These effects were not confined to the PAG, however, as stimulation in the lateral reticular formation of the midbrain also inhibited responses to both sensory modalities, with a relatively greater effect on heat (Carstens, Klumpp & Zimmermann, 1980a; Carstens, Bihl, Irvine & Zimmermann, 1981a).

Analysis of the effect of the inhibition on the temperature-response relationship of dorsal horn neurones suggested that different inhibitory systems were activated from these two regions. Stimulation in the lateral reticular formation shifted the temperature-response curves of neurones to the right whereas PAG stimulation reduced the slope of these curves. Although these studies showed a partial selectivity, Duggan and Griersmith (1979a) observed a non-selective reduction to both noxious heat and hair deflection responses of dorsal horn neurones by stimulation near the DRN, adjacent to the ventral PAG.

With the medullary raphé nuclei of the cat, a similar picture has emerged, with electrical stimulation of the central inferior raphé nucleus (CI), particularly the NRM, reducing the responses of dorsal horn neurones to noxious cutaneous stimuli (Le Bars, Menetrey & Besson, 1976b; Basbaum, Clanton & Fields, 1976; Guilbaud et al., 1977; Fields, Basbaum, Clanton & Anderson, 1977; Griersmith et al., 1981). With respect to modality selectivity, Belcher, Ryall and Schaffner (1978) reported that CI stimulation produced a greater incidence, degree and duration of inhibition on cells responsive to a noxious stimulus (bradykinin injection into the receptive field circulation) than on non-responsive cells, while examinations of multireceptive neurones during CI stimulation have shown a selective reduction of responses to noxious, but not innocuous, cutaneous stimuli (Guilbaud et al., 1977; Duggan & Griersmith, 1979a). One study, however, of spinothalamic neurones of the cat found that NRM stimulation produced a biphasic effect,

first excitation then a prolonged depression, on responses to mechanical pressure, including non-noxious intensities of this peripheral stimulus (McCreery, Bloedel & Hames, 1979). In a comparative study of different raphé nuclei, the NRM was found the most effective for stimulation-produced inhibition of dorsal horn neuronal responses to impulses in C fibres (Griffith & Gatipon, 1981). This region was equieffective with the ventral PAG, but similar stimulation in the DRN was less effective.

With trigeminal neurones of the cat, stimulation in PAG and NRM regions has usually produced non-selective inhibition. With neurones in nucleus reticularis dorsalis (probably analogous to lamina V of the spinal dorsal horn), non-selective inhibition of responses to nociceptive (tooth pulp) input and non-noxious mechanical stimulation was observed with stimulation in the ventral PAG, but for cells in subnucleus caudalis (analogous to lamina I) nociceptive responses were selectively reduced (Yokota & Hashimoto, 1976). In contrast, other studies, classifying subnucleus caudalis neurones as either nociceptive or non-nociceptive, have described inhibition of both neurone types following PAG and NRM stimulation (Andersen, Lund & Puil, 1978a; Hu & Sessle, 1979; Dostrovksy, 1980; Sessle, Hu, Dubner & Lucier, 1981), although one report did note inhibition of excitation by impulses in Aβ, Aδ, and C fibres (Sessle et al., 1981). One study of trigeminal nuclei oralis and caudalis found that PAG stimulation non-selectively inhibited multireceptive cells, but was relatively ineffective against the non-nociceptive responses of neurones without a

nociceptive input (Sessle, Dubner, Greenwood & Lucier, 1976). Other studies of these nuclei found that NRM stimulation powerfully inhibited nociceptive neurones with little or no effect on non-nociceptive neurones, although multireceptive cells were apparently not examined (Lovick, West & Wolstencroft, 1977; Lovick & Wolstencroft, 1979).

In rats, PAG stimulation inhibited the nociceptive responses of many multireceptive dorsal horn neurones, although the effects of stimulation on non-nociceptive responses of the same neurones were not tested (Bennett & Mayer, 1979). Cells responding only to non-noxious inputs, however, were not inhibited. Also in rats, PAG stimulation selectively inhibited activity in ascending spinal cord axons evoked by electrical stimulation of sural Aδ and C-fibre afferents, with no effect on Aβ activity (Jurna, 1980). Stimulation of NRM in this species, however, inhibited spinal dorsal horn neurones, but short-latency (Aα) responses as well as noxious heat and C-fibre responses were reduced (Rivot et al., 1979; Rivot, Chaouch & Besson, 1980). As in cats, trigeminal neurones excited by tooth pulp stimulation were inhibited by NRM stimulation (Dickenson, Hellon & Woolf, 1981).

In the monkey also, the predominant effect of NRM stimulation has been non-selective inhibition. With excitation of spinothalamic neurones by impulses evoked by electrical stimulation of the sural nerve, NRM stimulation inhibited responses to impulses in C and Aδ-fibres to a greater extent than those to impulses in large myelinated A-fibres (Beall, Martin, Applebaum & Willis, 1976; Willis, Haber & Martin, 1977;

Gerhart, Wilcox, Chung & Willis, 1981a). When natural cutaneous stimuli were examined, however, both nociceptive (noxious heat, pinch) and non-nociceptive (light touch, hair deflection) responses of most neurones were equally inhibited by NRM stimulation (Willis et al., 1977; Gerhart et al., 1981a).

Electrical stimulation of other less-studied supraspinal regions known to elicit analgesia has also produced inhibition of spinal neurones. The responses of dorsal horn neurones of the cat to noxious cutaneous heat were inhibited by stimulation at medial hypothalamic sites, especially the PVG region (Carstens, 1982), and in medial preoptic and ventromedial septal regions (Carstens, Mackinnon & Guinan, 1982). In the monkey, stimulation of the ventral posterolateral thalamic nuclei inhibited multireceptive spinothalamic tract neurones, although both nociceptive and non-nociceptive responses were reduced (Gerhart, Yezierski, Wilcox, Grossman & Willis, 1981b).

Another region subjected to investigation has been the NGC adjacent to the NRM. In cats, electrical stimulation of this nucleus reduced the responses of spinothalamic tract neurones to both noxious and non-noxious cutaneous (mechanical) stimuli (McCreery & Bloedel, 1975) or produced a biphasic excitation-inhibition effect (McCreery et al., 1979). In the rabbit, NGC stimulation reduced the nociceptive responses (intra-arterial bradykinin) of lamina V neurones (Takagi, Doi & Kawasaki, 1975). In the monkey, both excitation and inhibition of spinothalamic tract neurones by NGC stimulation was reported (Haber,

Martin, Chatt & Willis, 1978), the inhibition usually being non-selective for many types of cutaneous stimuli (Haber, Martin, Chung & Willis, 1980), although excitation by impulses in sural C primary afferents was relatively more susceptible than Aαβ excitation (Gerhart et al., 1981a). Finally, electrical stimulation of the nucleus reticularis magnocellularis (NMC), which is lateral to NRM and ventral to NGC, inhibited the responses of nociceptive dorsal horn neurones of the cat (Fields et al., 1977). (This nucleus in the cat corresponds approximately to the NPGC in the rat).

Thus there is abundant evidence for the existence of descending inhibitory systems which, when activated, are capable of inhibiting the firing of spinal neurones, although there is disagreement with regard to sensory modality selectivity. Perhaps a surprising aspect is that inhibition is so readily obtained from so many areas of the brain, an observation which will be subsequently discussed. Nevertheless these systems appear to provide reasonable neural correlates for the phenomenon of stimulation-produced analgesia.

Tonic Supraspinal Inhibition

In any consideration of descending inhibition, a distinction should be drawn between inhibition which is tonically present and that which is evoked by electrical stimulation at supraspinal sites. The preceding discussion has emphasized the latter, but numerous investigators have drawn attention to tonic inhibition of supraspinal origin which can control the firing of spinal neurones, including dorsal horn neurones excited by noxious cutaneous stimuli.

The studies of Lundberg and colleagues described tonic descending inhibition of activity in flexion reflex pathways (Eccles & Lundberg, 1959; Holmqvist & Lundberg, 1959) and obtained some evidence that this tonic effect was exerted on interneurones (Carpenter, Engberg, Funkenstein & Lundberg, 1963). This tonic inhibition was more pronounced in decerebrate than in barbiturate-anaesthetized cats (Holmqvist & Lundberg, 1959), affected transmission to several ascending pathways from flexion reflex afferents (Holmqvist et al., 1960), and was reduced by lesions in the raphé nuclei of the pons and medulla (Engberg et al., 1968a).

Several groups have demonstrated the presence of tonic descending inhibition of the firing of dorsal horn neurones in either decerebrate or anaesthetized cats. Such inhibition is readily demonstrated by comparing the activities of these neurones in the presence and absence of reversible block of spinal conduction by localized cooling of the spinal cord cephalic to the recording site. With decerebrate cats, Wall (1967) reported that cold block (thoracolumbar junction) expanded the receptive fields and increased the excitabilities of lumbar neurones of laminae IV, V and VI, with increased responses to both noxious and non-noxious cutaneous stimuli (Hillman & Wall, 1969). The findings of Wall (1967) were confirmed by Zieglgansberger and Herz (1971) but later studies noted that cold block preferentially increased the responses of lamina V neurones to a noxious stimulus (intra-arterial injection of bradykinin) with little effect on non-nociceptive responses (Besson, Guilbaud & Le Bars, 1975). Investigations

with anaesthetized preparations have produced similar results. When multireceptive dorsal horn neurones in barbiturate-anaesthetized cats were examined, cold block at L_1 increased their responses to noxious cutaneous heat and to impulses evoked electrically in primary afferent C fibres together with spontaneous firing, but non-nociceptive responses were little or not affected (Handwerker et al., 1975; Duggan et al., 1977c, 1981; Griersmith et al., 1981). In a study of "Class 3" cells of lamina I in chloralose-anaesthetized cats, Cervero et al. (1976) similarly found increased nociceptive responses following cold block, although these neurones did not respond to non-noxious cutaneous stimuli.

Tonic descending inhibition has been detected on spinocervical tract neurones in both anaesthetized and decerebrate cats. Initial observations found these cells, many of which are located in lamina IV, much more responsive to cutaneous stimuli in the spinal cat compared with decerebrate or anaesthetized preparations (Brown & Franz, 1969). Cold block experiments determined that descending inhibition was present on multireceptive spinocervical tract neurones (Zieglgansberger & Herz, 1971; Brown, 1971; Cervero et al., 1977) and exerted mainly on excitation by noxious stimuli and by impulses in unmyelinated (C) fibres (Brown, 1971).

In the anaesthetized monkey, limited spinal cord lesions have revealed tonic descending inhibition of spinothalamic tract neurones. The spontaneous activity of these neurones and their responses to impulses in Aδ sural primary afferents were

increased following lesions of the upper cervical dorsolateral fasciculus, although bilateral interruption was required (Willis et al., 1977).

In the anaesthetized rat also, tonic descending inhibition of dorsal horn neurones has been described. Necker and Hellon (1978) observed increased responses of these cells to noxious cutaneous heat when the cold block procedure was used, although spontaneous firing was infrequently elevated and non-nociceptive responses were not examined.

Tonic descending inhibition as revealed by cold block is not present on all dorsal horn neurones. For example, Cervero, Molony and Iggo (1979) could not detect such inhibition on neurones of the substantia gelatinosa in the cat.

Nevertheless, the available evidence based on conventional extracellular sampling techniques indicates that a large majority of neurones of laminae I, IV, V and VI are tonically inhibited from supraspinal sites. Moreover, this inhibition appears to be a relatively selective process in that excitation by noxious but not innocuous cutaneous stimuli is powerfully inhibited, suggesting a tonic control of the spinal transmission of nociceptive information.

It is not known to what extent this inhibition is present in conscious animals, but a number of investigators have described hyperalgesia following spinal or supraspinal lesions, suggesting a tonically active supraspinal system for suppressing the perception of pain.

In rats, the level of responsiveness to painful stimuli is often measured by determining the tail flick latency to a

noxious heat stimulus. Following acute spinalization at the thoracic level, several groups have observed a marked decrease in this latency (Grossmann, Jurna, Nell & Theres, 1973; Woolf, Mitchell & Barrett, 1980; Zemlan, Corrigan & Pfaff, 1980), although an earlier report found normal latency but a more vigorous tail flick response in acute spinal rats (Irwin, Houde, Bennett, Hendershot & Seevers, 1951). Berge (1982) found that the decreased tail flick latency following spinal transection in rats was permanent, and it seems clear that this nociceptive reflex is under tonic inhibitory control. Interestingly, a recent study in rats described reduced latency of paw withdrawal from noxious heat following spinal lesions confined to the DLF, more extensive lesions being ineffective (Davies, Marsden & Roberts, 1983). Tail flick latency was previously reported to be unaltered by DLF lesions (Hayes, Price, Bennett, Wilcox & Mayer, 1978). In the rat, the NRM has been proposed as a source of tonic inhibition of nociceptive processes since inactivation of this region, either irreversibly by lesions (Proudfit & Anderson, 1975; Proudfit, 1981) or reversibly by microinjection of local anaesthetic (Proudfit, 1980), shortened tail flick latency. A recent study, however, reported unchanged latency following considerable (>70%) destruction of the NRM (Prieto, Cannon & Liebeskind, 1983). The adjacent NGC and NPGC appear not to contribute to tonic descending inhibition since bilateral lesions of these regions failed to alter nociceptive (tail flick, hot plate) thresholds in rats (Mohrland, McManus & Gebhart, 1982). Other reports suggest that tonic inhibition

of nociception may arise from higher levels in this species. Lesions of the caudal or rostral PAG or in the caudal medial thalamus significantly decreased tail flick latency (Rhodes, 1979), and lesions of the medial hypothalamus consistently produced hyperalgesia (Vidal & Jacob, 1980).

Cats were found to be hyperalgesic for up to nine months (the longest observation period) following lumbar intrathecal administration of alumina cream (Kennard, 1950). This procedure, which presumably affected an unknown amount of the spinal cord, preserved proprioception and motor function but all forms of tactile stimuli appeared to become painful. Also in cats, enhanced responsiveness to painful pin-prick (but not to noxious heat) was produced by lesions of the central tegmental fasciculi and, in a small number of animals, of dorsal parts of the cervical spinal cord (Melzack, Stotler & Livingston, 1958). This finding received no support from a subsequent study, however, where central tegmental fasciculus lesions did not alter pain sensitivity (Kelly & Glusman, 1968). Both groups observed, however, that PAG lesions did not produce hyperalgesia in this species, but rather analgesia (Melzack et al., 1958) or normalgesia (Kelly & Glusman, 1968). Collectively these findings are not conclusive in indicating the presence of tonic descending inhibition in the conscious cat.

Limited destruction of the spinal cord of monkeys has also increased responses to painful stimulation. Ipsilateral lesions of the dorsolateral column decreased the latency and increased the force of escape from a noxious electrical

stimulus to hindlimbs, although thresholds were not altered (Vierck, Hamilton & Thornby, 1971). In this species, unilateral PAG destruction did not alter sensitivity to painful stimuli (Poirier, Bouvier, Olivier & Boucher, 1968).

Pathways

The neuronal pathways mediating stimulation-produced analgesia and descending inhibition of dorsal horn neurones have been investigated by a variety of techniques. There is evidence that some descending controls are conveyed to the spinal cord by the DLF. Initial studies noted that inhibition of the nociceptive responses of lumbar dorsal horn neurones by NRM stimulation in the cat was much less commonly observed caudal and ipsilateral to a unilateral DLF lesion compared with the intact side (Basbaum et al., 1976; Fields et al., 1977). Lesions of the ventrolateral funiculus, or indeed of the whole cord excluding the ipsilateral DLF, did not affect the NRM-induced inhibition. These results were confirmed in the monkey by Willis et al. (1977), who found that such inhibition of spinothalamic tract neurones was reduced by a unilateral DLF lesion and abolished by bilateral interruption. Interestingly, the latter procedure also released spinothalamic tract cells from tonic descending inhibition, as evidenced by increases in spontaneous firing, in the size of the cord dorsum N_3 wave (associated with Aδ fibre activity), and in sural-evoked Aδ responses.

Similarly, analgesia from electrical stimulation of the PAG in the rat requires an intact DLF. Unilateral

interruption of the DLF ipsilateral to the PAG stimulating electrode abolished analgesia in the ipsilateral hindlimb and reduced analgesia in the contralateral hindlimb (Basbaum, Marley, O'Keefe & Clanton, 1977).

Anatomical investigations have described pathways from the effective supraspinal regions to the spinal cord. An early study examining retrograde cellular changes in the raphé nuclei of the cat following lesions of their efferent fibres noted a significant spinal projection from the NRM, with lesser contributions from raphé nuclei pallidus, obscuris and pontis (Brodal, Taber & Walberg, 1960). Some of these descending fibres were localized to the DLF. Using a fluorescence technique with limited cord transection experiments, Dahlstrom and Fuxe (1964, 1965) extensively documented descending monoaminergic systems in the rat and other species. They described of abundance of 5-HT-containing neurones localized to the raphé nuclei and projecting via the DLF to the dorsal horn and the sympathetic lateral column, thus supporting the results of Brodal et al. (1960).

Autoradiographic studies in the cat have confirmed the raphé-spinal connection. Tritiated leucine, injected into the NRM, traced the descending fibres from this nucleus in the dorsal part of the lateral funiculus, with terminations in the dorsal horn, particularly lamina I to II and V to VI (Basbaum et al., 1976). Another group, however, reported descending tracts from NRM to be localized mainly in ventral and lateral funiculi (Bobillier, Segiun, Petitjean, Salvert, Touret & Jouvet, 1976). This may have been due to diffusion of [^{14}C]-

leucine to other medullary nuclei such as NGC and NMC, since in a subsequent investigation comparing NRM, NGC and NMC, the NGC projection (to several cranial motor nuclei) was via ventrolateral and ventral funiculi, while the NMC projection was similar to NRM but via both dorsolateral and ventrolateral funiculi and ipsilateral rather than bilateral (Basbaum, Clanton & Fields, 1978).

Using retrograde transport of horseradish peroxidase (HRP), several groups have corroborated these findings. Administration of HRP into the DLF at all spinal levels labelled many cells in NRM and NMC, with progressively less labelling for more caudal HRP administration, in both rats (Leichnetz, Watkins, Griffin, Murfin & Mayer, 1978; Basbaum & Fields, 1979; Watkins, Griffin, Leichnetz & Mayer, 1980) and cats (Kuypers & Maisky, 1975, 1977; Martin, Jordan & Willis, 1978; Basbaum & Fields, 1979; Tohyama, Sakai, Salvert, Touret & Jouvet, 1979a; Tohyama, Sakai, Touret, Salvert & Jouvet, 1979b). In many of these experiments, the spread of HRP was often extensive so that a whole spinal quadrant rather than individual funiculi was labelled. More definitive experiments have therefore employed selective cord lesions cephalic to the HRP injection site and sparing particular funiculi. Using this technique, projection of the NRM via the DLF was confirmed, although other raphé nuclei (such as pallidus and obscuris) were found to descend in the ventral quadrant (Martin et al., 1978; Basbaum & Fields, 1979). Kuypers and Maisky (1977), however, maintained that the predominant spinal projection of the medullary raphé was by the ventral and ventrolateral funiculi, with a small

projection within the DLF, although these authors did not differentiate between the different raphé nuclei.

The raphé-spinal connection has also been demonstrated in monkeys (Kneisley, Biber & La Vail, 1978) and the North American opossum (Martin, Cabana, Ditirro, Ho & Humbertson, 1982).

Thus the evidence for a medullary raphe-spinal dorsal horn pathway can only be described as substantial, and it is reasonable to conclude that this pathway is the substrate for inhibition and analgesia produced in a number of species by electrical stimulation of the raphé nuclei.

In contrast, many anatomical studies have obtained no evidence for a direct neuronal pathway from the midbrain PAG to the spinal cord. This observation has been made both for orthograde transport of tritiated leucine (Edwards, 1975; Ruda, 1975) and for retrograde transport of HRP (Kneisley et al., 1978; Tohyama et al., 1979a). A direct PAG-spinal projection has been demonstrated by these techniques, however, in several species including rat, cat, monkey and opossum, but this was predominantly confined to more cephalic segments, with only a very few cells projecting to lumbosacral cord levels (Kuypers & Maisky, 1975; Saper, Loewy, Swanson & Cowan, 1976; Loewy & Saper, 1978; Castiglioni, Gallaway & Coulter, 1978; Basbaum & Fields, 1979; Martin, Humbertson, Laxson, Panneton & Tschismadia, 1979a). One investigation noted a spinal projection of neurones of the DRN (Tohyama et al., 1979b).

Because of this general paucity of PAG neurones found projecting directly to the lumbosacral cord, it has been

proposed that the analgesic and inhibitory effects of electrical stimulation in the PAG are relayed to the spinal cord through neurones of the medullary raphé (reviewed by Basbaum & Fields, 1978; Fields & Basbaum, 1978). There is certainly considerable anatomical and physiological evidence for such a connection.

Early anatomical studies in the cat, tracing the course of degenerating fibres following PAG lesions, failed to find evidence for a descending pathway to the medullary raphé (Hamilton & Skultety, 1970; Hamilton, 1973). The existence of this connection, however, was clearly established in subsequent investigations employing HRP administration into the medullary raphé nuclei (particularly NRM) in rats (Gallagher & Pert, 1978; Senba, Takagi, Shiosaka, Sakanaka, Inagaki, Takatsuki & Tohyama, 1981; Beitz, 1982a; Carlton, Leichnetz, Young & Mayer, 1983), cats (Abols & Basbaum, 1981) and monkeys (Yezierski, Bowker, Kevetter, Westlund, Coulter & Willis, 1982a; Chung, Kevetter, Yezierski, Haber, Martin & Willis, 1983). Electrophysiological experiments have supported the anatomical data. In awake rats, analgesia (noxious pinch, footshock) produced by electrical stimulation of the PAG was associated with elevations in spontaneous multiple unit activity, and decreases in noxious-evoked activity, recorded in NRM (Oleson et al., 1978). With single unit recording, a proportion of neurones in the NRM which were activated antidromically by spinal cord stimulation were also excited by electrical stimulation of the PAG in cats (Fields & Anderson, 1978; Lovick, West & Wolstencroft, 1978a) and rats (Pomeroy &

Behbehani, 1979; Behbehani, 1982). Similarly, NRM stimulation antidromically activates neurones in the PAG in both species (Shah & Dostrovsky, 1980). In rats, microinjection of glutamate into the PAG is associated not only with increased firing of NRM neurones, but with an elevated threshold for a flexion reflex to noxious heating of the skin, and this elevation is prevented by NRM lesions (Behbehani & Fields, 1979).

The possibility has also been raised that the effects of PAG stimulation are relayed through the NGC, which also projects to the spinal cord (Wolstencroft, 1964). In rats, stimulation in NGC antidromically activated a number of PAG neurones (Harris & Sinclair, 1981a), and PAG stimulation has both excited and inhibited NGC neurones (Mohrland & Gebhart, 1980; Harris & Sinclair, 1981b). Studies with HRP, however, both in this species (Gallagher & Pert, 1978) and the monkey (Chung et al., 1983) detected relatively few PAG neurones projecting to the NGC, and bilateral lesions of NGC failed to influence antinociception (tail flick, hot plate) produced by PAG stimulation (Mohrland et al., 1982).

It has been suggested (Carstens, 1982) that inhibition of dorsal horn neurones by electrical stimulation of structures rostral to the PAG, such as the hypothalamus and periventricular gray regions, may be mediated by a PVG-PAG-NRM-spinal sequential pathway. Even though a direct hypothalamic-spinal projection has been demonstrated (Kuypers & Maisky, 1975), there is abundant evidence for a dense projection from the hypothalamus to the mesencephalic PAG. Early degeneration

experiments showed a periventricular system of fibres from the posterior hypothalamus to the PAG in the cat (Bucher & Burgi, 1953). Efferents from various hypothalamic nuclei to the PAG were later demonstrated using tritiated amino acid autoradiography in rats, cats and monkeys (Conrad & Pfaff, 1976; Saper, Swanson & Cowan, 1979; Veazey, Amaral & Cowan, 1982), and HRP studies in these species verified this observation (Grofova, Ottersen & Rinvik, 1978; Morrell, Greenberger & Pfaff, 1981; Beitz, 1982b; Mantyh, 1982). Finally, in rats, neurones of the ventromedial hypothalamus can be antidromically activated by PAG stimulation (Pittman, Blume, Kearney & Renaud, 1979; Sakuma & Pfaff, 1982), and stimulation of the ventromedial hypothalamic nucleus facilitated the firing of PAG neurones (Sakuma & Pfaff, 1980).

Notwithstanding the data cited above, a recent study has suggested that the direct PAG-spinal projection may have been underestimated. In rats, cats and monkeys, cervical or lumbar injections of the sensitive retrograde tracer, wheat germ agglutinin-HRP conjugate, labelled many neurones in the PAG and, in cats, in the dorsal raphé also (Mantyh & Peschanski, 1982). Thus the effects of PAG stimulation observed in the spinal cord may be mediated by both a direct pathway and an indirect pathway synapsing in the medulla.

Mechanisms of Descending Inhibition

There are comparatively few observations on the possible mechanisms by which descending inhibition might occur in the dorsal horn. It is likely, however, that the mechanism(s)

which result in selective inhibition of nociceptive responses of dorsal horn neurones differ from those producing non-selective inhibition, where responses to all excitatory inputs are reduced.

As discussed in the previous section of the thesis, selective inhibition could occur in a number of ways. One possible mechanism is a presynaptic action on the terminals or fibres of nociceptive, but not non-nociceptive, primary afferents, and supraspinal influences on primary afferent terminal excitability have been described. Negative dorsal root potentials, which are an indication of depolarization of the terminals of primary afferents (Schmidt, 1971), were produced in the lumbar spinal cord of decerebrate cats by CI stimulation, but the length constant of these potentials indicated that the fibres depolarized were large myelinated afferents (Proudfit & Anderson, 1974; Proudfit, Larson & Anderson, 1980). This was confirmed using the intraspinal stimulation technique for single sural fibres with both NRM and NGC stimulation in cats and monkeys, with no selectivity shown between large myelinated Aαβ fibres and small myelinated nociceptive Aδ fibres (Martin, Haber & Willis, 1979b). Electrical stimulation of NRM, NGC and PAG has also produced excitability changes in the terminals of tooth pulp afferents (which appear to be largely Aδ fibres) in brain stem trigeminal nuclei of the cat. There is general agreement that such stimulation increases terminal excitability, and a presynaptic inhibition of orofacial sensory input has been inferred from these results (Lovick et al., 1977; Lovick, West & Wolstencroft, 1978b; Hu,

Dostrovsky & Sessle, 1978; Dostrovsky, Sessle & Hu, 1981; Lovick, 1981).

Studies of the intraspinal excitability of sural A- and C-fibre populations have reported decreased excitability during reversible (cold block) or irreversible (transection) spinalization, suggesting the presence of a tonic supraspinal influence on both A- and C-fibre primary afferent terminals (Calvillo, 1978; Calvillo et al., 1982). Although such a mechanism is consistent with tonic descending inhibition of dorsal horn neurones, tonic inhibition is relatively selective for nociceptive rather than large myelinated fibre inputs (Besson et al., 1975; Handwerker et al., 1975; Duggan et al., 1977c). In contrast, a study of single lumbar C-fibres in the cat found that NRM stimulation produced increased thresholds for antidromic activation in half the fibres examined, although only one-quarter of those so affected were identified as nociceptive afferents (Hentall & Fields, 1979). As emphasized previously, however, the relationship between terminal excitability and presynaptic inhibition of transmitter release from unmyelinated fibres is not certain, so the functional consequences of such effects are not known. The possibility of artefactual alterations in terminal excitability secondary to circulatory changes (for which brain stem stimulation and spinalization are well known) should also be borne in mind.

Selective inhibition could also be produced by postsynaptic inhibition of superficial dorsal horn neurones excited only by nociceptive afferents and projecting to deeper multireceptive neurones, but there is little evidence for this

proposal. Such nociceptive-only "Class 3" cells have been described in lamina I (Christensen & Perl, 1970; Cervero et al., 1976). In one study, neurones of the substantia gelatinosa were found to be inhibited by electrical stimulation of the DLF (Cervero et al., 1979), which contains raphé-spinal fibres (Martin et al., 1978; Basbaum & Fields, 1979), but excitation of neurones of laminae I to III by DLF stimulation has also been reported (Dubuisson & Wall, 1980). More neurophysiological studies of these superficial neurones are needed to clarify this possibility.

Finally, selective inhibition could occur by postsynaptic inhibition on dendrites adjacent to sites of nociceptive afferent input, as proposed earlier to explain the selective effects of M-ENKA administered between the cell bodies of dorsal horn neurones and the substantia gelatinosa (Figure 17). Again there is little supporting evidence.

Non-selective inhibition is most likely to be produced by a somatic hyperpolarization. Stimulation of NRM blocked the antidromic activation of spinothalamic tract cells of the monkey (Giesler, Gerhart, Yezierski, Wilcox & Willis, 1981) and of medullary dorsal horn neurones of the cat (Shah & Dostrovsky, 1982), suggesting postsynaptic inhibition. Direct evidence in the form of inhibitory postsynaptic potentials (IPSPs) induced by NRM stimulation was also obtained (Giesler et al., 1981).

Stimulation in the brain stem will activate many neurones and fibres of passage and therefore it is not surprising that in investigations of the mechanisms of descending inhibitions,

a variety of effects has been observed. The possibility that the descending fibres are not themselves inhibitory, but excitatory to inhibitory interneurones which produce the observed effects, has not been investigated. Clearly, further studies will be required to resolve the relative importance of these proposed mechanisms.

Transmitters and Pharmacology of Supraspinal Inhibition

Experiments aimed at identifying the transmitters involved in these descending controls have been of three types:

(a) Histochemical studies of the cells of origin of descending fibres to determine if a particular substance is contained within these cells;

(b) The microelectrophoretic administration of suspected mediators of descending inhibition near the cells inhibited;

(c) The administration of substances which interfere with the storage, synthesis or action of suspected transmitters in attempts to similarly modify the descending controls. With systemic administration, there is the problem of uncertainty as to the site of action of an observed effect.

It is not intended to review this literature at length, but to emphasize its main conclusions.

(a) Opioid Peptides

As with segmental inhibition (discussed previously), the evidence for opioid peptide participation in spinal inhibition of supraspinal origin, including stimulation-produced analgesia, is inconsistent. Brain stem neurones containing

immunoreactive enkephalin and projecting to the lumbar cord have been described (Hokfelt et al., 1979; Bowker et al., 1981): enkephalin release could be from these fibres or from spinal interneurones activated by other descending fibres. The actions of enkephalins on dorsal horn neurones are consistent with those of an inhibitory transmitter (Chapter III). There is evidence that analgesia produced in humans by electrical stimulation of PAG-PVG regions is associated with significant increases in ventricular CSF levels of enkephalin-like material (Akil et al., 1978b) and immunoreactive β-endorphin (Akil, Richardson, Barchas & Li, 1978a; Hosobuchi, Rossier, Bloom & Guillemin, 1979b). Studies are divided, however, with regard to the ability of opioid antagonists to block such analgesia.

With systemic naloxone, analgesia produced by CI stimulation was clearly reduced or abolished in cats (Oliveras et al., 1977), but in rats, analgesia from stimulation of NPGC rather than NRM was attenuated (Satoh et al., 1980). Other studies in the rat, however, found analgesia from NRM and NPGC, but not NGC, reversible by naloxone administered systemically (Zorman et al., 1981), or by lumbar, but not cervical, intrathecal administration (Zorman et al., 1982), implicating an action by opioid peptides at the lumbar cord level. Naloxone antagonism of analgesia from electrical stimulation of the PAG has been reported as either partial or absent in rats (Akil et al., 1976; Yaksh, Yeung & Rudy, 1976; Pert & Walter, 1976), cats (Gebhart & Toleikis, 1978) and monkeys (Hayes et al., 1979). One study in the rat, however, reported analgesia from electrical stimulation of the DRN-ventral PAG region to be

reduced by naltrexone (Swajkoski et al., 1981). Another group found that intravenous naloxone, after a 20 minute delay, abolished an analgesic response to microinjection of glutamate into the PAG (Behbehani & Fields, 1979), a surprising result in view of the rapidity with which intravenous naloxone antagonizes the central actions of opioid peptides. In humans, naloxone reversal of analgesia from stimulation of PVG regions was substantial (Adams, 1976; Hosobuchi et al., 1977; Boivie & Meyerson, 1982).

In neurophysiological experiments, attempts to reduce descending inhibition with systemic naloxone have given variable results. In the cat, inhibition of the nociceptive responses of dorsal horn neurones produced by electrical stimulation in the PAG (Carstens, Klumpp & Zimmermann, 1979a), lateral mesencephalic reticular formation (Carstens & Zimmermann, 1980) and DRN and medullary raphé (Duggan & Griersmith, 1979a) was not affected by this antagonist. Also in the cat, naloxone had little or no effect on inhibition of trigeminal neurones (Sessle et al., 1981) or PAD of tooth pulp afferents (Hu et al., 1978; Lovick et al., 1978b) produced by PAG or NRM stimulation. In the rat, naloxone did not block inhibition of nociceptive transmission in ascending axons by PAG stimulation (Jurna, 1980), but reduced the inhibitory effects of NRM stimulation on C-fibre responses of dorsal horn neurones (Rivot et al., 1979). In cats, tonic descending inhibition of dorsal horn neurones was not reduced by systemic naloxone (Duggan et al., 1977c; Sinclair et al., 1980).

(b) 5-Hydroxytryptamine (5-HT)

There is a large literature which implicates this indoleamine in the spinal transmission of nociceptive information and its descending control (reviewed by Messing & Lytle, 1977; Basbaum & Fields, 1978). The projection of 5-HT-containing neurones of the medullary raphé nuclei via the DLF to the spinal dorsal horn has been well documented by anatomical techniques (Dahlstrom & Fuxe, 1964, 1965; Bowker et al., 1981; reviewed by Bowker, Westlund, Sullivan & Coulter, 1982). Recent studies have described a proportion of 5-HT-containing neurones of the PAG and DRN projecting to the medullary raphé (Yezierski et al., 1982a; Beitz, 1982a; Beitz, Shepard & Wells, 1983), implicating 5-HT at more than one level of descending control.

With microelectrophoretic administration of 5-HT near spinal neurones, excitation has sometimes been observed (Weight & Salmoiraghi, 1966; Belcher et al., 1978), but depression of cell firing has been the usual finding (Engberg & Ryall, 1966; Randic & Yu, 1976; Jordan, Kenshalo, Martin, Haber & Willis, 1978, 1979), especially of nociceptive responses (Randic & Yu, 1976; Belcher et al., 1978; Headley et al., 1978). Administration in the substantia gelatinosa selectively reduced nociceptive, but not non-nociceptive, responses of laminae IV and V neurones (Headley et al., 1978), and this effect was blocked by prior electrophoresis of methysergide (Griersmith & Duggan, 1980). These actions are consistent with those of an inhibitory transmitter released by descending fibres. In addition, electrophoretic 5-HT decreased the excitability of

primary afferent terminals (Carstens et al., 1981c). It has been suggested, however, that this may be secondary to a 5-HT uptake process following extracellular administration from micropipettes (Curtis, Leah & Peet, 1983). A presynaptic action of 5-HT on primary afferents receives little support from ultrastructural studies, which have described 5-HT-containing terminals in laminae I and II forming primarily axo-dendritic synapses, but axo-axonic connections were not found (Ruda, Allen & Gobel, 1981; Ruda, Coffield & Steinbusch, 1982).

Although there is some doubt regarding the adequacy of putative 5-HT antagonists at central 5-HT receptors (Haigler & Aghajanian, 1977), systemic administration of these drugs has reduced analgesia produced by lumbar intrathecal administration of 5-HT (Yaksh & Wilson, 1979) and that evoked by electrical stimulation of the DRN (Hayes, Newlon, Rosecrans & Mayer, 1977), NRM (Satoh et al., 1980) and locus coeruleus and substantia nigra (Sandberg & Segal, 1978). The problem in such experiments is knowing where the drug acts. Depletion of brain 5-HT by p-chlorophenylalanine (pCPA) also reduced analgesia from ventral PAG stimulation (Akil & Mayer, 1972; Akil & Liebeskind, 1975), although selective spinal 5-HT depletion by desipramine/intrathecal 5,7-dihydroxytryptamine did not (Johannessen, Watkins, Carlton & Mayer, 1982). Tolerance to stimulation-produced analgesia has been reversed by administration of 5-HT precursors: 5-hydroxytryptophan reversed tolerance to CI stimulation in cats (Oliveras et al., 1978) and L-tryptophan, to PAG stimulation in humans (Hosobuchi, 1978, 1980). Consistent with these findings, NRM stimulation was

shown to enhance 5-HT metabolism in the spinal cord (Bourgoin, Oliveras, Bruxelle, Hamon & Besson, 1980; Rivot, Chiang & Besson, 1982).

In neurophysiological studies, inhibition of dorsal horn neurones by DRN stimulation was reduced by systemic administration of LSD (which may depress midbrain raphé neurones as well as act as a 5-HT antagonist) (Liebeskind et al., 1973; Guilbaud, Besson, Oliveras & Liebeskind, 1973), but methysergide failed to reduce such inhibition from medullary raphé stimulation (Belcher et al., 1978; Griersmith et al., 1981). A subsequent study reported that reduction of NRM-induced inhibition by 5-HT antagonists depended on the stimulus parameters and antagonist dose (Yezierski, Wilcox & Willis, 1982b), but inhibition from PAG stimulation was convincingly reduced by these antagonists (Carstens, Fraunhoffer & Zimmermann, 1981b; Yezierski et al., 1982b). Depletion of 5-HT by pCPA also reduced inhibition of dorsal horn neurones from stimulation of NRM (Rivot et al., 1980) and PAG (Carstens et al., 1981c).

Tonic descending inhibition of dorsal horn neurones in cats appears not to involve 5-HT. This inhibition was not altered by administration of pCPA or fluoxetine (a specific 5-HT neuronal uptake inhibitor) (Soja & Sinclair, 1980), nor by methysergide administered electrophoretically near dorsal horn neurones, in the substantia gelatinosa, or topically to the cord surface (Griersmith et al., 1981). Systemically, methysergide depressed cell responses, even in the spinal animal, making it difficult to gauge its effects on tonic descending

inhibition. Although comparable experiments have not been done in rats, there is evidence for serotonergic involvement in tonic descending inhibition of nociception in this species since hyperalgesia was produced by intrathecal (Proudfit & Hammond, 1981) and systemic (Berge, 1982) administration of 5-HT antagonists. This accords with earlier results showing hyperalgesia in rats following inactivation of the serotonin-rich NRM (Proudfit & Anderson, 1975; Proudfit, 1980, 1981).

(c) Amino Acids

Since the evidence is substantial that GABA and glycine are widely used inhibitory transmitters in the central nervous system (Curtis & Johnston, 1974), these amino acids have been considered as possible transmitters released from spinal neurones by descending fibres. In the spinal cord, GABA levels are high in the dorsal horn (Berger, Carter & Lowry, 1977), where terminals containing glutamate decarboxylase (the GABA synthesising enzyme) form a variety of synapses (McLaughlin et al., 1975). Electrophoretic administration of the GABA antagonist bicuculline and the glycine antagonist strychnine near dorsal horn neurones, however, did not reduce their inhibition by CI stimulation (Belcher et al., 1978; Johnston & Davies, 1981), but electrophoretic bicuculline did antagonize NRM-induced inhibition of medial brain stem reticular neurones (Lovick & Wolstencroft, 1980b) and NRM-induced increases in trigeminal Aδ terminal excitability (Lovick, 1981). Intravenous and electrophoretic bicuculline and strychnine also failed to reduce tonic descending inhibition of feline dorsal horn neurones (Duggan et al., 1981).

(d) Catecholamines

When administered electrophoretically in the substantia gelatinosa or near multireceptive laminae IV and V neurones of the cat, noradrenaline was comparatively potent in reducing nociceptive responses (Headley et al., 1978). Lumbar intrathecal administration of noradrenaline and adrenaline to rats produced antinociception in doses much less than those of 5-HT (Kuraishi, Harada & Takagi, 1979; Reddy, Maderdrut & Yaksh, 1980), and in cats intrathecal noradrenaline was similarly analgesic (Reddy & Yaksh, 1980). There is evidence, however, that noradrenaline may act to reduce some supraspinal controls. Analgesia produced by PAG stimulation was enhanced by disulfiram-induced selective depletion of brain noradrenaline in rats (Akil & Liebeskind, 1975) and reduced by intraventricular noradrenaline administration in cats (Dubuisson & Melzack, 1977). In addition, microinjection of noradrenaline antagonists into the NRM of rats produced hypoalgesia (Hammond, Levy & Proudfit, 1980), which was blocked by intrathecal phentolamine (Sagen & Proudfit, 1981), suggesting that a descending noradrenergic inhibitory system was activated by antagonism of noradrenaline action in the NRM region. Tonic descending inhibition of feline dorsal horn neurones was not reduced by systemic administration of the noradrenergic antagonists phentolamine, yohimbine, alprenolol or propranolol (Duggan, 1982), but was reduced by the noradrenaline uptake blockers nisoxetine and desipramine, whereas reserpine-induced depletion of central noradrenaline increased this inhibition (Soja & Sinclair, 1983). Difficult

to reconcile with these findings is the report that intrathecal phentolamine produced hyperalgesia in rats (Proudfit & Hammond, 1981).

The investigations cited in this literature review emphasize the substantial difficulties encountered in attempts to clearly implicate a particular putative neurotransmitter in the descending control of spinal nociceptive processes. In many cases it is not possible to explain why conflicting results have been obtained by different investigators. Anaesthesia and/or species differences are not sufficient to explain, for example, the differing effects of opioid antagonists on inhibition of spinal neurones from supraspinal stimulation. Electrical stimulation will almost certainly activate fibres of passage as well as cell bodies in the vicinity of the electrode tip, and slight differences in tip location may result in different investigators studying separate and/or overlapping descending systems not necessarily using the same transmitter(s). The interpretation of observed effects is also complicated by the phenomenon of co-existence of multiple putative neurotransmitters within neurones, including supraspinal neurones projecting to the spinal cord (Johansson, Hokfelt, Pernow, Jeffcoate, White, Steinbusch, Verhofstad, Emson & Spindel, 1981). Nevertheless, the available data support the conclusion that putative transmitters such as opioid peptides and 5-HT do play some role in the descending control of the spinal transmission of nociceptive information.

The experiments described in the second part of this

thesis investigated some important questions about supraspinal inhibition of dorsal horn neurones in the cat. As discussed previously, the excitation of many dorsal horn neurones by impulses in unmyelinated primary afferent fibres is subject to a powerful tonic inhibition from supraspinal sites in both anaesthetized and decerebrate cats. This inhibition is relatively selective since when natural cutaneous stimuli are used, responses to noxious stimuli are reduced with little effect on non-nociceptive responses. The supraspinal source(s) of this inhibition is not known. In the first of this series of investigations therefore (section A), brain stem regions were selectively destroyed in a systematic search to define the site of origin of this tonic inhibition. This search initially concentrated on those regions such as the PAG, DRN and NRM, areas which, when electrically stimulated, inhibit dorsal horn neurones. In the rat, the NRM and the PAG have been implicated in tonic inhibition of nociception by the observations that NRM (Proudfit & Anderson, 1975; Proudfit, 1981) and PAG (Rhodes, 1979) lesions produced hyperalgesia. In the present experiments, the degree of tonic descending inhibition on laminae I, IV and V neurones was measured by reversible cold block of spinal conduction cephalic to the recording site, as described in Methods, while electrocoagulating supraspinal regions.

In the next group of investigations (sections B, C and D), studies were undertaken on various aspects of inhibition of dorsal horn neurones produced by spinal and supraspinal electrical stimulation. The first study (B) in this series

aimed to examine possible mechanisms of descending inhibition by recording intracellularly from dorsal horn neurones. Successful intracellular recording from these neurones, however, is not compatible with changes in blood pressure, for which both spinal cold block (removing tonic inhibition) and brain stem stimulation (producing inhibition) are well known. Since the integrity of the DLF is a requirement for both stimulation-produced inhibition (Fields et al., 1977) and tonic descending inhibition (Willis et al., 1977), strongly suggesting that the relevant fibres descend in this part of the cord, it was proposed to electrically stimulate the DLF caudal to a cord transection in order to activate the descending fibres. Although this technique would probably activate fibres of diverse supraspinal origin, dividing the spinal cord removes tonic descending inhibition and hence the effects of stimulation may be more readily detected.

The second study (C) in this series was prompted by the observation of Oliveras et al. (1974a), that the midbrain regions producing inhibition of dorsal horn neurones when electrically stimulated in anaesthetized cats were much more extensive than the sites producing analgesia when similarly stimulated in conscious animals. As previously pointed out, inhibition of dorsal horn neurones observed in neurophysiological experiments can be more readily related to behavioural analgesia when such inhibition is selective for excitation by noxious rather than non-noxious cutaneous stimuli. With this in mind, the effects of electrical stimulation in the PAG were compared with those elicited from

more ventral sites in the midbrain tegmentum on the responses of multireceptive dorsal horn neurones, recorded extracellularly, to impulses in a variety of primary afferent fibres. Early in these experiments it was noted that electrical stimulation in the PAG produced a rapid, short-lasting increase in the CO_2 levels of expired air and irregularities in blood pressure. Since in a paralysed, artificially ventilated animal a relatively rapid and reversible increase in expired CO_2 most probably results from an increased pulmonary blood flow (and hence an increased cardiac output), it was decided to study these circulatory changes concurrently with inhibition of dorsal horn neurones.

The search for the supraspinal source of tonic descending inhibition of dorsal horn neurones had found that this inhibition was reduced or abolished only by bilateral lesions in the ventrolateral medulla in the region of the caudal lateral reticular nuclei. The importance of these lateral reticular areas in the control of dorsal horn neurones was further investigated in the third study (D) in this series on stimulation-produced inhibition. These experiments examined the effects of electrical stimulation of both these and adjacent regions on the responses of multireceptive dorsal horn neurones to impulses in both small and large diameter primary afferent fibres.

The effects of PAG stimulation on dorsal horn neurones are thought to be relayed to the spinal cord largely through neurones of the NRM (Basbaum & Fields, 1978). Since the preceding experiments had emphasized the importance of lateral

reticular areas in descending inhibition, the fifth and final study (E) of supraspinal control of dorsal horn neurones compared the relative importance of both raphé and lateral reticular regions of the medulla for inhibition produced by PAG stimulation. In these experiments the medullary regions were coagulated while observing PAG-induced inhibition of the responses of dorsal horn neurones to impulses in unmyelinated primary afferents.

RESULTS

A. THE BRAIN STEM ORIGIN OF TONIC DESCENDING INHIBITION

In these experiments, extracellular recordings were obtained from lumbar dorsal horn neurones of laminae I, III-IV and V excited by impulses in unmyelinated primary afferent fibres. Because of the circulatory changes produced by the spinal cold block technique, these impulses were evoked by electrical stimulation of the ipsilateral common tibial nerve with a strength suprathreshold for C fibres (commonly 100T) rather than by noxious heating of a digital pad. The degree of tonic descending inhibition present on an individual neurone was determined by comparing the number of action potentials evoked by impulses in C-fibre primary afferents during normal spinal conduction to the number evoked during cold block of spinal conduction.

With cold block an unknown volume of spinal cord is cooled sufficiently to block impulse conduction. In each experiment therefore, the duration of cooling was determined by the time required for the increase in cell firing to impulses in C fibre afferents to stabilize. This was usually 4 to 6 minutes but cold block was commonly continued for 8 to 10 minutes and cell responses measured at two fixed intervals after commencement of cooling. By having fixed periods of cooling and making observations at fixed times after the onset of cooling, descending inhibition could be repeatedly measured, intermittently with electrocoagulation of brain stem regions.

In four animals (14% of the total), tonic descending inhibition of dorsal horn neurones could not be detected by cold block and thus brain stem lesions were not performed in these experiments.

(a) Lesions Not Reducing Tonic Descending Inhibition

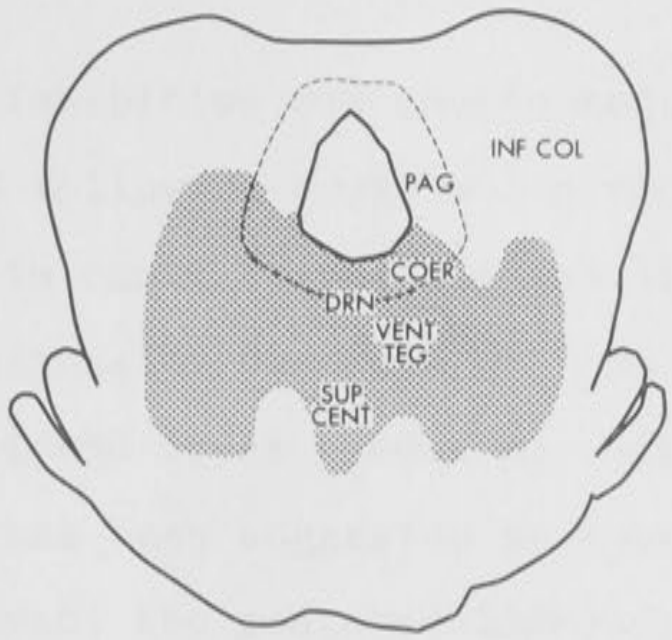
Initially, electrocoagulation was performed in those regions which, when electrically stimulated, have been shown to produce inhibition of nociceptive dorsal horn neurones and analgesia in conscious animals. The areas of destruction in these experiments were usually extensive since, when lesions at particular sites failed to reduce descending inhibition, further lesions were made in adjacent areas. Table III summarizes the results of twelve experiments in which a number of brain stem regions were extensively coagulated without reducing the tonic descending inhibition of dorsal horn neurones. In many experiments, lesions of varying proportions of these structures were found histologically, but only those experiments where complete or very extensive destruction was apparent are included in Table III.

The failure of extensive coagulation in the midbrain to reduce tonic descending inhibition is typified in Figure 18. With this lamina IV neurone, the removal of tonic descending inhibition by cold block of spinal conduction increased the number of action potentials evoked by impulses in C fibre afferents from 17.4 ± 0.7 S.E.M. (n = 16) to 82.5 ± 1.8. In this experiment the initial lesions were stereotactically aimed at the ventral PAG but were extended to more ventral and

TABLE III BRAIN STEM REGIONS DESTROYED WITHOUT REDUCING TONIC DESCENDING INHIBITION

Region	Number of Experiments
Midbrain	
Ventral periaqueductal gray	4
Dorsal raphé nucleus	4
Nucleus caeruleus (locus coeruleus) (bilateral)	2
Brachium conjunctivum (bilateral)	2
(ipsilateral)	1
Central tegmental field (bilateral)	3
Paralemniscal tegmental field (bilateral)	3
Pons-medulla	
Inferior central raphé nucleus	11
Trapezoid body nucleus (ipsilateral)	2
Superior olivary nuclei (ipsilateral)	2
Nucleus praepositus hypoglossi (ipsilateral)	2
(contralateral)	1
Hypoglossal nucleus (ipsilateral)	2
Dorsal motor nucleus of vagus (ipsilateral)	2
Nucleus ambiguus (ipsilateral)	2
Gigantocellular tegmental field (NGC) (bilateral)	2
(unilateral)	4
Magnocellular tegmental field (NMC) (bilateral)	1
(unilateral)	4
Lateral tegmental field (bilateral)	2
(unilateral)	4

Regions are named using the terminology of Berman (1968).



CONTROL

POST-LESIONS

NORMAL SPINAL CONDUCTION

SPINAL CONDUCTION BLOCKED

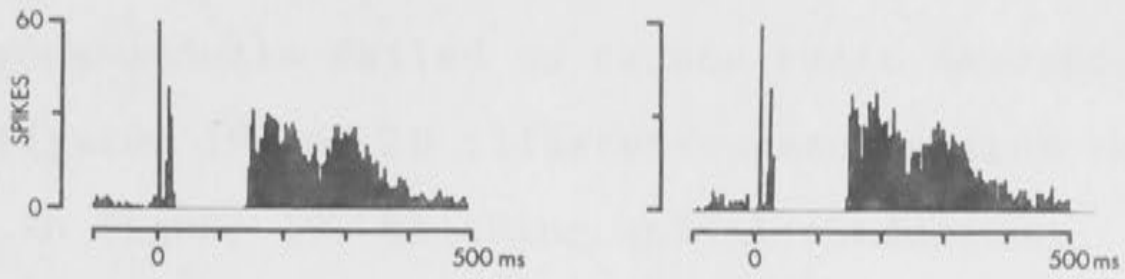


Fig. 18. Failure of extensive coagulation in the midbrain to reduce tonic descending inhibition of a lamina IV neurone. The stippled area marks the extent of coagulation apparent in a 15 µm transverse paraffin section of the midbrain at AP -1, midway through the lesion. Each peristimulus time histogram (memory address dwell time 5 msec) represents the summed responses of the neurone to 16 successive stimuli (0.5 msec pulses, 0.2 Hz), delivered at time 0 to the ipsilateral tibial nerve at a strength adequate to excite unmyelinated primary afferents. In this and subsequent figures, the large increases in the responses during cold block of spinal conduction at the first lumbar segment are due to removal of tonic descending inhibition on the dorsal horn neurones. The atlas of Berman (1968) was used for this and subsequent diagrams. PAG, periaqueductal gray; DRN, dorsal raphé nucleus; SUP CENT, superior central raphé nucleus; COER, locus coeruleus; VENT TEG, ventral tegmentum; INF COL, inferior colliculus.

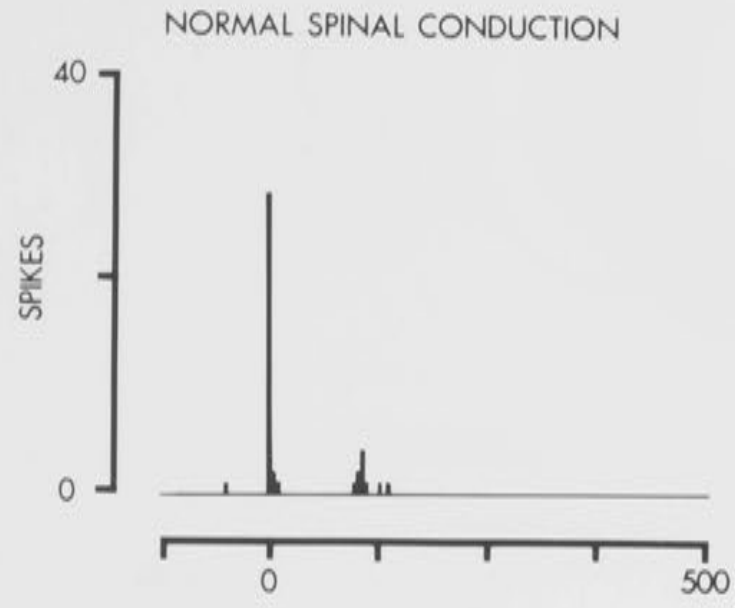
lateral areas when inhibition was unaffected. The illustrated histograms compiled following coagulation show no reduction but rather an increase in tonic descending inhibition. Such increases were occasionally observed but not systematically examined in terms of the areas producing them.

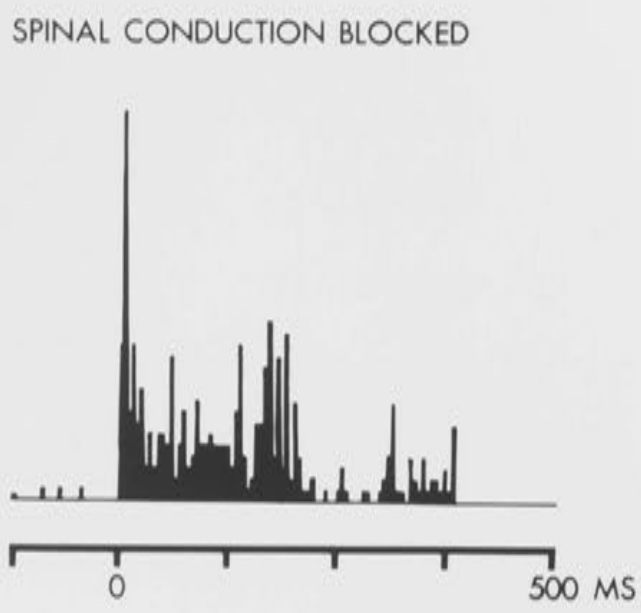
Since the NRM has been suggested to tonically inhibit nociception in the rat, the pontomedullary raphé was lesioned in many experiments. In each case an attempt was made to lesion the midline from 1 mm above the basiocciput (to avoid the basilar artery) up to the floor of the fourth ventricle and the adjacent areas 1 to 1.5 mm on either side of the midline. The antero-posterior (AP) extent of coagulation varied but it was common to destroy 8 to 12 mm of midline tissue centred on AP-7. In all eleven experiments, extensive coagulation of the raphé of the pons-medulla failed to reduce tonic descending inhibition. Figures 19 and 20 illustrate results from two such experiments. In Figure 19, blocking spinal conduction increased the number of C afferent-evoked action potentials of the lamina IV neurone from 9.1 ± 0.7 S.E.M. (n = 16) to 28.2 ± 0.8. After destruction of the pontine and medullary raphé (as shown in the photograph of the transversely sectioned brain stem), descending inhibition was unchanged. In Figure 20, the gated C fibre-evoked response of the lamina IV neurone increased from 1.6 ± 0.4 S.E.M. (n = 16) with normal spinal conduction, to 20.8 ± 0.6 in the cold blocked state. Extensive coagulation in the medulla including the raphé and adjacent regions (shown in transverse section at AP-7) failed to reduce tonic descending inhibition on this neurone.

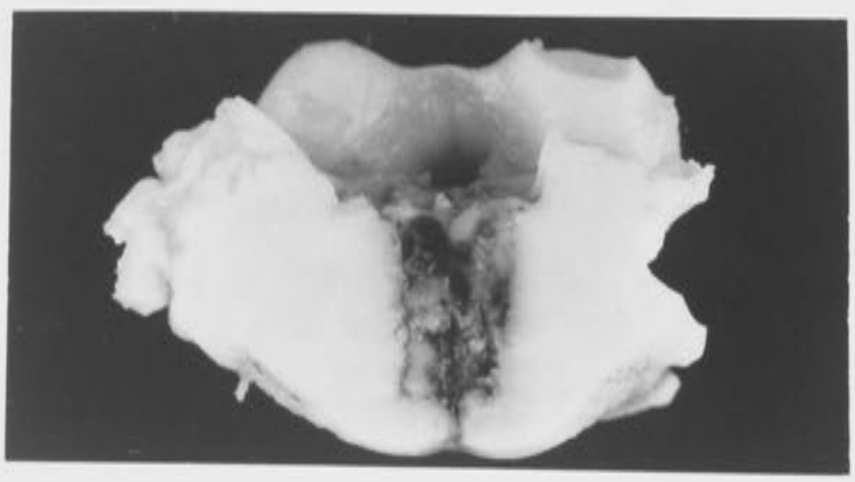
Fig. 19. Failure of medullary raphé lesions to influence tonic descending inhibition of a lamina IV neurone. The photograph shows the extent of raphé destruction exposed by a transverse section through the fixed brain stem, midway through the lesion (approximately Horsley-Clarke AP -6). As in Figure 18, peristimulus histograms (memory address dwell time 5 msec) are the summed responses to 16 successive stimuli (0.5 msec pulses, 0.3 Hz), at time 0, to the ipsilateral tibial nerve at a strength adequate to excite C fibre afferents.

A. Control responses
B. Responses following coagulation of the raphé

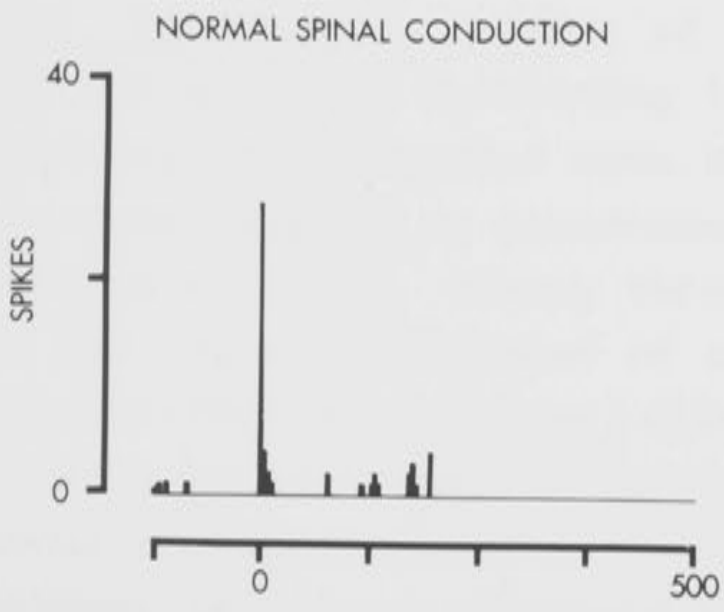
A CONTROLS

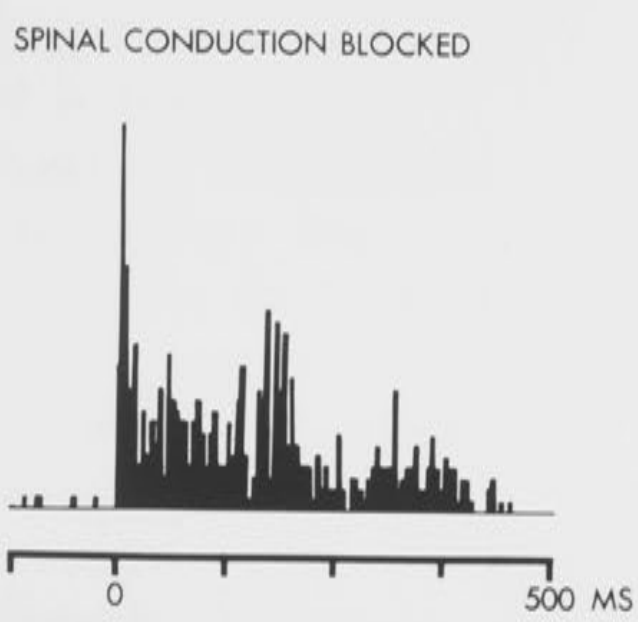






B AFTER RAPHE LESIONS





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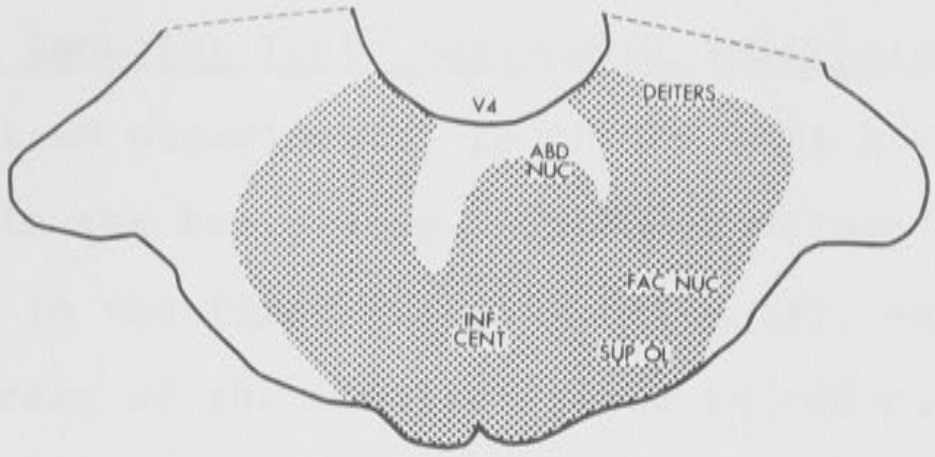
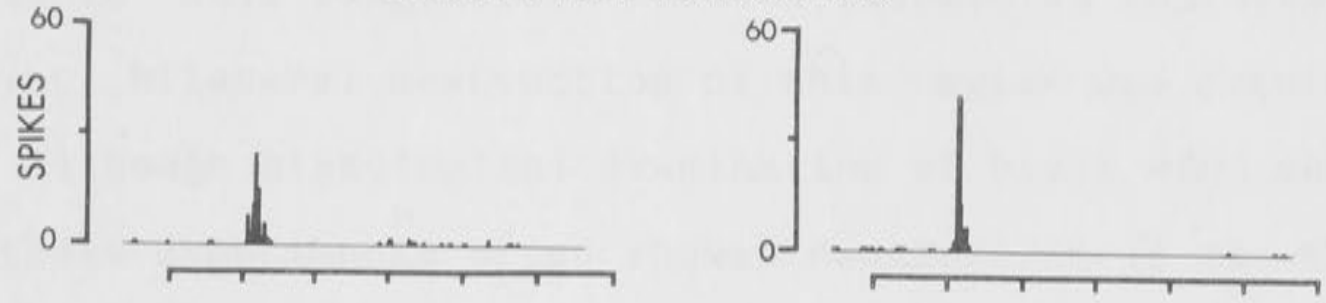


CONTROLS

POST-LESION

NORMAL SPINAL CONDUCTION



SPINAL CONDUCTION BLOCKED

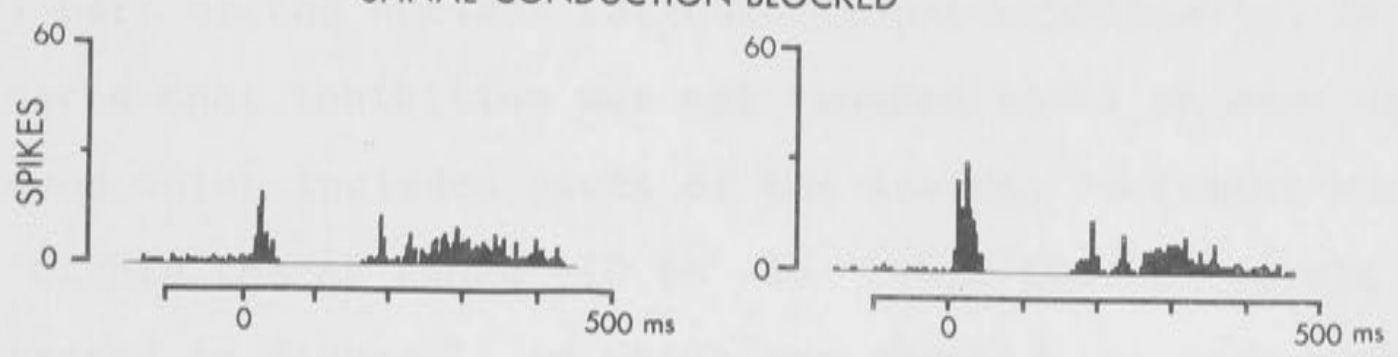


Fig. 20. Lack of effect of extensive coagulation in the medulla on tonic descending inhibition of a lamina IV neurone. The stippled area shows the extent of coagulation apparent in a 15 µm transverse paraffin section of the medulla at AP -7, midway through the lesion. As in Figures 18 and 19, the responses of the neurone to 16 successive stimuli (0.5 msec pulses, 0.3 Hz) to the ipsilateral tibial nerve have been represented by peristimulus histograms (memory address dwell time 5 msec). The tibial nerve stimuli were suprathreshold for C fibres and delivered at time 0. INF CENT, inferior central raphé nucleus; SUP OL, superior olive; FAC NUC, facial nucleus; ABD NUC, abducens nucleus; DEITERS, lateral vestibular nucleus of Deiters; V4, fourth ventricle.

(b) Lesions Reducing Tonic Descending Inhibition

In thirteen experiments, tonic inhibition was reduced by coagulation in the brain stem. These experiments fall into two categories. In the first (nine experiments), extensive lesions in several areas of the medulla failed to reduce inhibition but when the coagulating electrodes were more than 3 mm lateral from the midline, close to the ventral surface and caudal (AP -10 to -16), coagulation reduced descending inhibition. Moreover, bilateral destruction of this region was required. Thus, although histological examination of brain stem sections from these experiments often showed destruction of the NRM, NGC, paramedian reticular nucleus, the inferior olive, the hypoglossal nucleus, dorsal motor nucleus of the vagus and the medial part of the nucleus reticularis parvocellularis, it was considered that inhibition was not reduced until an area was destroyed which included parts of the lateral reticular nuclei (LRN) within the AP range -10 to -16. This type of result is illustrated in Figure 21 in which are plotted the mean numbers of C fibre-evoked action potentials to 16 successive stimuli to the tibial nerve. The extent of descending inhibition is illustrated by the shaded bars. In this experiment, an initial series of lesions contralateral to the recording site, although extensively damaging the lateral medulla, did not reduce descending inhibition. Immediately the coagulating electrodes were positioned 4 mm from the midline on the ipsilateral side and 1 mm above the ventral surface of the brain stem, a small reduction of inhibition occurred even prior to coagulation of

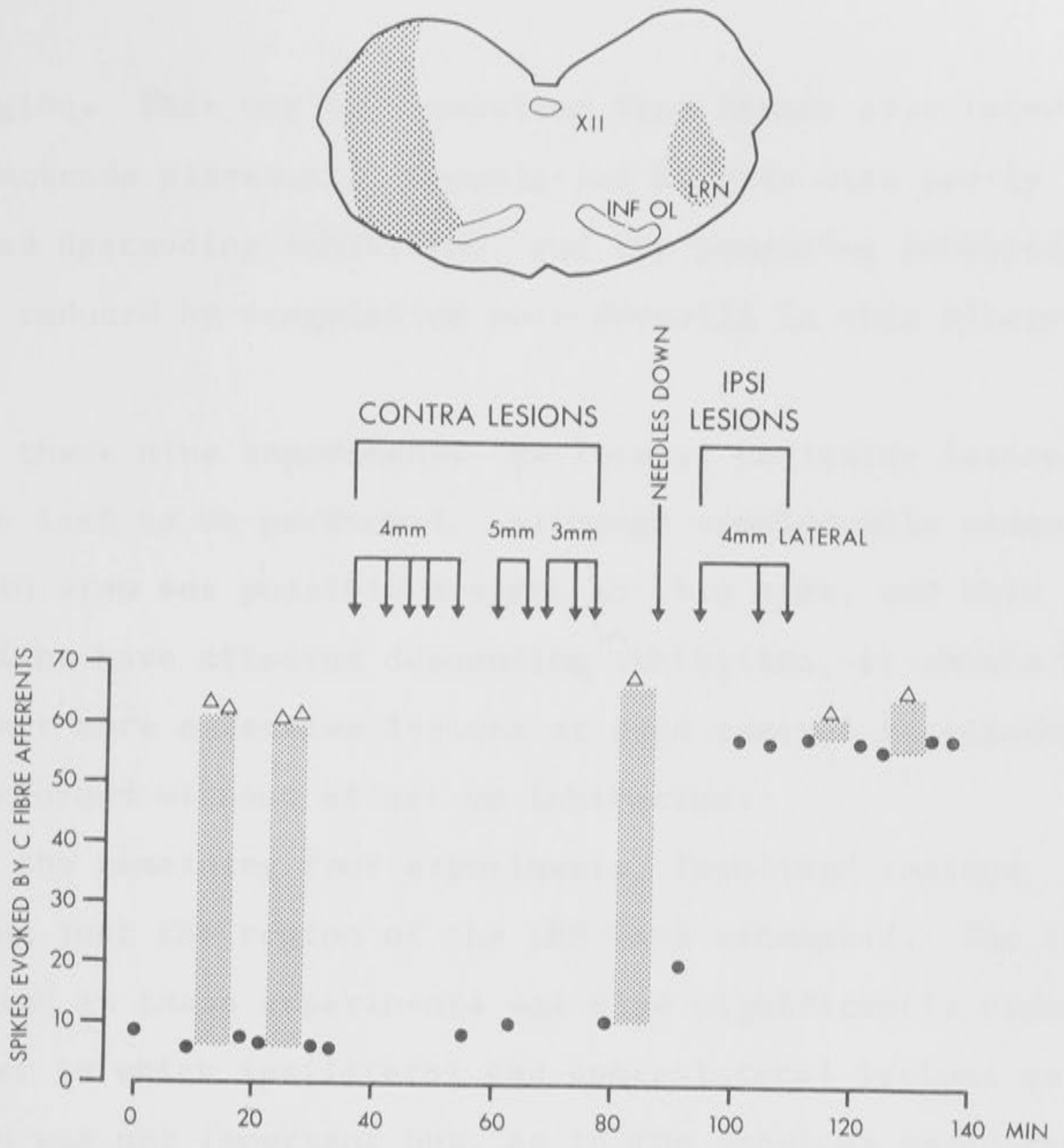


Fig. 21. Reduction of tonic descending inhibition of a lamina V neurone by bilateral lesions in the ventrolateral caudal medulla. The stippled areas show the extent of coagulation apparent in a 15 µm transverse paraffin section of the medulla at AP -14.5. The mean numbers of C fibre-evoked action potentials to 16 successive stimuli to the tibial nerve are plotted with respect to time. Filled circles: means of 16 gated C fibre responses with normal spinal conduction; open triangles, means of 16 gated C fibre responses during cold block of spinal conduction (L_1). The standard errors of the means were all less than 2 action potentials and hence have not been plotted. The height of the shaded bars measures tonic descending inhibition present on this neurone. This inhibition was not affected by extensive coagulation in the contralateral medulla (3 to 5 mm from the midline), but was reduced by subsequent placement of the coagulating electrodes in the ipsilateral medulla ("Needles Down") and almost abolished following ipsilateral coagulation (4 mm from midline). INF OL, inferior olive; XII, hypoglossal nucleus; LRN, lateral reticular nucleus.

this region. This may have resulted from trauma associated with electrode placement. Coagulation at this site nearly abolished descending inhibition, and the remaining inhibition was not reduced by coagulation more dorsally in this electrode track.

In these nine experiments the lateral reticular lesions were the last to be performed. Although considerable oedema of the brain stem was possibly present at this time, and this alone might have affected descending inhibition, it should be noted that more extensive lesions at more rostral locations were performed without effect on inhibition.

In the remaining four experiments, localized lesions involving just the region of the LRN were attempted. The tonic inhibition in these experiments was also significantly reduced. The order in which ipsilateral and contralateral lesions were produced was not important but, as in the previous experiments where extensive coagulation was performed, bilateral destruction was required. Results from one experiment in which lesions reducing descending inhibition were confined largely to the LRN is illustrated in Figure 22.

In this experiment the lesions were all at sites 4 mm lateral over the AP range -13.5 to -16.5. Some reduction in inhibition was produced by initial lesions 0.5 mm dorsal to the base of the cranium but a large rapid reduction occurred only with bilateral lesions 1.5 mm dorsal to the base, at AP -16.5. The requirement for lesions to be at least 1 mm dorsal to bone was a constant feature of these experiments. Collectively the results indicated that bilateral medullary lesions in the

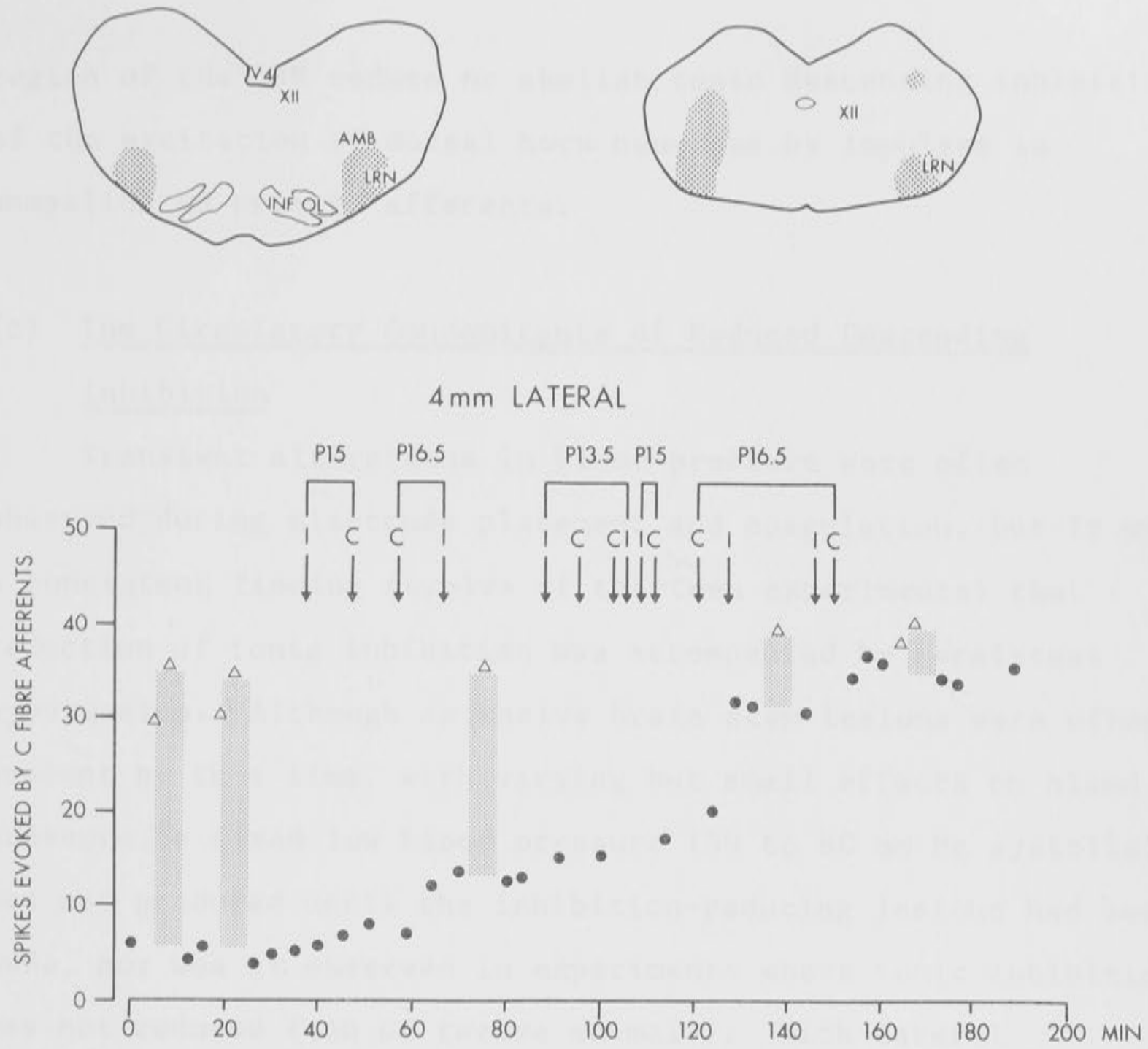


Fig. 22. Reduction of tonic descending inhibition of a lamina V neurone by bilateral lesions confined mainly to the regions of the lateral reticular nuclei. The stippled areas show the regions of coagulation seen in 15 µm transverse paraffin sections of the medulla at AP -13.5 and AP -16.5. The stippled area on the left at AP -16.5 includes a ventral coagulated region and dorsal haemorrhage, probably caused by introduction of the electrodes. The mean numbers of C fibre-evoked action potentials are plotted as for Figure 21, with the standard errors of the means similarly omitted. In this experiment, only 2 coagulating electrodes were used. Rapid and significant reductions in inhibition occurred with bilateral coagulation at AP -16.5. These sites were at 0.5 mm dorsal to the base of the cranium (at 60 minutes) and 1.5 mm dorsal (at 125 minutes). INF OL, inferior olive; AMB, nucleus ambiguus; LRN, lateral reticular nucleus; XII, hypoglossal nucleus; V4, fourth ventricle; I, ipsilateral; C, contralateral.

region of the LRN reduce or abolish tonic descending inhibition of the excitation of dorsal horn neurones by impulses in unmyelinated primary afferents.

(c) The Circulatory Concomitants of Reduced Descending Inhibition

Transient alterations in blood pressure were often observed during electrode placement and coagulation, but it was a consistent finding (twelve of thirteen experiments) that reduction of tonic inhibition was accompanied by persistent hypotension. Although extensive brain stem lesions were often present by this time, with varying but small effects on blood pressure, a fixed low blood pressure (50 to 80 mm Hg systolic) was not produced until the inhibition-reducing lesions had been made, nor was it observed in experiments where tonic inhibition was not reduced (ten of twelve animals). With lateral reticular sites, transient hypertension was frequently observed during the passage of the coagulating current but this reverted to hypotension within 5 minutes of tissue destruction. These results (Table IV) show a significant correlation between hypotension and reduction of tonic inhibition (χ^2, Yates correction = 11.58; $P<0.001$).

B. STIMULATION-PRODUCED INHIBITION. I. LATERAL SPINAL FUNICULI

In these experiments, intracellular recordings were obtained from lumbar dorsal horn neurones of laminae III-IV, V

TABLE IV THE HYPOTENSIVE EFFECT OF BRAIN STEM LESIONS REDUCING TONIC DESCENDING INHIBITION

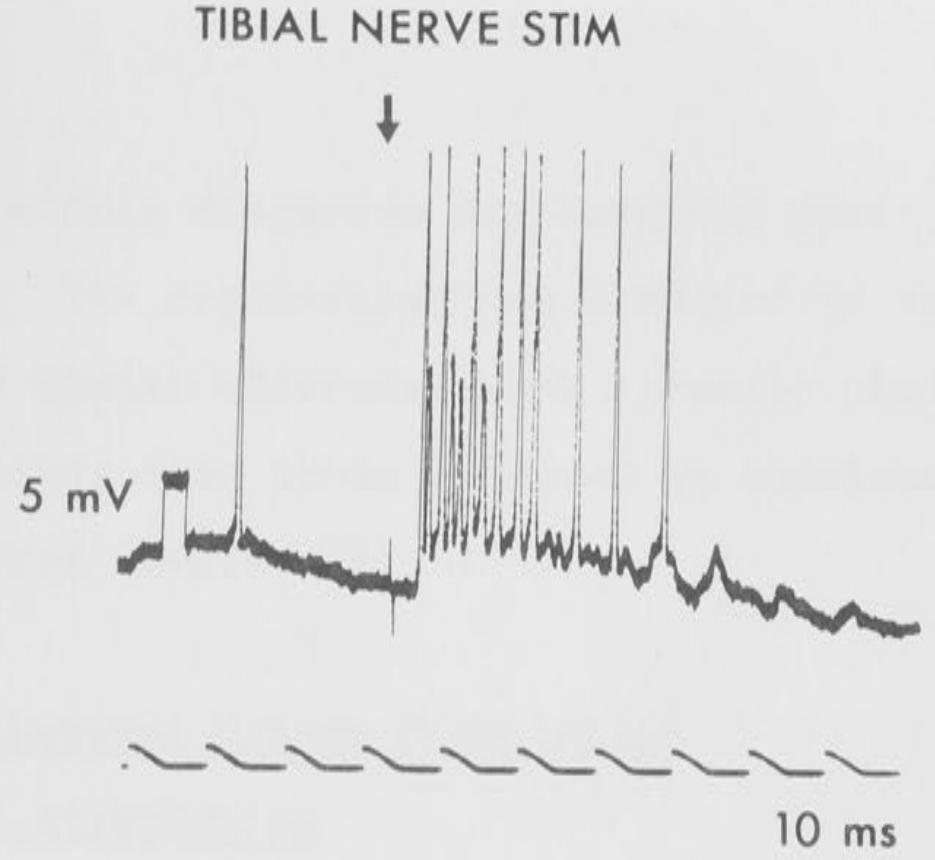
		TONIC DESCENDING INHIBITION		
		Reduced	Not Reduced	
BLOOD PRESSURE	Hypotension (decrease of >10 mm Hg)	12	2	14
	Normotension	1	10	11
		13	12	25

χ^2, Yates correction = 11.58; $P<0.001$.

and VI excited by impulses evoked by electrical stimulation of tibial primary afferent fibres. It was difficult to obtain stable intracellular recordings from these neurones and data analysis has been restricted to those in which the resting membrane potential did not decrease more than 10 mV over the period of observations. Of thirty-one neurones, nineteen had a resting membrane potential of greater than 40 mV while twelve had resting membrane potentials of 30 to 40 mV for the period of observations. Intracellular recordings were obtained from neurones for up to 65 minutes with a mean recording time of 9.6 minutes. Eleven were in lamina III-IV, ten in lamina V, seven in lamina VI and the location not determined with three. The spinal cord was transected at the thoraco-lumbar junction in all experiments.

(a) Intracellular Responses to Tibial Nerve Stimulation

Twenty-eight of thirty-one neurones responded to impulses in large myelinated primary afferent fibres with a burst of action potentials (latency 3.5 to 5.0 msec) superimposed on a large depolarization which was followed by a prolonged (112 msec ± 12 S.E.M.) hyperpolarization (see Figures 23 and 28). Two neurones responded with a depolarization-hyperpolarization sequence without firing, while one showed a hyperpolarization alone (4 msec latency). When the stimulus to the tibial nerve was adequate to excite unmyelinated fibres, two-thirds of neurones responded with either a burst of late action potentials (latency 150 to 325 msec, see Figures 23 and 28B) or



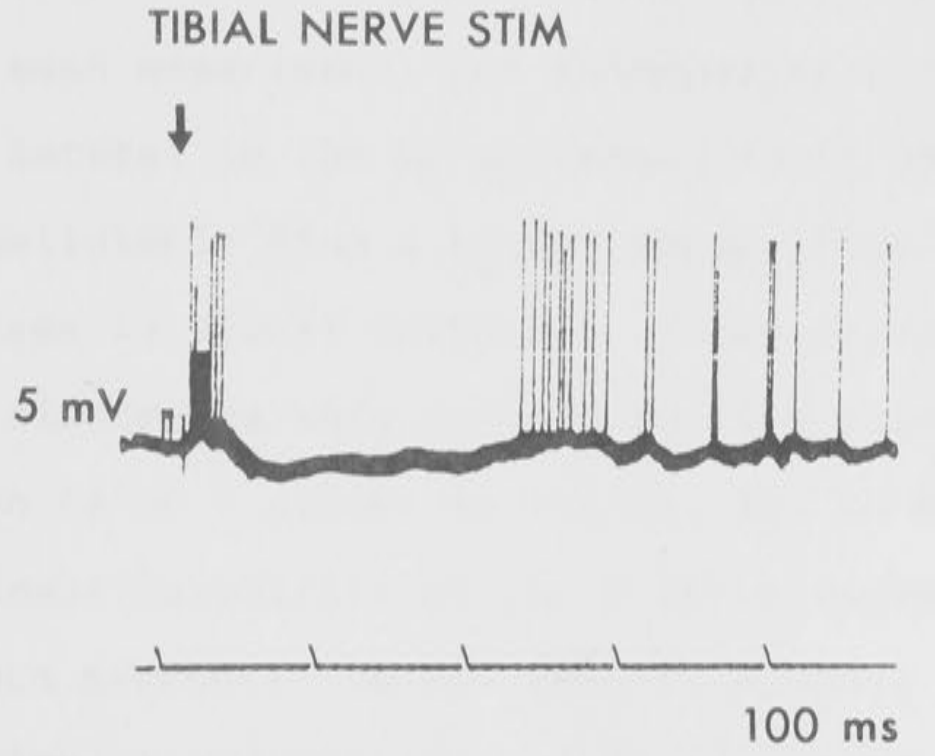


Fig. 23. Intracellular recordings from a lamina V neurone, illustrating potential changes and action potentials evoked by a single stimulus (0.5 msec pulse) to the ipsilateral tibial nerve. In the upper record, the stimulus was adequate to excite only myelinated primary afferents, producing a group of action potentials superimposed on a depolarizing potential (latency 3.3 msec). When the tibial stimulus was adequate to also excite unmyelinated primary afferents, the longer latency response to impulses in these fibres was recorded (lower record).

a low amplitude widely dispersed depolarizing potential of similar latency. The depolarizations produced by impulses in large myelinated tibial afferents were commonly observed to be of greater amplitude than those produced by impulses in unmyelinated fibres (Figure 28).

(b) Effects of Lateral Column Stimulation

(i) Site of stimulation

In these experiments it was proposed to electrically stimulate the DLF to activate descending fibres which convey supraspinal inhibition to the lumbar dorsal horn. At the commencement of each experiment, the stimulating electrodes were introduced lateral to the L_2-L_3 dorsal roots while recording extracellularly from a L_6-L_7 lamina IV or V neurone excited by impulses in tibial C-fibres. Under microscopic observation the electrodes were lowered until a site was found where stimulation (6 or 7 pulses at 310 Hz, 100 to 600 μA) produced significant inhibition of the C fibre-evoked response of the dorsal horn neurone. It was rare to produce inhibition with a single stimulus and therefore a brief tetanus, timed to occur just before the C-response (as described in Methods), was used. The stimulating electrodes were always placed ipsilateral to the recording site: in some experiments electrodes were similarly positioned on both sides of the cord. Insertion of the stimulating electrodes usually caused some dimpling of the cord and hence it was difficult to gauge their depth below the dorsal surface. When steel electrodes were

used, however, Prussian blue reactions showed that in nearly all cases the tips were at the junction of the dorsal and ventral quadrants of the lateral columns.

(ii) Potential changes evoked in dorsal horn neurones

The predominant effect of ipsilateral lateral column stimulation was a hyperpolarization. This was observed during intracellular recording with nineteen dorsal horn neurones and examples are shown in Figures 25, 26 and 27. This was rarely a simple response as in nearly all instances ripples were present on the initial downward deflection of the hyperpolarization. The mean duration of this potential change was 110 msec ± 13 S.E.M. and its peak amplitude varied from 2 to 13 mV (mean 9.2 ± 0.8 S.E.M.), the larger values being recorded in cells with relatively low resting membrane potentials. Since a tetanic stimulus of the lateral columns was used, latency measurements of the hyperpolarization were taken from the first pulse of the train although in some instances this may have underestimated the conduction time. The mean latency was 11.9 msec ± 1.2 S.E.M. For a distance of 80 mm and assuming no interposed neurones this gives a conduction velocity in the descending fibres of 6.7 metres/second. With an additional eight neurones a distinct depolarization preceded a prolonged hyperpolarization, and in five this depolarization was sufficient to evoke action potentials (Figures 24 and 28). Two neurones responded to lateral column stimulation with a depolarization alone. Antidromic activation was uncommon (two neurones) and this was

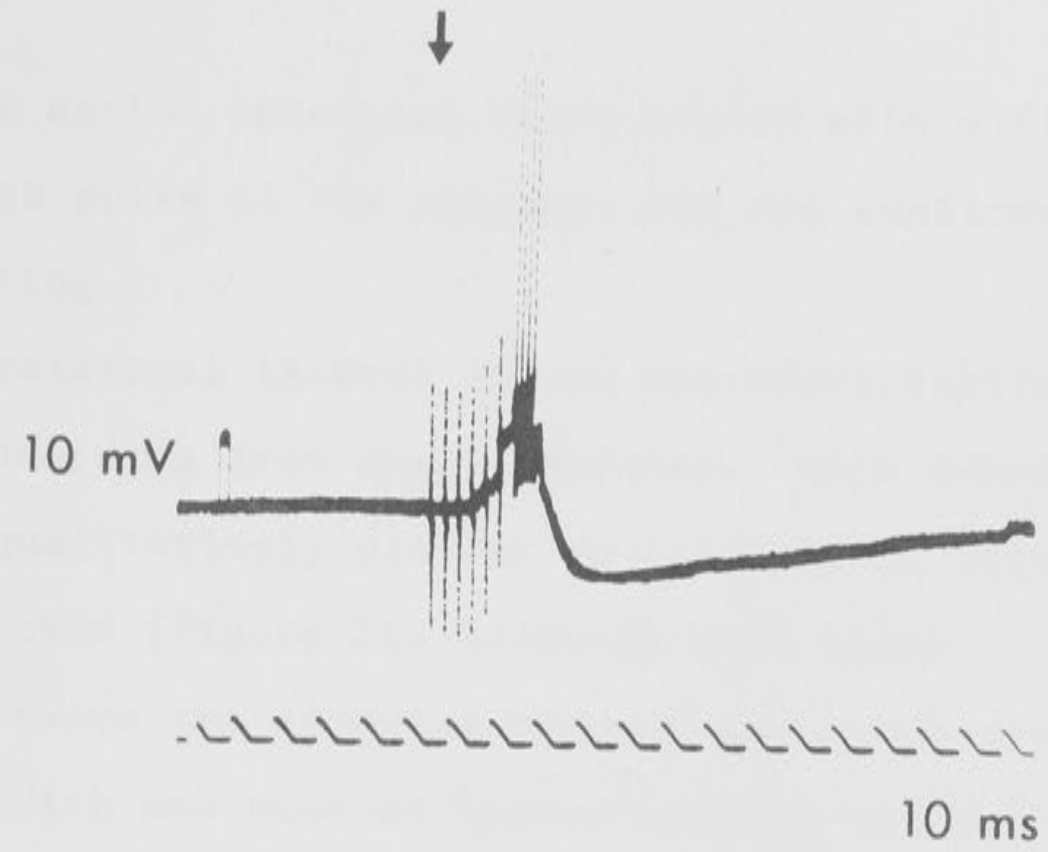


Fig. 24. Intracellular records of a depolarization-hyperpolarization sequence evoked in a lamina V neurone by tetanic stimulation (six 0.5 msec pulses at 310 Hz, 900 μA) of the ipsilateral and contralateral lateral spinal funiculi (LAT COL STIM). In both cases the depolarization was of sufficient amplitude to produce firing of the neurone.

presumed by an action potential being evoked with a fixed latency by each pulse of the tetanus, but not confirmed by collision testing.

The contralateral lateral column was electrically stimulated while recording from eight neurones. With seven the effects were qualitatively similar to ipsilateral lateral column stimulation (Figure 24) although with three, approximately twice the stimulus current was necessary to elicit them. With one neurone hyperpolarized by ipsilateral stimulation, no effect of contralateral stimulation was observed.

(iii) Membrane conductance during hyperpolarization

As described previously for motoneurones, membrane conductance was determined by measuring the potential produced across the cell membrane by intracellular injection of current pulses. Unfortunately the electrical properties of the type of micropipette required for successful penetrations of dorsal horn neurones were less than satisfactory for this procedure, since significant bridge imbalance was usually produced by passing currents in excess of 0.5 nA through the intracellular electrode. Hence meaningful measurements of membrane conductance were obtained with only three neurones, and with all three this conductance was increased during lateral column-induced hyperpolarization. One of these results is illustrated in Figure 25.

To obtain further information on the nature of the hyperpolarization, recordings were obtained from ten neurones using potassium chloride-containing microelectrodes. These

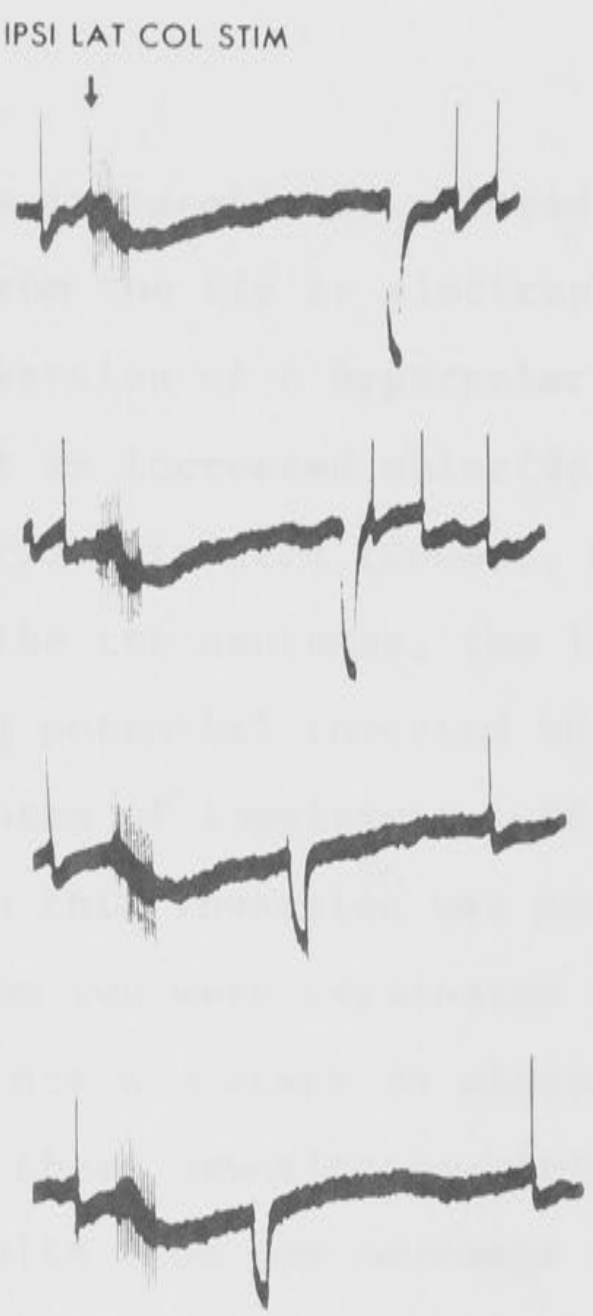


Fig. 25. Increased membrane conductance of a lamina V neurone during hyperpolarization induced by tetanic stimulation (twelve 0.5 msec pulses at 310 Hz, 600 μA) of the ipsilateral lateral spinal funiculus (IPSI LAT COL STIM). A 3 nA, 20 msec hyperpolarizing current pulse was passed across the cell membrane at various times after the tetanic stimulus. Values of membrane resistance calculated from these records (to the nearest 0.5 MΩ) are (from the top): 7.5, 7.0, 4.0, 3.5 and 1.0 MΩ. Thus conductance is clearly increased in the lower 3 records. The spontaneously occurring action potentials have been truncated.

electrodes can increase intracellular chloride concentrations (either by diffusion from the tip or electrophoretic ejection) and the decrease or inversion of a hyperpolarization by this procedure suggests that an increased chloride conductance occurs during the hyperpolarization (Coombs, Eccles & Fatt, 1955). With seven of the ten neurones, the lateral column-induced hyperpolarizing potential inverted to a depolarizing potential within 3 minutes of impalement. Of the remaining three neurones in which this inversion was not observed, intracellular recordings from two were terminated within one minute of impalement. It was not necessary to electrophoretically eject chloride ions in these experiments, diffusion apparently being sufficient. Results from two neurones are illustrated in Figures 26 and 27. In Figure 26, the inversion occurred without manipulation of membrane potential; in Figure 27, diffusion of chloride ions from the electrode diminished but did not invert the hyperpolarization, but the passage of hyperpolarizing currents then produced a clear depolarization.

(c) Interaction of Lateral Column- and Tibial Nerve-evoked Effects

The excitation of dorsal horn neurones by impulses in both A and C primary afferents of the tibial nerve was inhibited when preceded by the hyperpolarization induced by lateral column stimulation. When measured as a percentage reduction in the number of tibial-evoked action potentials, this inhibition was more effective in reducing excitation by C than by A primary afferents. This relative selectivity of inhibition is

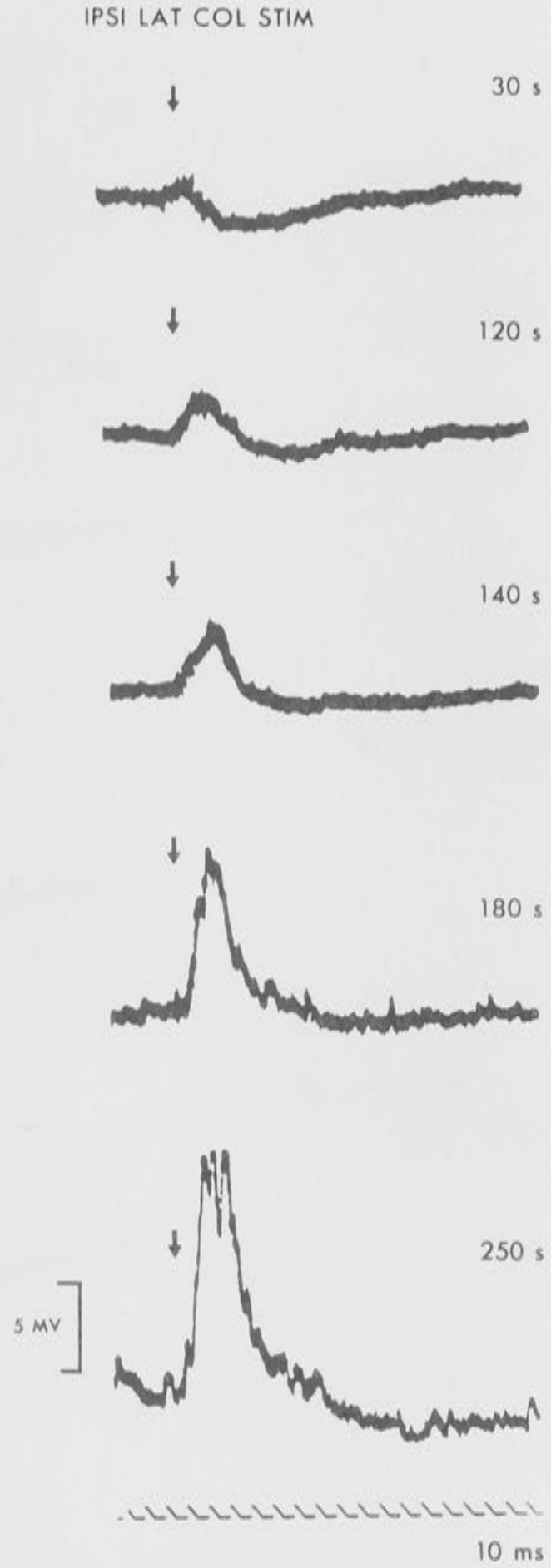


Fig. 26. The effect of a 2 M KCl-containing electrode on the hyperpolarization of a lamina VI neurone induced by tetanic stimulation (six 0.5 msec pulses at 310 Hz, 300 μA) of the ipsilateral lateral spinal funiculus (IPSI LAT COL STIM). Records were obtained at various times (indicated in seconds) after impalement of the neurone. The 1.3 mV hyperpolarization recorded 30 seconds after impalement inverted to a 13.5 mV depolarization at 250 seconds. At the latter time, lateral column stimulation then evoked a burst of action potentials (not illustrated). The stimulus artefacts have been omitted for clarity.

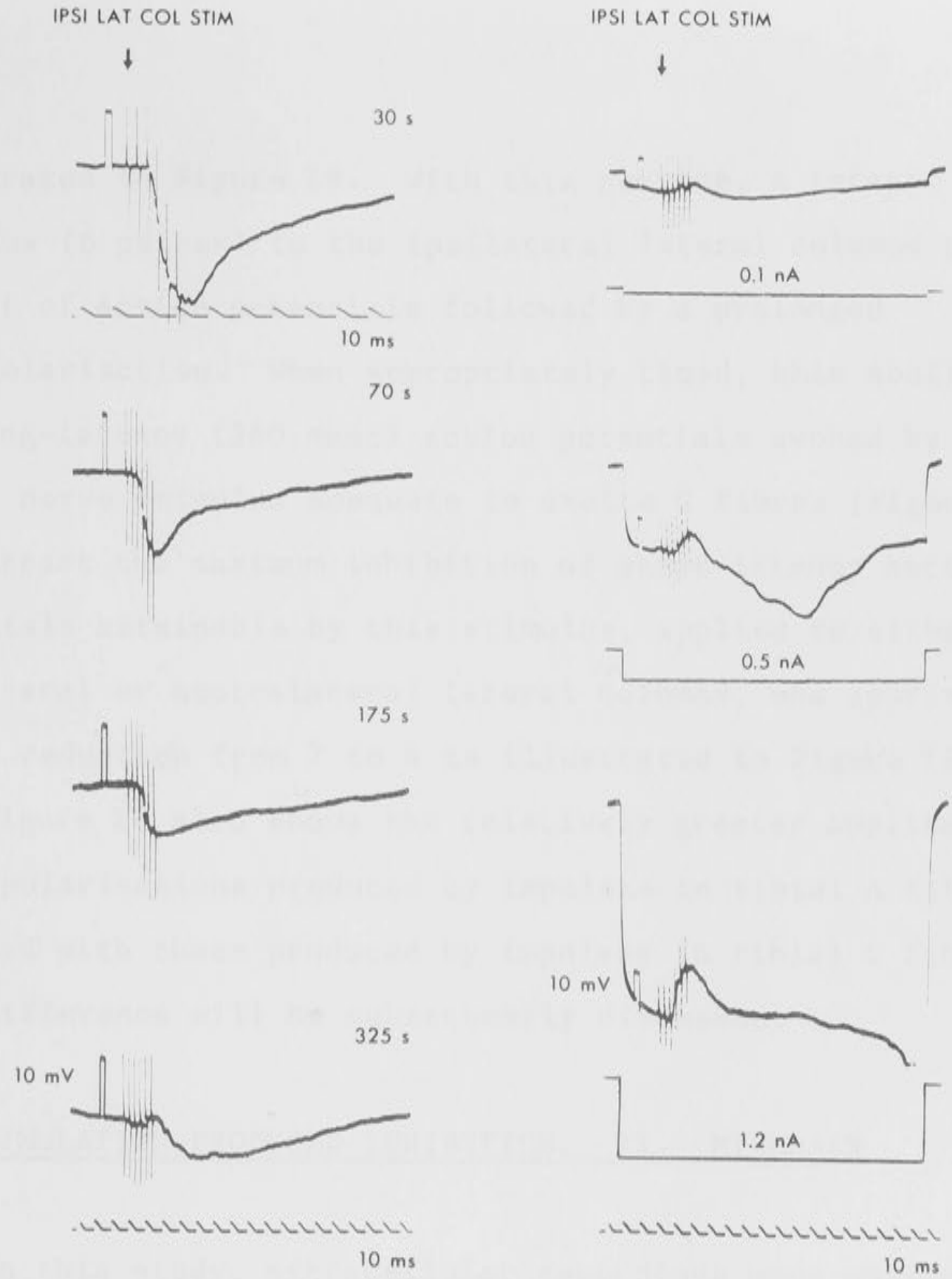


Fig. 27. Diminution of the hyperpolarization produced in a lamina V neurone by tetanic stimulation (six 0.5 msec pulses at 310 Hz, 300 μA) of the ipsilateral lateral spinal funiculus (IPSI LAT COL STIM). Records were obtained at various times (indicated in seconds) after impalement of the neurone with a 2 M KCl-containing electrode. It was necessary to hyperpolarize this cell by the passage of current to observe a clear depolarization. Three currents 0.1, 0.5 and 1.2 nA are illustrated.

illustrated in Figure 28. With this neurone, a tetanic stimulus (6 pulses) to the ipsilateral lateral columns produced a burst of action potentials followed by a prolonged hyperpolarization. When appropriately timed, this abolished the long-latency (360 msec) action potentials evoked by a tibial nerve stimulus adequate to excite C fibres (Figure 28B). By contrast the maximum inhibition of short-latency action potentials attainable by this stimulus, applied to either ipsilateral or contralateral lateral columns, was approximately 50% (a reduction from 7 to 4 is illustrated in Figure 28A).

Figure 28 also shows the relatively greater amplitude of the depolarizations produced by impulses in tibial A fibres compared with those produced by impulses in tibial C fibres. This difference will be subsequently discussed.

C. STIMULATION-PRODUCED INHIBITION. II. MIDBRAIN

In this study, extracellular recordings were obtained from thirty dorsal horn neurones during electrical stimulation in the midbrain. One was in lamina I, thirteen in laminae III-IV, fifteen in lamina V and one in lamina VI. These experiments initially selected neurones only on the basis of excitation by impulses in unmyelinated primary afferents, but it was soon found that this response was inhibited by stimulation in many areas of the midbrain. In view of this widespread distribution of effective sites, and since inhibition which is selective for nociceptive but not non-nociceptive responses is more readily associated with antinociception, it was decided to search for

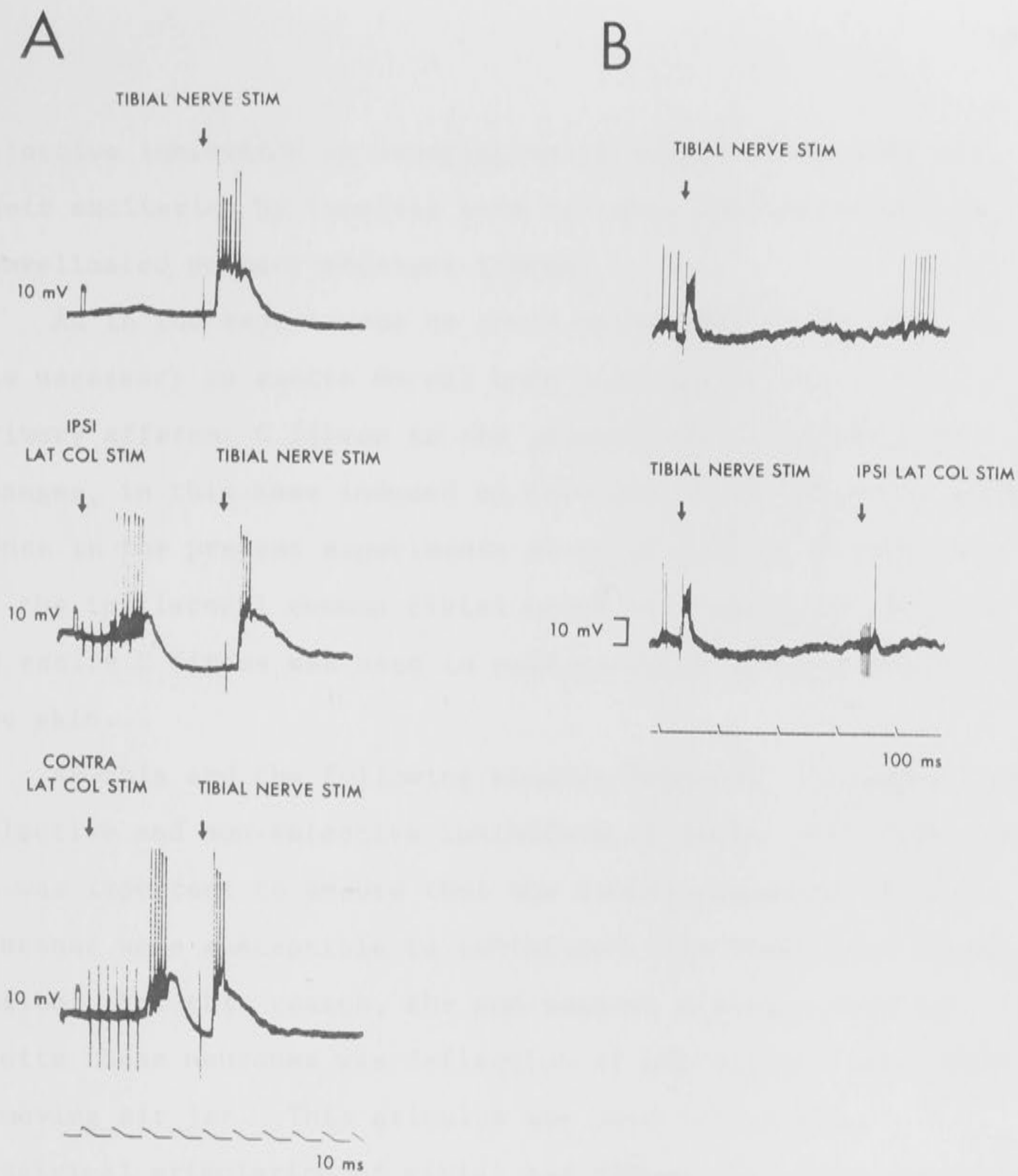


Fig. 28. Intracellular recordings from a lamina V neurone illustrating synaptic excitation by a single stimulus (0.5 msec pulse) to the ipsilateral tibial nerve and its inhibition by a preceding tetanic stimulus (six 0.5 msec pulses at 310 Hz, 900 μA) to the ipsilateral and contralateral lateral spinal columns (LAT COL STIM). The records show the differential effect of lateral column stimulation in inhibiting action potentials evoked by impulses in myelinated (A) and unmyelinated (B) primary afferents. Results from this neurone are also shown in Figure 24.

selective inhibition by studying multireceptive neurones and their excitation by impulses both in large myelinated and in unmyelinated primary afferent fibres.

As in the experiments on tonic descending inhibition, it was necessary to excite dorsal horn neurones by impulses in primary afferent C fibres in the presence of cardiovascular changes, in this case induced by stimulation in the brain stem. Hence in the present experiments also, electrical stimulation of the ipsilateral common tibial nerve at a strength adequate to excite C fibres was used in preference to noxious heating of the skin.

As this and the following studies proposed to examine both selective and non-selective inhibition of dorsal horn neurones, it was important to ensure that the evoked responses of these neurones were susceptible to inhibition from brain stem stimulation. For this reason, the non-noxious stimulus used to excite these neurones was deflection of peridigital hairs with a moving air jet. This stimulus was used rather than electrical stimulation of tibial Aαβ fibres since the previous series of experiments (section B) had found that such stimulation evokes a large depolarizing potential in dorsal horn neurones, and the resultant action potentials were more difficult to inhibit with a somatic hyperpolarization than those produced by the EPSPs from the relatively widely dispersed C fibre volley evoked electrically. This produced an apparent selectivity of inhibition (Figure 28). In the present experiments, this was confirmed when it was found that stimulation in some midbrain regions similarly inhibited responses

evoked by electrical stimulation of tibial C fibre afferents and by just-threshold stimulation of large myelinated tibial afferents, but failed to reduce responses evoked by suprathreshold stimulation of the latter. The responses evoked by hair deflection appeared not to be similarly resistant to inhibition since stimulation of these midbrain regions produced comparable inhibition of both hair deflection and C fibre-evoked responses of dorsal horn neurones.

(a) Stimulation in the Periaqueductal Grey (PAG)

Of the thirty neurones studied in these experiments, twenty-nine were observed during electrical stimulation in the PAG. Such stimulation reduced the responses of all of these cells to impulses in C fibre primary afferents. The duration of this inhibition following each stimulus train in the midbrain was not systemically studied, but in all cases where clear inhibition was observed, it was characterized by a rapid onset/offset. Thus the first gated tibial C response following commencement of the stimulus trains (at 0.2-0.5 Hz) was usually inhibited, and the first C response following cessation of the trains had usually returned to control levels. Examples are shown in Figures 29, 31 and 32.

Of the fifteen neurones in which responses to hair deflection were also studied, the effects of PAG stimulation were selective in twelve. Thus stimulus currents producing 50 to 100% inhibition of C responses had no effect on excitation by hair deflection. This selective effect of PAG stimulation is illustrated in Figure 29, which also shows that the onset and

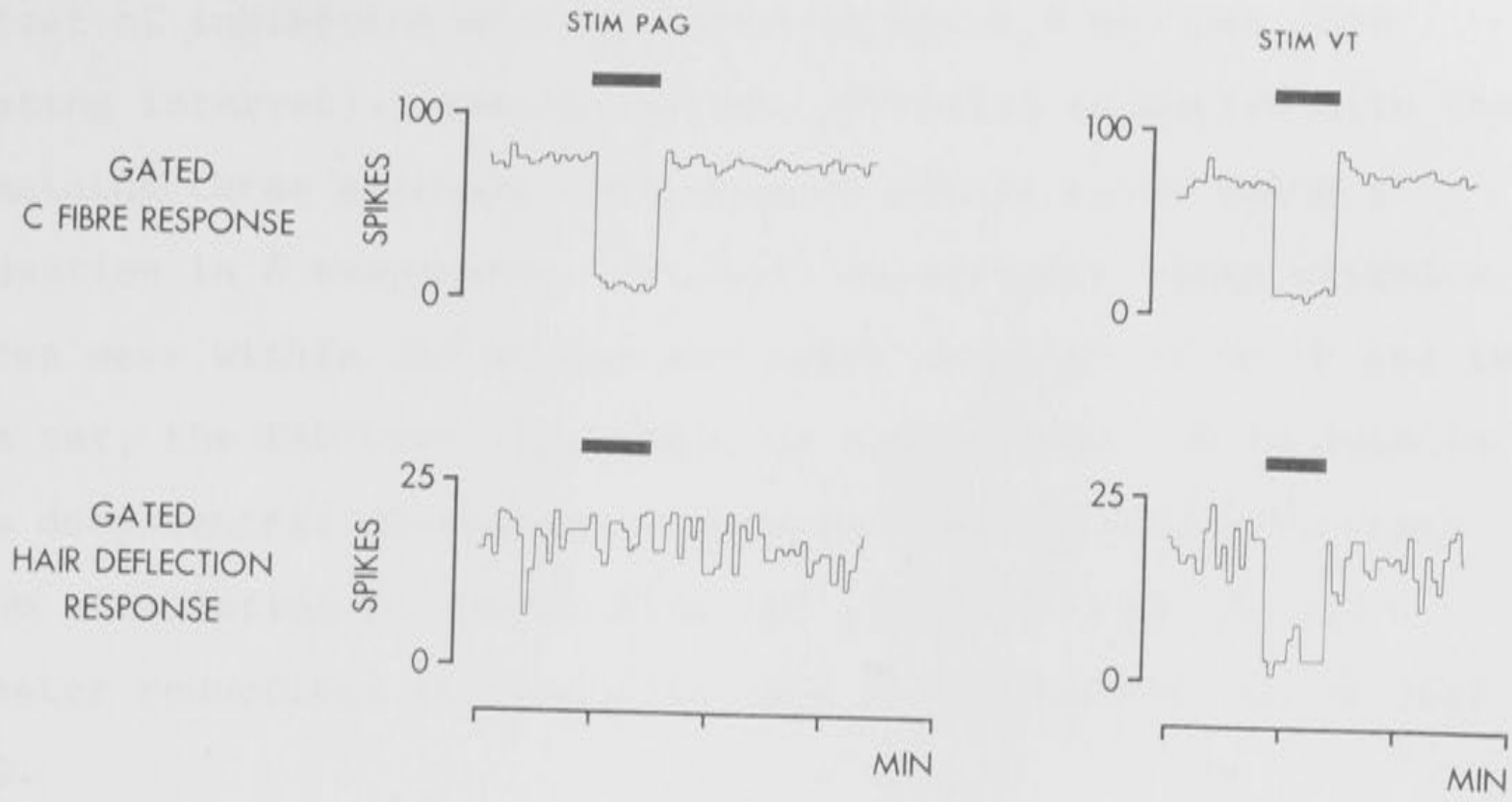


Fig. 29. Selective and non-selective inhibition of a lamina I neurone from electrical stimulation in the periaqueductal grey (PAG) and the ventral tegmentum (VT). The upper records show the number of action potentials evoked by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve at 0.3 Hz. The lower records show the number of action potentials evoked by movement of peridigital hairs of the ipsilateral hind limb by the oscillation of an air jet across part of the neurone's receptive field at 0.3 Hz. The black bars indicate the periods of electrical stimulation in the PAG (Horsley-Clarke co-ordinates AP 0, V + 1) and the VT (AP 0, V -1) by a tetanic train (twenty-eight 0.5 msec pulses at 310 Hz, 200 μA) repeated at 0.3 Hz. In this and subsequent figures, the last pulse of each train occurred approximately 10 msec before the onset of the counting gate for each peripheral stimulus, and stimulation sites were confirmed histologically by reference to iron or tungsten deposits from the stimulating electrodes.

offset of inhibition were complete within 3.3 seconds (the testing interval). Inhibition was partially selective with the remaining three neurones, the greater effect still being a reduction in C responses. In these experiments, most stimulus sites were within the antero-posterior range AP +4 to -2 and in the cat, the PAG over this range is approximately 4 mm deep in the dorsoventral direction. Although inhibition was obtained from stimulation in essentially all parts of this region, greater reductions in responses were obtained from the ventral PAG.

(b) Stimulation in the Ventral Tegmentum (VT)

In examining other regions of the midbrain, eighteen cells were studied during electrical stimulation at sites ventral to the PAG. Although this is a complex region containing many fibre tracts and cell groupings, it has been collectively termed the "ventral tegmentum" (VT) since inhibition from all sites stimulated differed from that produced by PAG stimulation. As the stimulating electrodes were advanced ventrally (the VT is approximately 5 to 7 mm deep at the areas studied), the percent inhibition was not constant at each site and it was common to observe zones not giving inhibition. In particular, inhibition of C responses was often reduced in the most ventral 2 mm of the tegmentum.

With all eighteen cells studied, excitation by C fibre primary afferents was reduced by electrical stimulation at sites ventral to the PAG. Thus when studying only this parameter (eight cells), inhibition was similar to that

produced by PAG stimulation. With ten neurones, however, responses to hair deflection were also studied and with all ten, the inhibition was non-selective in reducing both types of excitation. Figure 29 illustrates the shift from selective to non-selective inhibition of one neurone from stimulation in the PAG and a more ventral site in the VT. In Figure 30 the gated counts for two neurones have been converted to percent inhibition for stimulation at several sites. With Cell A, inhibition was completely selective with PAG stimulation, non-selective with stimulation in the upper VT and, with stimulation deep in the VT, there was little inhibition of C responses but powerful inhibition of excitation by hair deflection. With 0.5 mm electrode tips 3 mm apart, stimulation limited to small structures such as the DRN is probably not possible. Nevertheless, as shown for Cell B in Figure 30, it was clear that stimulation immediately below the PAG (an area adjacent to the DRN) produced non-selective inhibition.

(c) Stimulation in the Diencephalon

In the present experiments sites of stimulation were usually restricted to the range AP +4 to -2 of the mesencephalon, but with some neurones more rostral regions were also investigated. Electrical stimulation at many diencephalic sites between AP +14 and +6 inhibited the C responses of dorsal horn neurones. Since the stimulating electrodes were positioned across the midline and moved dorso-ventrally, these sites often included the hypothalamus and the PVG regions adjacent to the wall of the third ventricle. With some

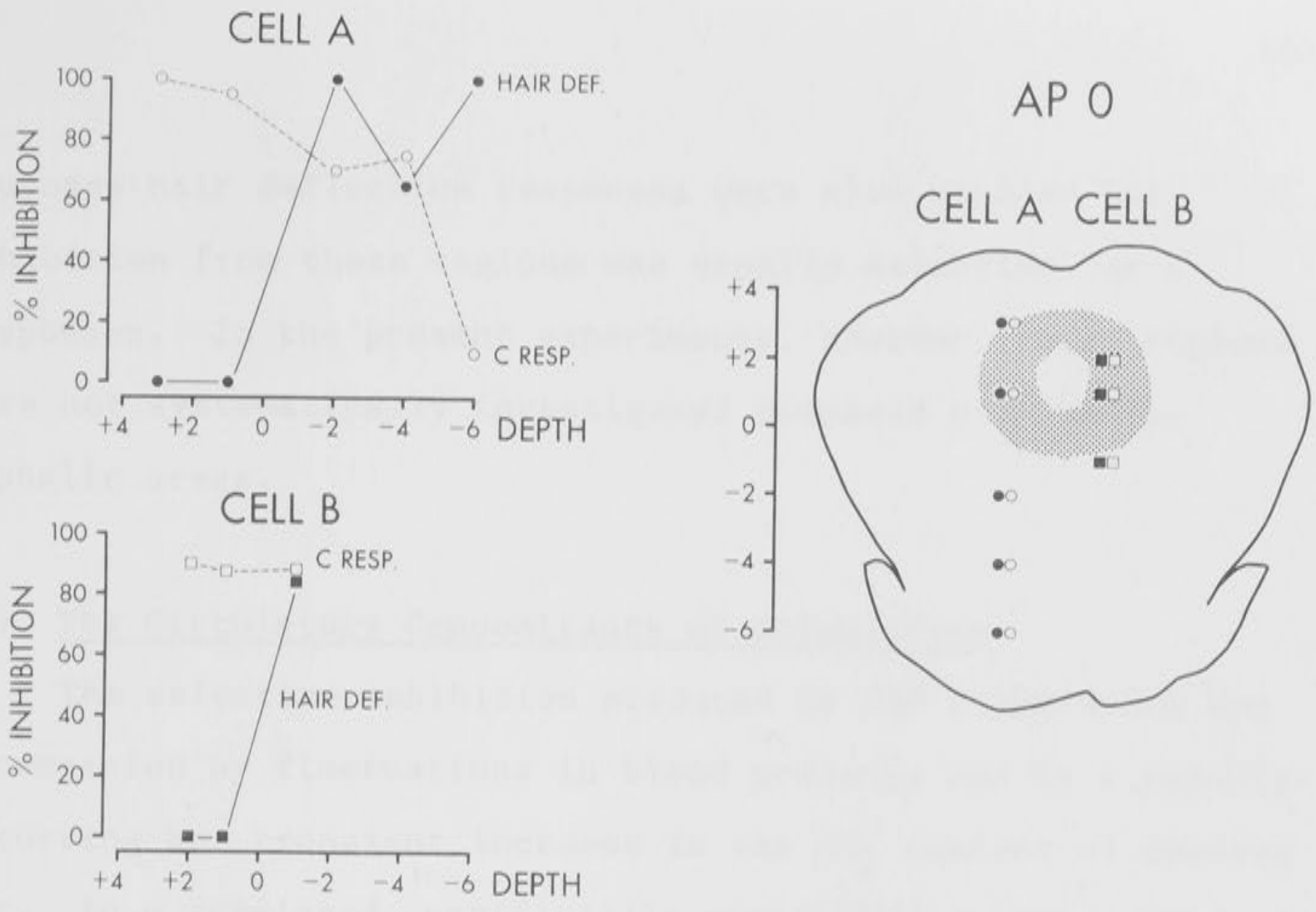


Fig. 30. Inhibition of dorsal horn neurones by electrical stimulation in the periaqueductal grey (PAG) and the ventral tegmentum (VT). Cell A was in lamina IV, cell B in lamina I. For each neurone the gated counts of excitation by impulses in unmyelinated primary afferents of the ipsilateral tibial nerve and by hair deflection, obtained during electrical stimulation in the midbrain, have been expressed as percent inhibition of controls and plotted according to the Horsley-Clarke vertical co-ordinates of the histologically confirmed stimulation sites. Filled symbols, inhibition of responses to hair deflection; open symbols, inhibition of responses to C primary afferent stimulation; stippled area, PAG.

Stimulus parameters:

Cell A, thirty-seven 0.5 msec pulses at 310 Hz, 250 μA, 0.3 Hz.

Cell B, twenty-eight 0.5 msec pulses at 310 Hz, 200 μA, 0.3 Hz.

neurones hair deflection responses were also studied but inhibition from these regions was usually selective for C responses. In the present experiments, however, these regions were not systematically investigated compared with mesencephalic areas.

(d) The Circulatory Concomitants of Stimulation

The selective inhibition produced by PAG stimulation was accompanied by fluctuations in blood pressure and by a rapidly-occurring but transient increase in the CO_2 content of expired air. In a paralysed, artificially ventilated animal, rapid and transient changes in expired CO_2 most probably result from changes in pulmonary perfusion, and hence of cardiac output. To detect possible alterations in the peripheral circulation concomitant with this increase in cardiac output, changes in skin surface temperature and muscle temperature were monitored during midbrain stimulation, as described in Methods.

(i) Changes in end-tidal CO_2 levels

The effects of PAG stimulation on end tidal CO_2 levels were measured while recording from twenty-eight neurones. Consideration of the data from stimulation at all sites in the PAG within the range AP +4 to -2 (and in some cases stimulation was repeated up to 11 times at any one site) gives a mean increase in CO_2 levels (expressed as a percentage of expired air) during stimulation of 0.18 ± 0.009 S.E.M. (n = 192). (The baseline variance in these records permitted measurements to a limit of 0.01%). Since end tidal CO_2 levels were maintained at

approximately 4% of expired air, this is a 4 to 5% increase in CO_2 levels. Examples of this effect, illustrating its short latency (seconds) and duration (rarely greater than 2 minutes), are shown in Figures 31 and 32.

With stimulation in the VT (all sites and including repeated stimulation at some sites) the increase in CO_2 was much less, the mean being 0.07 ± 0.008 S.E.M. (n = 138).

It is important to point out that a significant increase in end-tidal CO_2 occurred during stimulation in the PAG while recording from all twenty-eight cells. Moreover, if the analysis is restricted to multireceptive neurones (eight) in which responses to impulses in C fibre afferents and to hair deflection were adequately studied with stimulation both in the PAG and at ventral sites, the results are:-

PAG (selective inhibition), CO_2 increase (as a percentage of expired air): 0.19 ± 0.012 S.E.M. (n = 75);

VT (non-selective inhibition), CO_2 increase: 0.07 ± 0.008 S.E.M. (n = 68).

Although it is not possible to convert these figures to circulatory parameters, it is clear that stimulation at sites producing selective inhibition (PAG) produced a significantly greater increase in cardiac output than the same stimulation at ventral sites not producing this inhibition.

(ii) Changes in muscle temperature

Changes in muscle temperature were monitored during electrical stimulation in the midbrain while recording from nineteen neurones. As with analysis of CO_2 changes, the data have been collected separately for stimulation at all PAG sites and for more ventral sites.

Electrical stimulation in the PAG produced increases in muscle temperature: the mean change during such stimulation was an increase of 0.030°C ±0.004 S.E.M. (n = 121). (The mean has been expressed to the limit of measurement, 0.005°C). Such increases were of longer latency and of much longer duration than the increases in end-tidal CO_2, as shown in Figure 31. (Since these increases were measured with a glass-coated thermistor, however, such observations of latency and duration may not be comparable). With stimulation in the VT, the mean increase in muscle temperature was 0.005°C ± 0.001 S.E.M. (n = 79). Thus a significantly greater rise was produced by stimulation in the PAG. In only 3 of the 200 trials was a decrease in muscle temperature produced by stimulation in the brain stem. Figure 31 contrasts the effects on muscle temperature of stimulation in the PAG and just ventral to the PAG, both sites giving similar inhibition of C responses.

Considering results in relation to neurones, an increase in muscle temperature was observed while recording from eighteen of nineteen neurones with stimulation in the PAG. Complete data on stimulation in the PAG and at more ventral sites were obtained with eight multireceptive cells and with these the analysis shows:-

PAG (selective inhibition), mean increase: 0.035°C ± 0.006 S.E.M. (n = 73);

VT (non-selective inhibition), mean increase: 0.005°C ± 0.001 S.E.M. (n = 64).

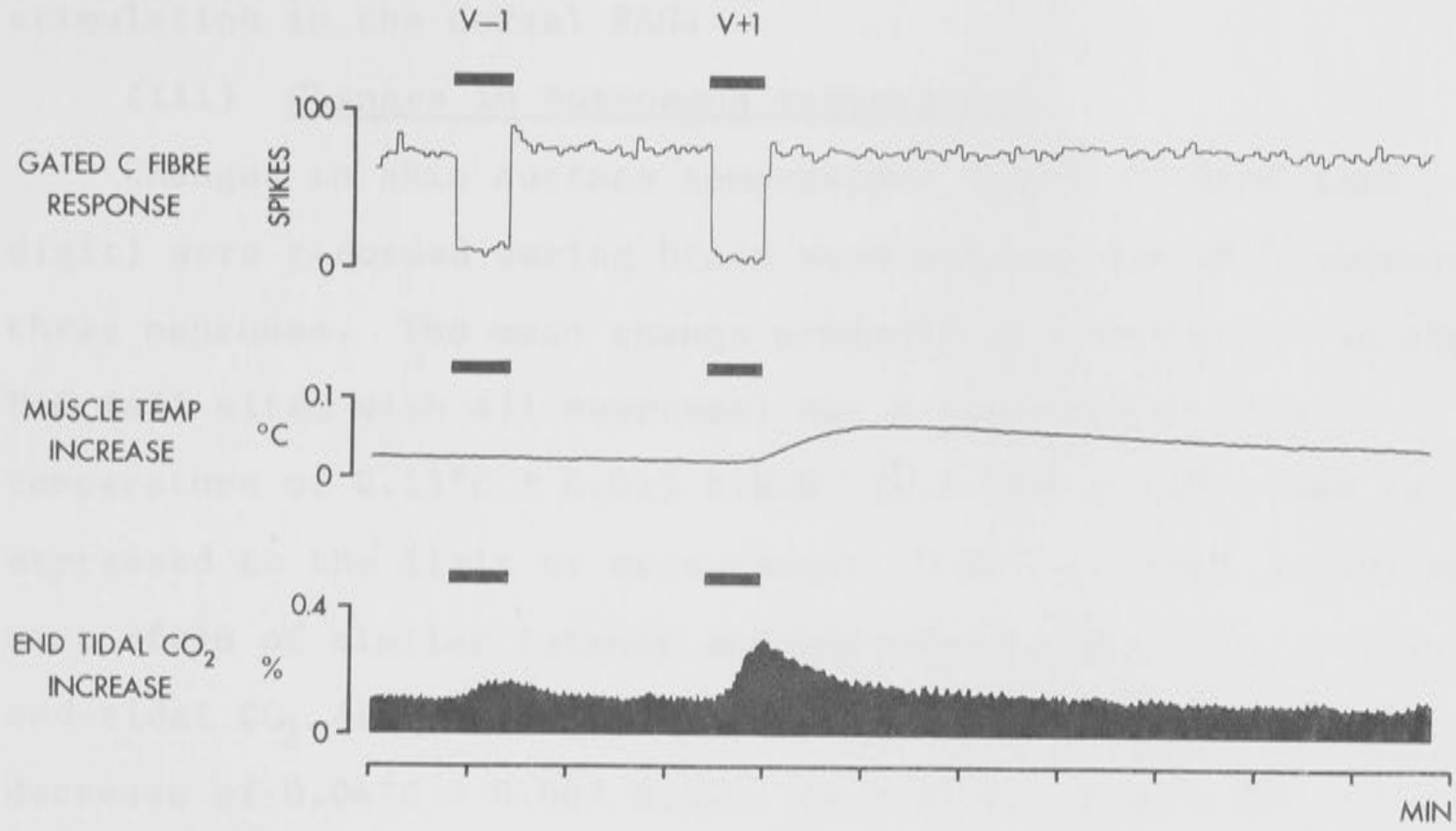


Fig. 31. The differential effects on C fibre responses, muscle temperature and end tidal CO_2 levels produced by electrical stimulation in the periaqueductal grey (PAG) and the ventral tegmentum (VT). The recordings are responses of a lamina I neurone to impulses in C primary afferents of the ipsilateral tibial nerve, temperature in the posterior muscle compartment of the right hind limb and CO_2 levels as a percentage of expired air. The lower two traces are high gain records and are calibrated as increases rather than in absolute values. The black bars mark the periods of stimulation in the upper VT (Horsley-Clarke AP 0, V -1) and in the ventral PAG (Horsley-Clarke AP 0, V +1) by the tetanic train (twenty-eight 0.5 msec pulses at 310 Hz, 200 µA, 0.3 Hz).

The greatest increase in muscle temperature produced by a single trial of midbrain stimulation was 0.25°C, from stimulation in the dorsal PAG.

(iii) Changes in cutaneous temperature

Changes in skin surface temperature (pinna or hind limb digit) were recorded during brain stem stimulation with twenty-three neurones. The mean change produced by stimulation in the PAG (all sites with all neurones) was a decrease in skin temperature of 0.13°C ± 0.015 S.E.M. (n = 157). (The mean is expressed to the limit of measurement, 0.01°C). Such decreases were often of similar latency and duration to the increases in end-tidal CO_2 (Figure 32). Stimulation in the VT gave a mean decrease of 0.04°C ± 0.007 S.E.M. (n = 111). Figure 32 illustrates the differing effects on skin temperature of stimulating in the PAG and more ventrally.

The effects on skin temperature were less constant than changes in CO_2 levels and muscle temperature. The figures above were derived from all sites (whether a decrease was produced or not) and include repeated stimulation at the same site. While recording from some neurones, however, a change in temperature did not occur with every period of stimulation at a given site. If a change is defined as a decrease in more than 60% of trials, this occurred with eighteen of twenty-three neurones with PAG stimulation. Restricting the analysis to multireceptive neurones (eight) with which data were obtained

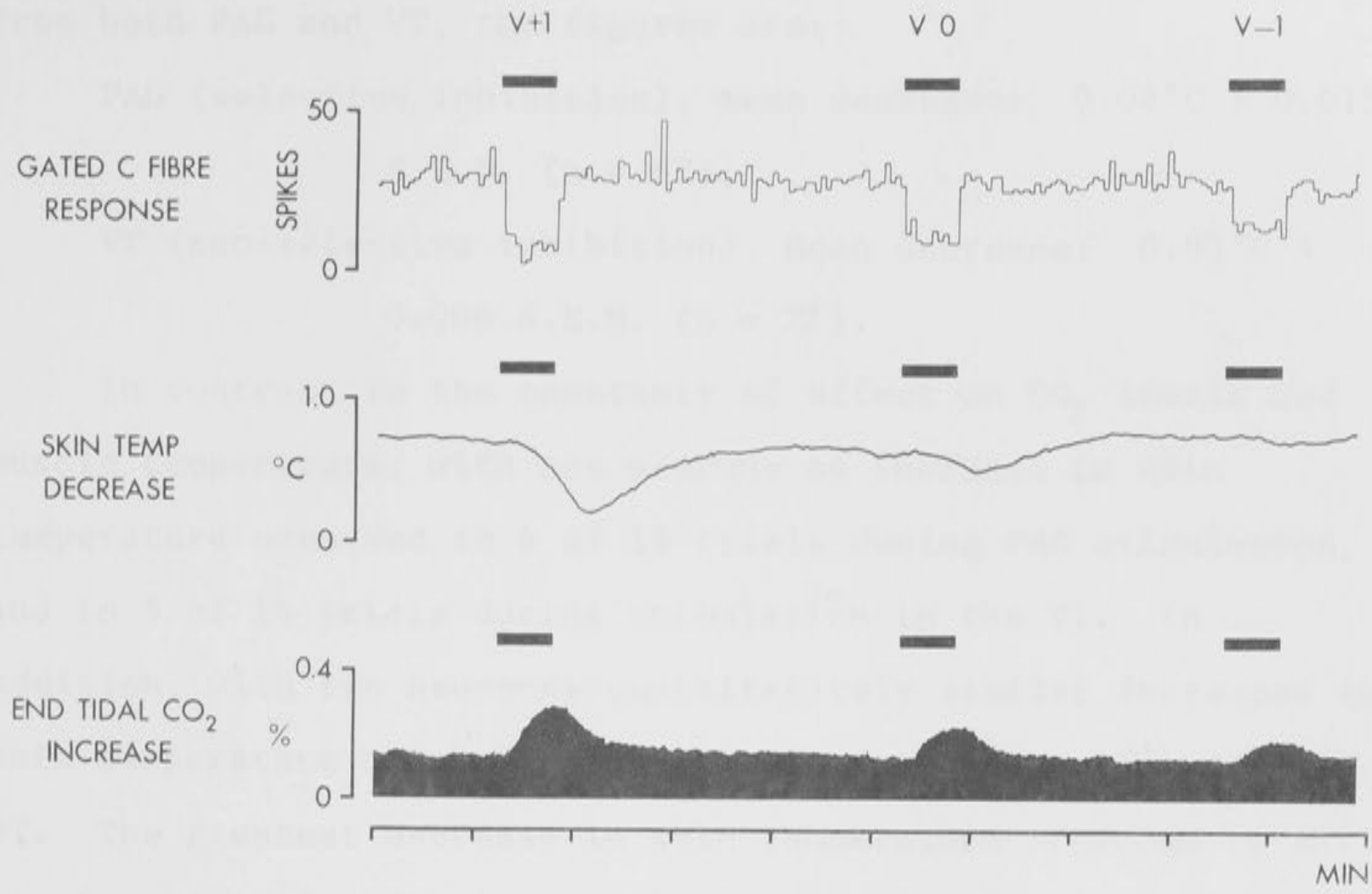


Fig. 32. The differential effects on C fibre responses, skin temperature and end tidal CO_2 levels from stimulation in the periaqueductal grey (PAG) and the ventral tegmentum (VT). The recordings are responses of a lamina IV neurone to impulses in C primary afferents of the ipsilateral tibial nerve, skin temperature detected by a thermistor placed on the skin of the central pad of the left hind limb and CO_2 levels as a percentage of expired air. As in Figure 31, the calibration of the lower two records is for changes rather than for absolute values. The black bars indicate the periods of electrical stimulation (thirty-seven 0.5 msec pulses at 310Hz, 300 μA, 0.3 Hz) at 3 sites, all at AP +2 : V +1, in the ventral PAG; VO, at the border of the PAG and the VT; and V -1, in the dorsal VT.

from both PAG and VT, the figures are:-

PAG (selective inhibition), mean decrease: 0.08°C ± 0.015 S.E.M. (n = 67);

VT (non-selective inhibition), mean decrease: 0.03°C ± 0.008 S.E.M. (n = 72).

In contrast to the constancy of effect on CO_2 levels and muscle temperature, with one neurone an increase in skin temperature occurred in 4 of 16 trials during PAG stimulation, and in 5 of 14 trials during stimulation in the VT. In addition, with two neurones quantitatively similar decreases in skin temperature occurred with stimulation in the PAG and the VT. The greatest decrease in skin temperature produced by a single trial of midbrain stimulation was 0.71°C, from stimulation in the ventral PAG.

Figure 33 summarizes the effects of stimulating in the PAG and the VT on end-tidal CO_2 levels, muscle and skin temperature.

(iv) Changes in blood pressure

In these experiments, stimulation in the midbrain produced changes in blood pressure. Stimulation in the PAG often produced transient hypertension, but decreases and biphasic effects were also observed. Stimulation in the VT produced lesser but equally inconsistent effects. The relationship, therefore, between such changes in blood pressure and the type of inhibition produced by midbrain stimulation was difficult to define.

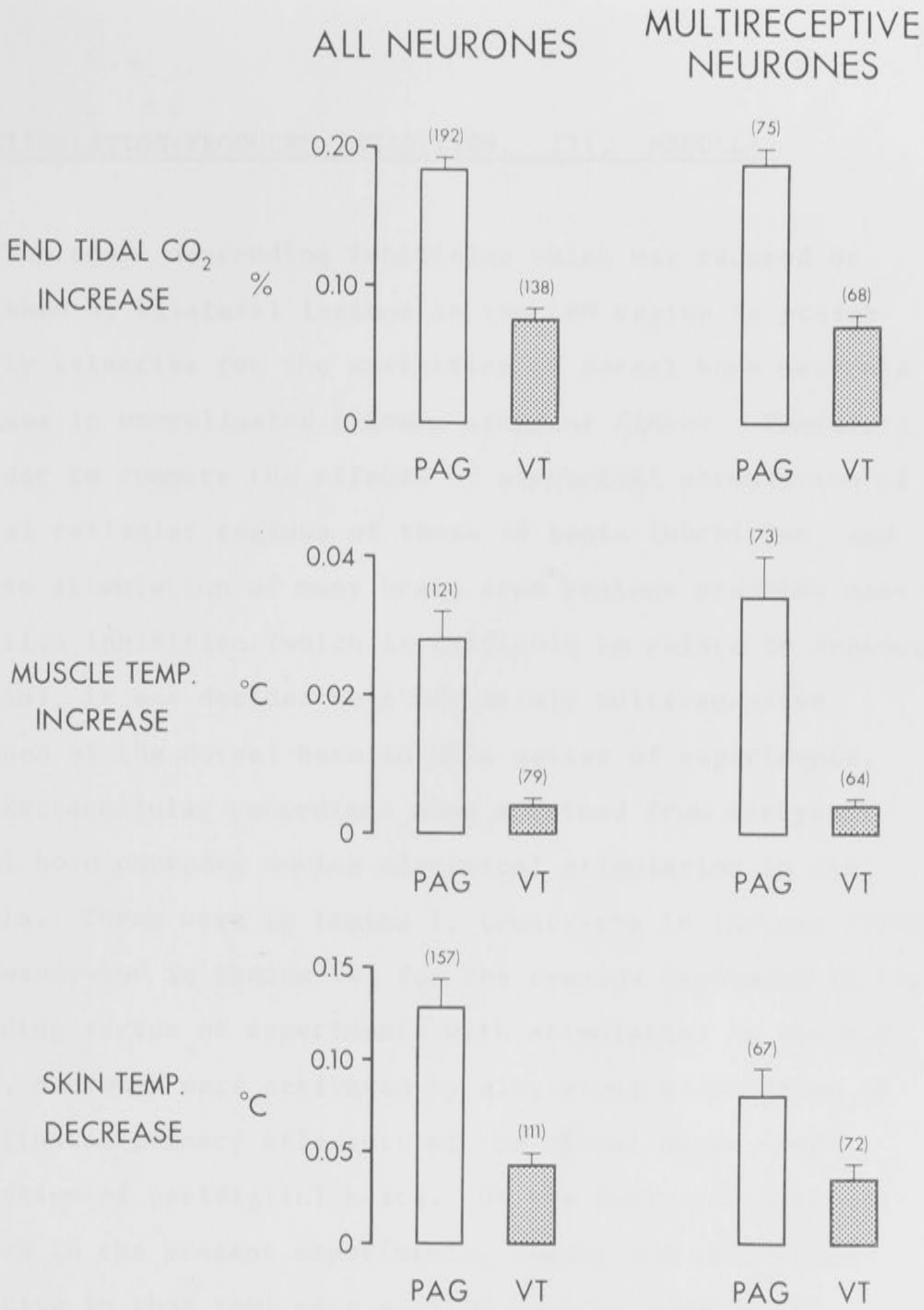


Fig. 33. Comparison of the effects of electrical stimulation in the periaqueductal grey (PAG) (open bars) and the ventral tegmentum (VT) (stippled bars) on end tidal CO_2, muscle and skin temperature. The bars represent the mean changes (and standard errors of the means) in these parameters. Results have been pooled for all neurones studied and for all multireceptive neurones in which excitation by impulses in C primary afferents and by hair deflection were concurrently studied. The numbers of stimulation trials (including repeated trials at some sites) are given in parentheses.

D. STIMULATION-PRODUCED INHIBITION. III. MEDULLA.

The tonic descending inhibition which was reduced or abolished by bilateral lesions in the LRN region is predominantly selective for the excitation of dorsal horn neurones by impulses in unmyelinated primary afferent fibres. Therefore, in order to compare the effects of electrical stimulation of lateral reticular regions of those of tonic inhibition, and because stimulation of many brain stem regions produces non-selective inhibition (which is difficult to relate to antinociception), it was decided to study mainly multireceptive neurones of the dorsal horn in this series of experiments.

Extracellular recordings were obtained from forty-six dorsal horn neurones during electrical stimulation in the medulla. Three were in lamina I, twenty-one in laminae III-IV, and twenty-two in lamina V. For the reasons expounded in the preceding series of experiments with stimulation in the midbrain, neurones were activated by electrical stimulation of unmyelinated primary afferents of the tibial nerve, and by deflection of peridigital hairs. Of the forty-six neurones studied in the present experiments, twenty-six were multireceptive in that they were excited both by impulses in unmyelinated primary afferents and by hair deflection. Of the remaining twenty neurones (in which only C responses were studied), ten were not activated by hair deflection and ten were not tested for hair deflection responses.

In these experiments, both bipolar (twenty-seven neurones) and monopolar (nineteen neurones) stimulation techniques were

used. As discussed previously (Methods, Section F(c)), the bipolar technique, while usually giving results similar to those obtained with monopolar stimulation, in some instances gave inhibition of dorsal horn neurones from stimulation at many widespread sites in the brain stem. Hence the results of experiments employing monopolar stimulation are presented below and the differences observed with bipolar stimulation will be detailed subsequently.

(a) Monopolar Stimulation in the Region of the Lateral Reticular Nucleus (LRN)

With seventeen of the nineteen neurones studied, monopolar electrical stimulation in the ipsilateral ventrolateral medulla in the region of the caudal LRN reduced responses to impulses in unmyelinated primary afferents. An example is illustrated in Figure 34. As with the inhibition produced by stimulation in the midbrain, this effect was also of rapid onset and did not outlast the period of stimulation (cf. Figure 29). With the other two neurones, stimulation in the LRN region did not influence C responses.

When multireceptive neurones were examined, the effects of LRN stimulation were essentially selective for C responses. Thus with seven of eight neurones tested, C fibre responses were inhibited at stimulus currents which were without effect on responses to hair deflection. An example of this selective inhibition is shown in Figure 34. With this neurone, selective inhibition of C responses was produced by stimulation at two ventral sites within 1.5 mm of the base of the brain (sites B

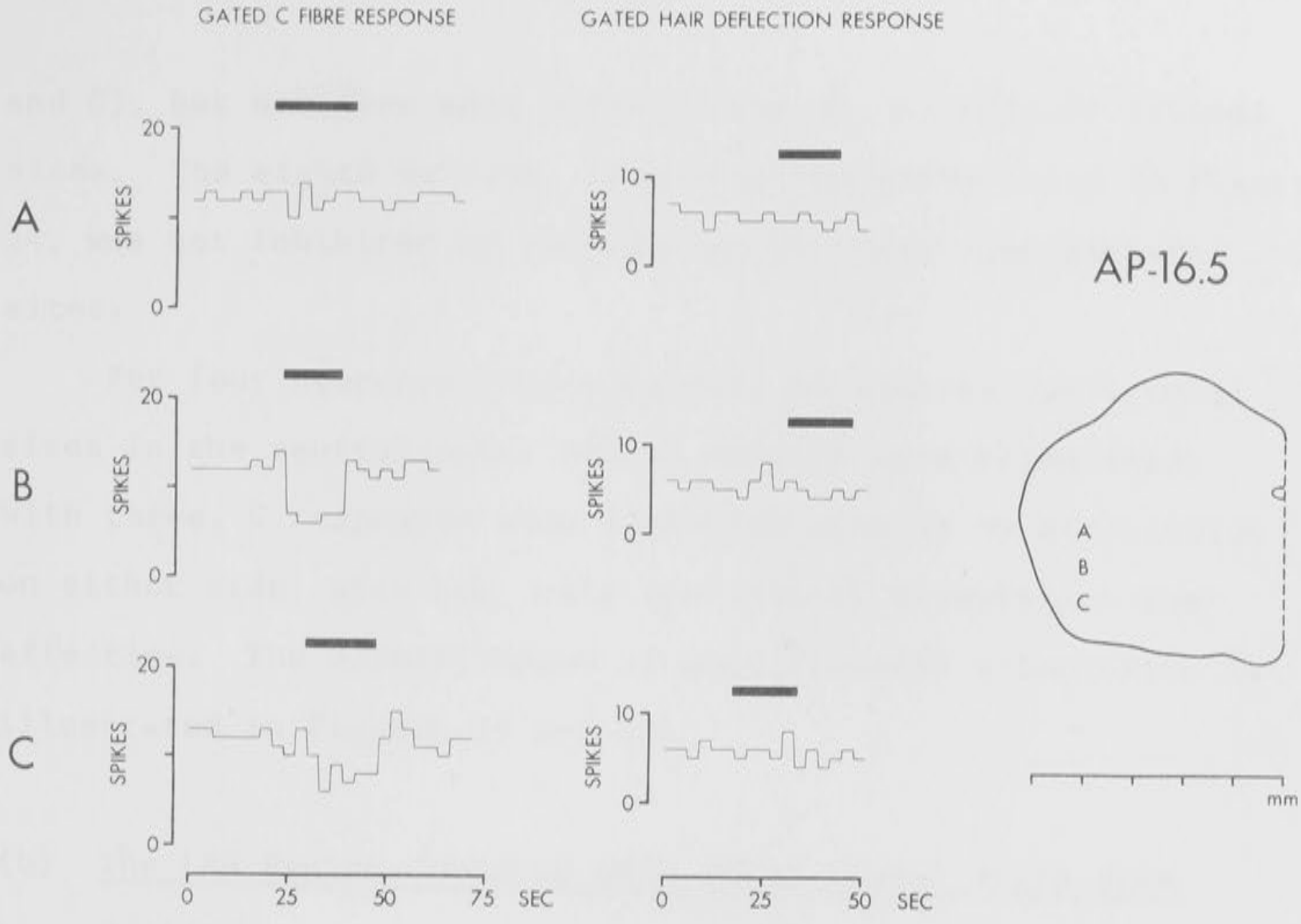


Fig. 34. Selective inhibition from electrical stimulation in the ventrolateral caudal medulla near the lateral reticular nucleus (LRN). All records were obtained from a lamina IV neurone. The recordings of the left are the number of action potentials evoked by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve at 0.3 Hz. The recordings on the right are the number of action potentials evoked by movement of peridigital hairs of the ipsilateral hind limb by an oscillating air jet (0.3 Hz). The black bars indicate the periods of monopolar electrical stimulation at sites A, B and C by a tetanic train (thirty-one 0.5 msec pulses at 310 Hz, 20 μA, 0.3 Hz).

and C), but not from more dorsal (site A), medial, or lateral sites. The eighth neurone, also from the animal used in Figure 34, was not inhibited by stimulation of these ventrolateral sites.

For four neurones, contralateral as well as ipsilateral sites in the ventrolateral caudal medulla were stimulated. With three, C responses were inhibited equally by stimulation on either side; with one, only ipsilateral stimulation was effective. The effectiveness of contralateral stimulation is illustrated in Figures 36 and 37.

(b) The LRN Region Compared with Other Caudal Brain Stem Regions

The significance of inhibition resulting from electrical stimulation of a particular region of the brain stem is increased if stimulation with similar currents in adjacent areas does not produce this effect. In many experiments therefore, a number of regions were stimulated in turn while recording from the same dorsal horn neurone. The extent of this mapping procedure varied according to the ability to maintain stable extracellular recording conditions. Electrode tracks were all at the same AP level, separated by 0.5-1.0 mm in the transverse plane and stimulation sites were 0.5 to 1.0 mm apart in the dorsoventral direction. In each case a stimulus current was used which, in the LRN region, produced significant inhibition of C fibre responses of the neurone being studied.

With all thirteen neurones studied in this way, inhibition of C responses was tested. Six of these were multireceptive neurones and excitation by hair deflection was also examined.

(i) Areas adjacent to the LRN

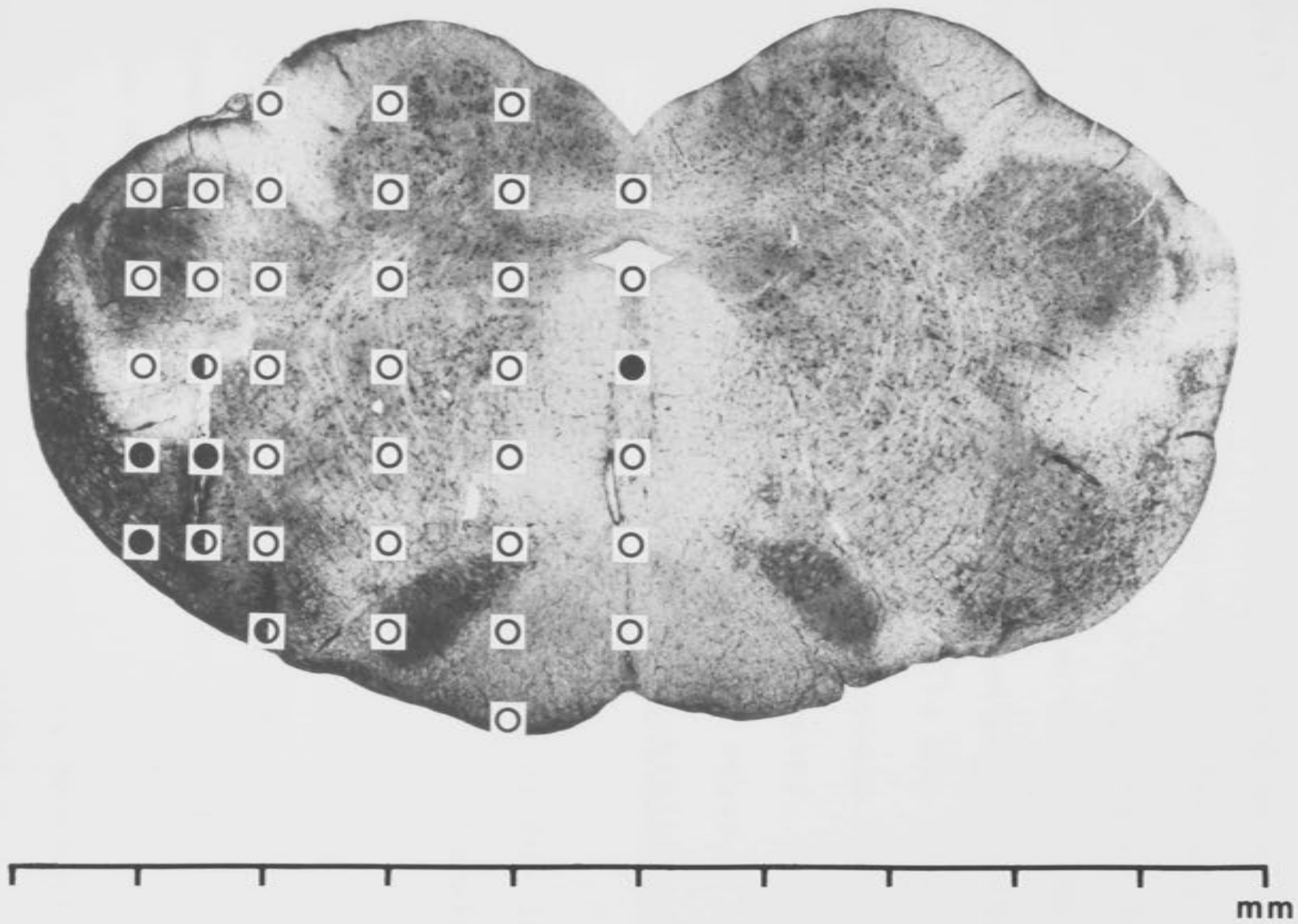
With twelve neurones (in ten animals), stimulus currents giving significant inhibition of C responses from stimulation near the LRN had little or no effect at more dorsal and medial sites, excluding the medullary raphé (see below). These regions included the lateral tegmental field, trigeminal nucleus, cuneate nucleus, hypoglossal nucleus, inferior olive and paramedian reticular nucleus. Figure 35 illustrates the effects on one neurone of this mapping procedure. With one neurone, inhibition was produced from many areas of the caudal medulla, not confined to the LRN region.

(ii) Raphé

With six neurones, the other medullary sites tested included the raphé. With four of these cells (from four animals), inhibition by similar currents was produced by monopolar stimulation of the region of the LRN and the raphé, but not of intervening and dorsal reticular areas. Such a result is also illustrated in Figure 35. With this neurone, a stimulus current of 5 μA gave powerful inhibition of C responses from 3 adjacent sites in the LRN region and one site in the raphé, and lesser inhibition from a further 3 ventrolateral sites. This current, however, was ineffective at the 31 other sites tested. With two neurones (two animals), stimulus currents which gave significant inhibition from the

Fig. 35. Inhibition of C responses of a dorsal horn neurone by monopolar stimulation in the ipsilateral caudal medulla. The marked sites were electrically stimulated by a tetanic train (thirty-one 0.5 msec pulses at 310Hz, 5 μA, 0.3 Hz) while recording from an ipsilateral lamina IV neurone excited by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve at 0.3 Hz. Stimulus sites were plotted onto the 15 μm paraffin section by reference to iron deposits. Potent inhibition, gated C fibre response reduced by 60-95%; slight inhibition, gated C fibre response reduced by 25-35%.

AP-15.5



mm

● POTENT INHIBITION

◐ SLIGHT INHIBITION

○ NO INHIBITION

lateral area were without effect in the raphé. This type of result is shown in Figure 36. In addition to the sites illustrated, midline stimulation was performed at 7 dorsoventral levels 3 mm rostral to this AP plane, with no effect.

With the six multireceptive neurones (in six animals), both C and hair deflection responses were tested during the stimulation-mapping procedure, and for all six the sites producing selective inhibition were confined to the LRN region. One such result is illustrated in Figure 36. With this neurone, selective inhibition of C responses was obtained by stimulation at only 5 of the 35 sites tested, with neither modality being inhibited at the other 30 sites.

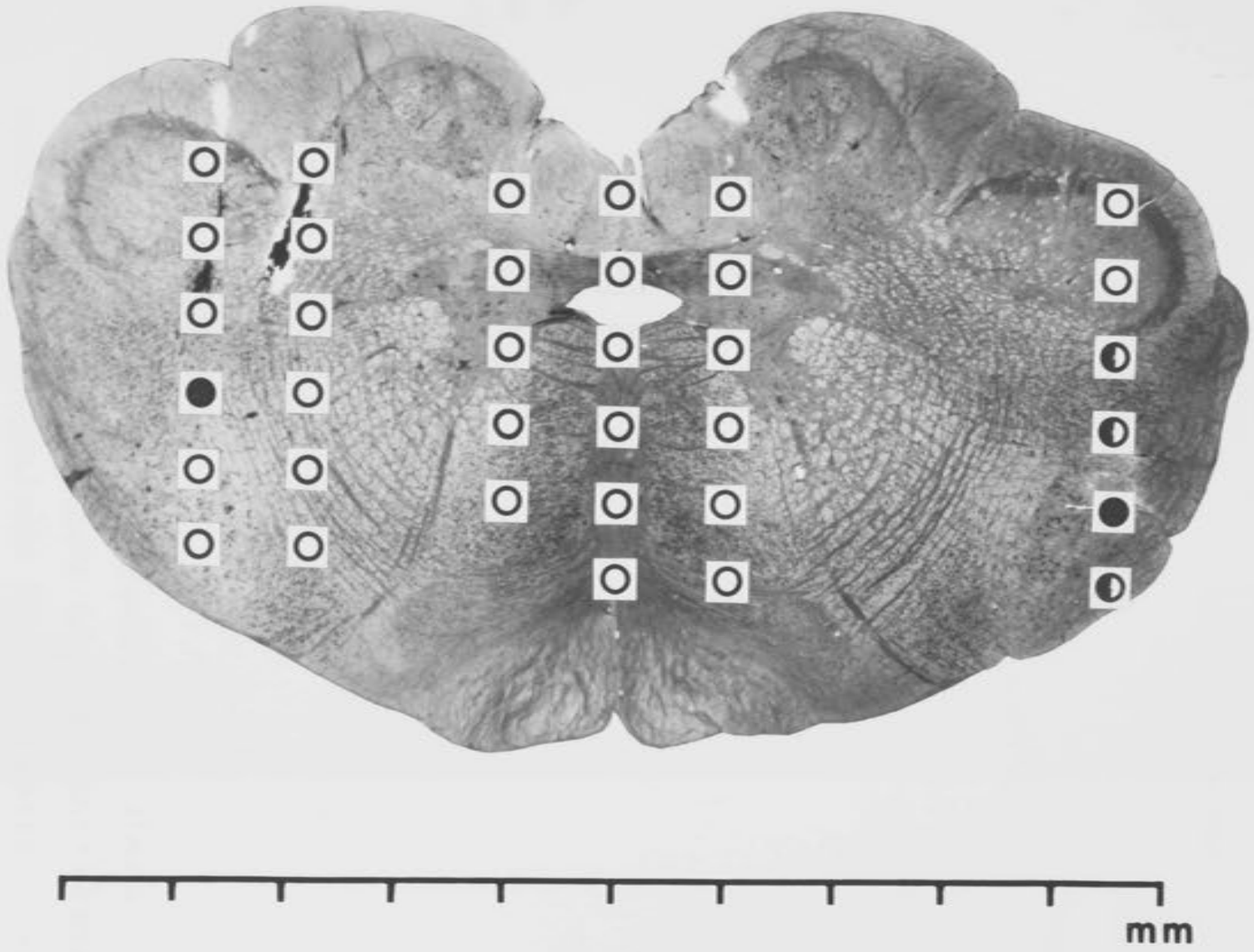
(iii) Antero-posterior extent of inhibition-producing sites

To determine the longitudinal extent of the lateral reticular regions producing inhibition when stimulated, limited results were obtained by stimulating more rostrally or caudally from a particularly effective site. This was done with 20 of such sites using monopolar stimulation with each of the 3 rostro-caudally aligned electrodes in turn while recording from nine neurones.

The commonest result (14 sites, five neurones) was that inhibition of C responses was most powerful with stimulation from the most caudal electrode of the 3. In fact with 8 of these sites, only the caudal electrode was effective. Results from one experiment are illustrated in Figure 37. With this neurone, powerful inhibition of C responses was obtained by stimulation with the most caudal electrode (AP -15.5), lesser

Fig. 36. Selective inhibition of C responses of a dorsal horn neurone by monopolar stimulation in the ipsilateral (left) and contralateral (right) caudal medulla. The marked sites were electrically stimulated by a tetanic train (sixty-two 0.5 msec pulses at 310 Hz, 30 μA, 0.2 Hz) while recording from a lamina V neurone The neurone was excited alternately by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve, and by deflection of peridigital hairs of the ipsilateral hind limb by a moving air jet, at 0.2 Hz. Stimulus sites were plotted onto the 50 μm frozen section by reference to iron deposits. Potent inhibition, gated C fibre response reduced by 60-80%; slight inhibition, gated C fibre response reduced by 20-35%. Responses to hair deflection were not inhibited by stimulation at any of the sites.

AP-16



● POTENT INHIBITION

◐ SLIGHT INHIBITION

○ NO INHIBITION

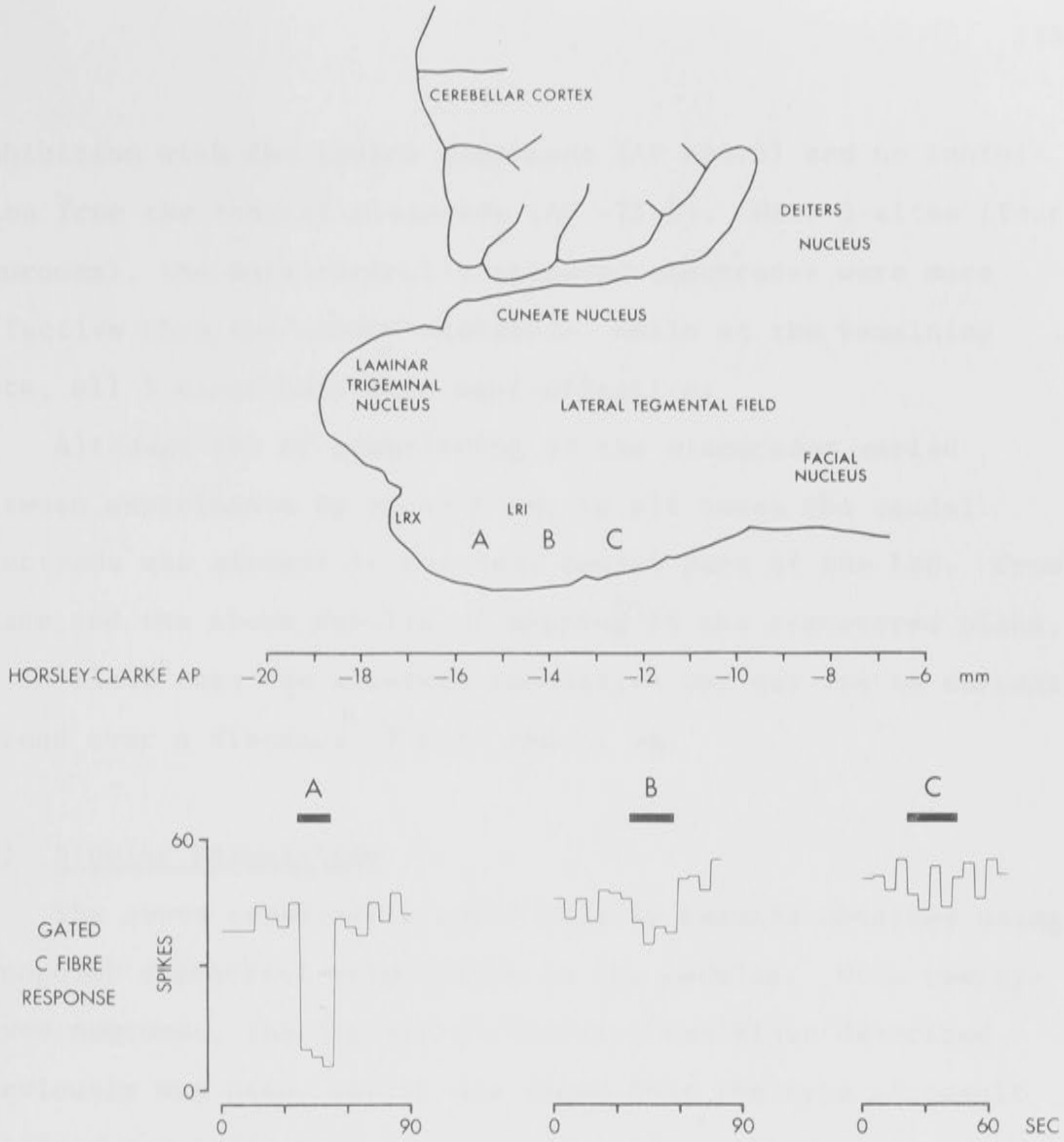


Fig. 37. Inhibition of C responses of a dorsal horn neurone by monopolar stimulation in the contralateral medulla. Records were obtained from a lamina V neurone. The recordings are counts of the number of action potentials evoked by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve at 0.3 Hz. The black bars indicate the times of electrical stimulation at sites A, B and C by a tetanic train (fifty-eight 0.5 msec pulses at 310 Hz, 30 μA, 0.3 Hz). Stimulus site locations in the parasagittal plane (4.5 mm lateral) were plotted by reference to iron deposits. LRI, lateral reticular nucleus, internal division; LRX, lateral reticular nucleus, external division.

inhibition with the centre electrode (AP -14.0) and no inhibition from the rostral electrode (AP -12.5). With 5 sites (four neurones), the more rostrally situated electrodes were more effective than the caudal electrode, while at the remaining site, all 3 electrodes were equi-effective.

Although the AP positioning of the electrodes varied between experiments by up to 2 mm, in all cases the caudal electrode was closest to the most caudal part of the LRN. From these and the above results of mapping in the transverse plane, it is clear that the observed inhibition was not due to current spread over a distance of more than 1 mm.

(c) Bipolar Stimulation

The above experiments refer only to results obtained using monopolar electrical stimulation in the medulla. With twenty-seven neurones, the bipolar stimulation technique described previously was used, and it was found that the type of result obtained was influenced by the stimulation method employed.

(i) Bipolar stimulation in the LRN region

As with monopolar stimulation, bipolar stimulation in the region of the ipsilateral LRN reduced excitation of dorsal horn neurones by impulses in primary afferent C fibres (twenty-three of twenty-seven neurones tested). A similar result was obtained with contralateral stimulation with four of five neurones (the fifth neurone not being affected by stimulation on either side). When multireceptive neurones were considered, however, stimulation in the ipsilateral LRN region selectively inhibited the C responses of only six of eleven neurones,

TABLE V THE EFFECT OF STIMULATION TECHNIQUE ON THE SELECTIVITY OF INHIBITION PRODUCED

STIMULATION METHOD	EFFECT OF STIMULATION IN LRN REGION			
	Selective inhibition of C responses	Non-selective inhibition	No effect	
Bipolar	6	5	0	11
Monopolar	7	0	1	8
	13	5	1	19

whereas both hair deflection and C responses were reduced with the other five. These results are summarized in Table V.

(ii) The LRN region and other brain stem regions

When comparing the LRN region to other brain stem regions with monopolar stimulation, the sites producing inhibition were confined to the LRN region (twelve of thirteen neurones). With bipolar stimulation, however, this result was obtained with only one of five neurones tested, the other four being inhibited from widespread areas of the brain stem. Thus the localization of effective sites to the LRN region appeared to be associated with the use of monopolar stimulation (Table VI).

(d) The Effects of Stimulation and Tonic Descending Inhibition

The lateral reticular regions which produced inhibition when stimulated were stereotactically the same as those identified in the earlier experiments (section A) as the source of tonic descending inhibition. Nevertheless, it was important to study the association between the presence of tonic inhibition and inhibition from stimulation in the region of the LRN and to ensure that destruction of the regions which were effective when stimulated reduced or abolished tonic inhibition. The extent of tonic descending inhibition present was determined by reversible cold block of spinal conduction for thirty-five neurones. With twenty-four, a significant increase in the gated C fibre-evoked response occurred, with relatively small and inconsistent increases in spontaneous firing. Of the eleven cells where tonic inhibition could not be detected, eight were from four animals and in all four this descending

TABLE VI THE EXTENT OF SITES PRODUCING INHIBITION OF C RESPONSES WITH BIPOLAR AND MONOPOLAR STIMULATION

STIMULATION METHOD	EXTENT OF SITES PRODUCING INHIBITION OF C RESPONSES		
	Confined to LRN region	Widespread	
Bipolar	1	4	5
Monopolar	12	1	13
	13	5	18

(In this Table, the expected values of less than 5 preclude the use of the χ^2 test).

control was not present on any neurone examined. As shown in Table VII, the inhibition of C responses produced by stimulation (monopolar and bipolar results combined) in the LRN region was strongly associated with the presence of tonic inhibition on the neurone.

In eight experiments bilateral coagulation of the effective ventrolateral sites was performed by passing high frequency current between adjacent stimulating electrodes. This procedure greatly reduced tonic descending inhibition in all eight cases. As in the previous experiments where brain stem coagulation reduced tonic inhibition (section A(b)), confined ventrolateral lesions, which included part of the caudal lateral reticular nuclei, were observed by histological examination.

(e) Blood Pressure

Changes in blood pressure were often observed during the periods of electrical stimulation in the ventrolateral caudal medulla. Pressor or biphasic effects were most commonly produced. These changes, however, were not systematically investigated in relation to inhibition of dorsal horn neurones.

E. INHIBITION FROM PAG STIMULATION: THE IMPORTANCE OF MEDULLARY RAPHE AND LATERAL RETICULAR REGIONS.

In this investigation, extracellular recordings were obtained from thirteen multireceptive dorsal horn neurones. One was in lamina I, nine in laminae III-IV and three in lamina

TABLE VII THE EFFECT OF STIMULATION (MONOPOLAR AND BIPOLAR) IN THE REGION OF THE LRN IN THE PRESENCE AND ABSENCE OF TONIC DESCENDING INHIBITION

	TONIC DESCENDING INHIBITION		
	Present	Not present	
Inhibition of C responses	24	5	29
EFFECT OF STIMULATION IN LRN REGION			
No inhibition	0	6	6
	24	11	35

(In this Table, the expected values of less than 5 preclude the use of the χ^2 test).

V. As in the preceding experiments employing brain stem stimulation and the spinal cold block technique, the responses of these neurones studied were excitation by impulses in unmyelinated afferents of the tibial nerve, and by deflection of peridigital hairs. The amount of tonic descending inhibition present on these neurones was determined by comparing their gated C fibre responses during normal spinal conduction with those during cold block of spinal conduction.

Earlier experiments (section C) had shown that electrical stimulation in the PAG (using the technique described in Methods) evokes a complex response characterized by selective inhibition of the excitation of multireceptive dorsal horn neurones by impulses in primary afferent C fibres, increased cardiac output and increased perfusion of muscle but not skin. Stimulation of tegmental areas ventral to the PAG non-selectively inhibited both C and hair deflection responses without circulatory effects. In the present experiments these effects produced by stimulation of these two regions (PAG and VT) were confirmed for each dorsal horn neurone. Henceforth, the only effect of PAG stimulation studied was inhibition of C fibre responses.

For each neurone studied, a PAG site was selected where stimulation produced significant inhibition of C responses, and the position of the stimulating electrodes was then not altered throughout the experiment. Such inhibition was measured with a range of stimulus currents at the chosen PAG site both prior to and following the introduction of the coagulating electrodes into the brain stem. This was done to take into account the

possibility that trauma produced by electrode placement in the medulla prior to coagulation may affect the inhibition of dorsal horn neurones evoked by PAG stimulation.

In these experiments the control C responses of the neurones studied (in the absence of PAG stimulation) were reduced by introducing the coagulating electrodes into the raphé, but were increased by removal of tonic descending inhibition by coagulation of LRN regions. In these circumstances of changing basal C response, an important consideration is the parameter used to measure inhibition from PAG stimulation. Clearly such inhibition can be expressed as a percentage of control C responses, or as the actual number of action potentials removed from the gated C response by PAG stimulation. With changes in baseline, measurements of inhibition based solely on percentage of controls can be misleading, so in these circumstances inhibition has been expressed both as percentage values and in terms of the number of action potentials removed.

(a) Lesions of the Medullary Raphé

Introduction of the coagulating electrodes into the raphé decreased the C responses of seven of eight dorsal horn neurones (mean reduction 42% ± 7 S.E.M. (n = 7)), while no change was observed with one. The level of C response attained during spinal cold block, however, was unchanged, indicating that this decrease in the basal C response was due to increased descending inhibition, presumably through exciting brain stem

neurones by mechanical deformation. This effect is shown in Figure 38.

With the array of 4 coagulating electrodes centred initially on Horsley-Clarke co-ordinates AP -7, V -9, LO, the midline was coagulated at 1 mm intervals over its dorso-ventral extent. The regions 1 mm on either side of the midline were then similarly destroyed. This coagulation in 3 parasagittal planes was then repeated rostrally with the electrodes centred on AP -5, and caudally, centred on AP -9.

In six of eight experiments, such lesions of the raphé reduced the inhibition of C responses of dorsal horn neurones produced by PAG stimulation. There was no change in one experiment. (Results from the eighth experiment have been excluded since inhibition was only temporarily reduced by raphé lesions, recovering with subsequent commencement of lateral reticular lesions). For each experiment, the reduction in inhibition was calculated by subtracting the inhibition (expressed as a percentage of control C responses) for a given stimulus current obtained following raphé lesions from that observed prior to lesions. These differences in percent inhibition were averaged, the mean reduction for the six experiments being 28.3% ± 5.4 S.E.M. (n = 6). In no instance was inhibition from PAG stimulation abolished by raphé lesions.

Results from one experiment are illustrated in Figure 38. Prior to raphé lesions, stimulation (200 μA) in the ventral PAG reduced the gated C response of the lamina IV neurone from 13.6 ± 0.2 S.E.M. to 6.5 ± 0.2 S.E.M. (n = 11), a reduction of 7.1 action potentials, or 52%. Insertion of the coagulating

Fig. 38. The effect of coagulation in the medulla on the inhibition of a lamina IV neurone produced by electrical stimulation in the PAG. The recordings show the number of action potentials evoked by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve at 0.6 Hz. The black bars indicate the periods of stimulation in the ventral PAG (AP 0, V 0) by a tetanic train (thirty-seven 0.2 msec pulses at 310 Hz, 0.6 Hz) at the stimulus currents shown, and the periods of cold block of spinal conduction (L_1) during which C responses were enhanced by removal of tonic descending inhibition.

A. Inhibition of C responses by PAG stimulation, and reduction of basal C responses by insertion of coagulating electrodes into the medullary raphé.

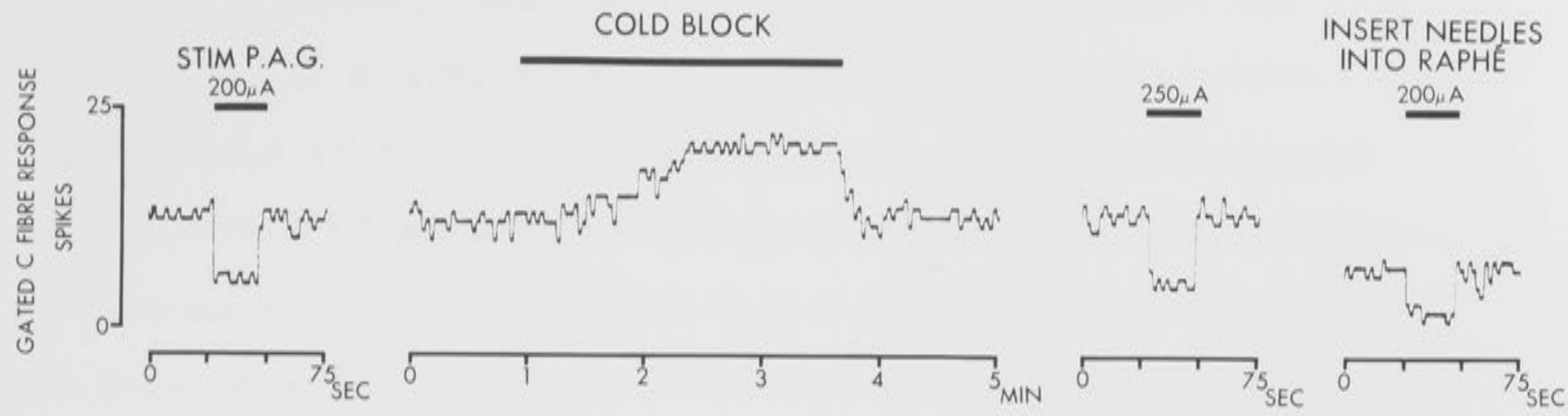
B. Inhibition of C responses by PAG stimulation following extensive coagulation (D) in the raphé.

C. Inhibition of C responses by PAG stimulation following raphé coagulation and limited lesions in the LRN regions. Following the lateral reticular lesions, the basal C responses were increased to the level attained during spinal cold block.

D. The photograph shows the extent of raphé destruction exposed by a transverse section through the fixed brain stem at approximately Horsley-Clarke AP -10.

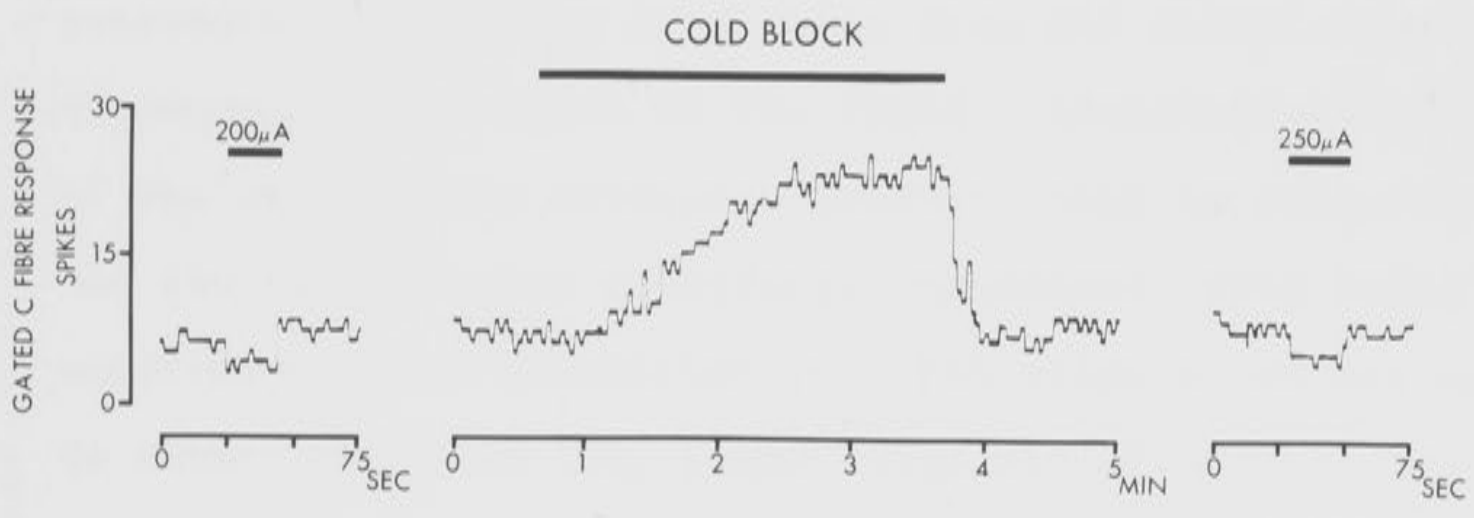
A

CONTROLS



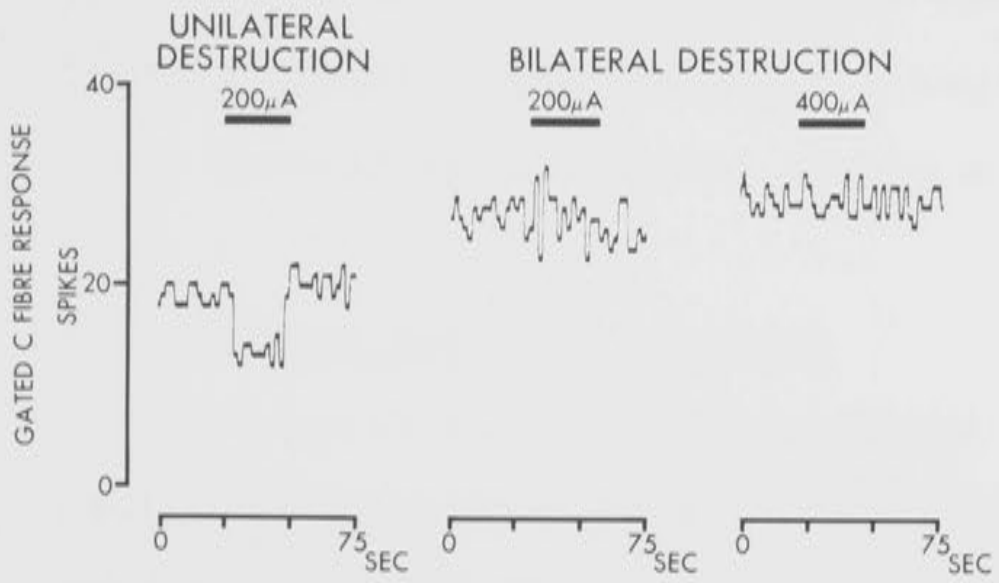
B

AFTER RAPHÉ LESIONS



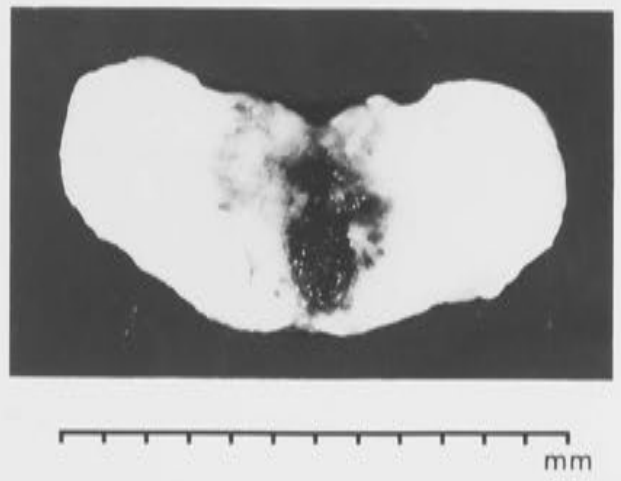
C

AFTER L.R.N. LESIONS



D

AP-10



electrodes into the raphé decreased the uninhibited response to 7.8 ± 0.2 S.E.M., and PAG stimulation then reduced the response to 3.0 ± 0.2 S.E.M. (n = 11), a reduction of 4.8 action potentials (62%) (Figure 38A). Following extensive raphé coagulation (Figure 38D), PAG stimulation was less effective, reducing the C response from 7.0 ± 0.2 S.E.M. to 4.7 ± 0.2 S.E.M. (n = 11), a reduction of 2.3 action potentials, or 33% (Figure 38B).

A possible reason for the limited effectiveness of this procedure in reducing inhibition from PAG stimulation could be inadequate destruction of the raphé. Histological examination of the brain stems revealed, however, that in only one instance was the raphé lesion considered incomplete, this being the experiment where inhibition from PAG stimulation was unchanged. As shown in Figure 38D, haemorrhage was often associated with raphé lesions.

The uninhibited C response was not elevated by raphé destruction in these experiments, confirming the previous finding (section A(a)) that this region does not contribute to tonic descending inhibition in the anaesthetized cat.

(b) Lateral Reticular Lesions

Bilateral lesions of the caudal LRN region reduced or abolished inhibition from PAG stimulation in nine of ten experiments. In five experiments the LRN regions alone were destroyed whereas in the other five the medullary raphé was lesioned prior to the lateral coagulation. The results

differed between the two groups of experiments. When the LRN regions alone were destroyed, the inhibition from PAG stimulation was abolished in one experiment, significantly reduced in three and unchanged in one, whereas coagulation of these lateral reticular regions after raphé destruction abolished the inhibition in all five experiments.

Bilateral destruction of the LRN regions reduced tonic descending inhibition of the neurones studied, confirming the previous results (section A(b)). This increased the number of action potentials evoked by each tibial stimulus. Thus after lesions of lateral reticular areas, inhibition from PAG stimulation was superimposed on an elevated C response and this alone may have altered the apparent effectiveness of PAG stimulation. Some of the results, however, argue against this explanation. In the experiment illustrated in Figure 38, ipsilateral coagulation of the LRN region after raphé destruction increased the basal C response from 8.3 ± 0.2 S.E.M. to 19.0 ± 0.3 S.E.M. (n = 11) action potentials. Despite this elevation, the effectiveness of PAG stimulation (200 μA), 31% inhibition, was similar to pre-lesion controls (33% inhibition). The absolute number of action potentials removed from the C response by PAG stimulation was even increased (5.8 ± 0.5 S.E.M. compared with 2.3 ± 0.4 S.E.M. previously). Although this experiment was atypical in that ipsilateral LRN lesions alone removed some tonic descending inhibition, a further increase in the basal C response, to 26.4 ± 0.4 S.E.M. (n = 11), occurred with positioning of the

electrodes in the contralateral LRN region. In this instance the trauma of this placement alone was sufficient to abolish both tonic descending inhibition and inhibition from PAG stimulation.

Such reduction of tonic inhibition by placing the coagulating electrodes in the LRN region after destruction of the opposite lateral area is occasionally observed. In another experiment, although tonic inhibition was abolished in this way, PAG stimulation still produced inhibition which was abolished by subsequent coagulation of the second LRN region. Since this coagulation did not elevate the C response further, the abolition of inhibition from PAG stimulation cannot be attributed to an altered level of C response.

It needs to be emphasized that lesions in the LRN region abolishing inhibition from PAG stimulation were relatively small compared with the extensive destruction of raphé areas. If lesion is defined as that produced by the passage of current between the 3 pairs of electrodes, then it was common to abolish PAG inhibition by one or two lesions of each lateral area. By contrast 45 such lesions were usually performed in destroying the raphé, with relatively modest effects on inhibition from the PAG.

Figure 39 shows results from one experiment where lateral reticular lesions alone significantly reduced inhibition from PAG stimulation. Stimulation in the ventral PAG at 100 μA reduced the gated C response of the lamina IV neurone by 19.7 ± 1.7 S.E.M. (n = 6) (41% inhibition), and at 150 μA, by 30.6 ± 0.9 S.E.M. (n = 5) (59% inhibition). After bilateral

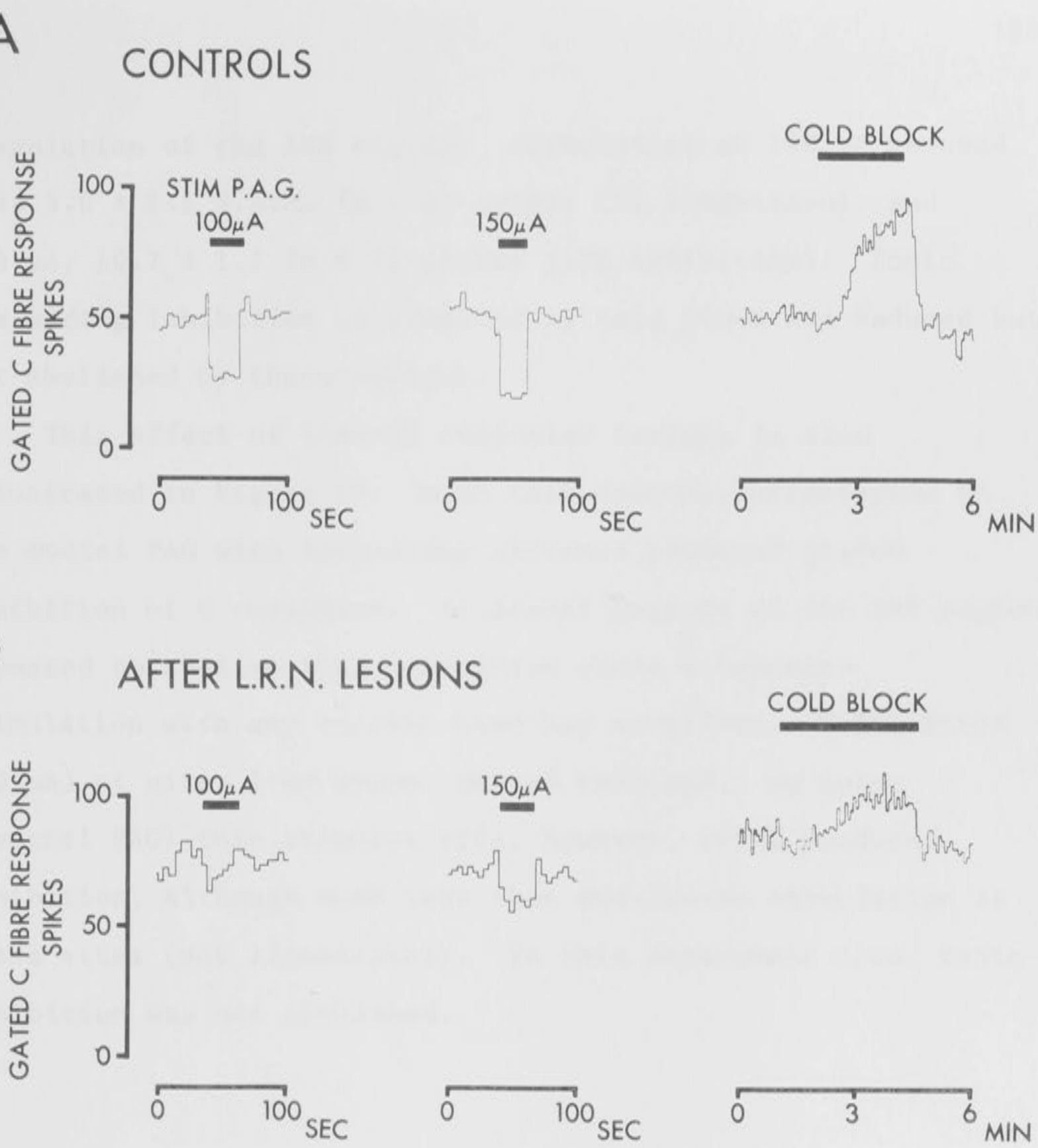


Fig. 39. The effect of bilateral coagulation in the LRN regions of the medulla on the inhibition of a lamina IV neurone by electrical stimulation in the PAG. As in Figure 38, the recordings show the number of action potentials evoked by impulses in C fibre primary afferents following electrical stimulation of the ipsilateral tibial nerve at 0.23 Hz. The black bars mark the periods of stimulation in the ventral PAG (AP +2, V +1) by a tetanic train (thirty-one 0.5 msec pulses at 310 Hz, 0.23 Hz) at the stimulus currents shown, and the periods of cold block of spinal conduction (L_1).

A. Inhibition of C responses by PAG stimulation and enhancement of C responses by removal of tonic descending inhibition during spinal cold block.

B. Inhibition of C responses by PAG stimulation and the remaining tonic inhibition following bilateral coagulation in the LRN regions.

coagulation of the LRN regions, stimulation at 100 μA removed only 5.0 ± 2.5 S.E.M. (n = 6) spikes (7% inhibition), and 150 μA, 10.7 ± 1.7 (n = 7) spikes (15% inhibition). Tonic descending inhibition as revealed by cold block was reduced but not abolished by these lesions.

This effect of lateral reticular lesions is also illustrated in Figure 40. With this neurone, stimulation in the medial PAG with increasing currents produced graded inhibition of C responses. Bilateral lesions of the LRN region appeared to abolish this inhibition since subsequent stimulation with any current used had no effect. Stimulation (50 μA) at sites 1 mm above (dorsal PAG) and 1 mm below (ventral PAG) this stimulus site, however, still produced inhibition, although much less than pre-lesion stimulation at these sites (not illustrated). In this experiment also, tonic inhibition was not abolished.

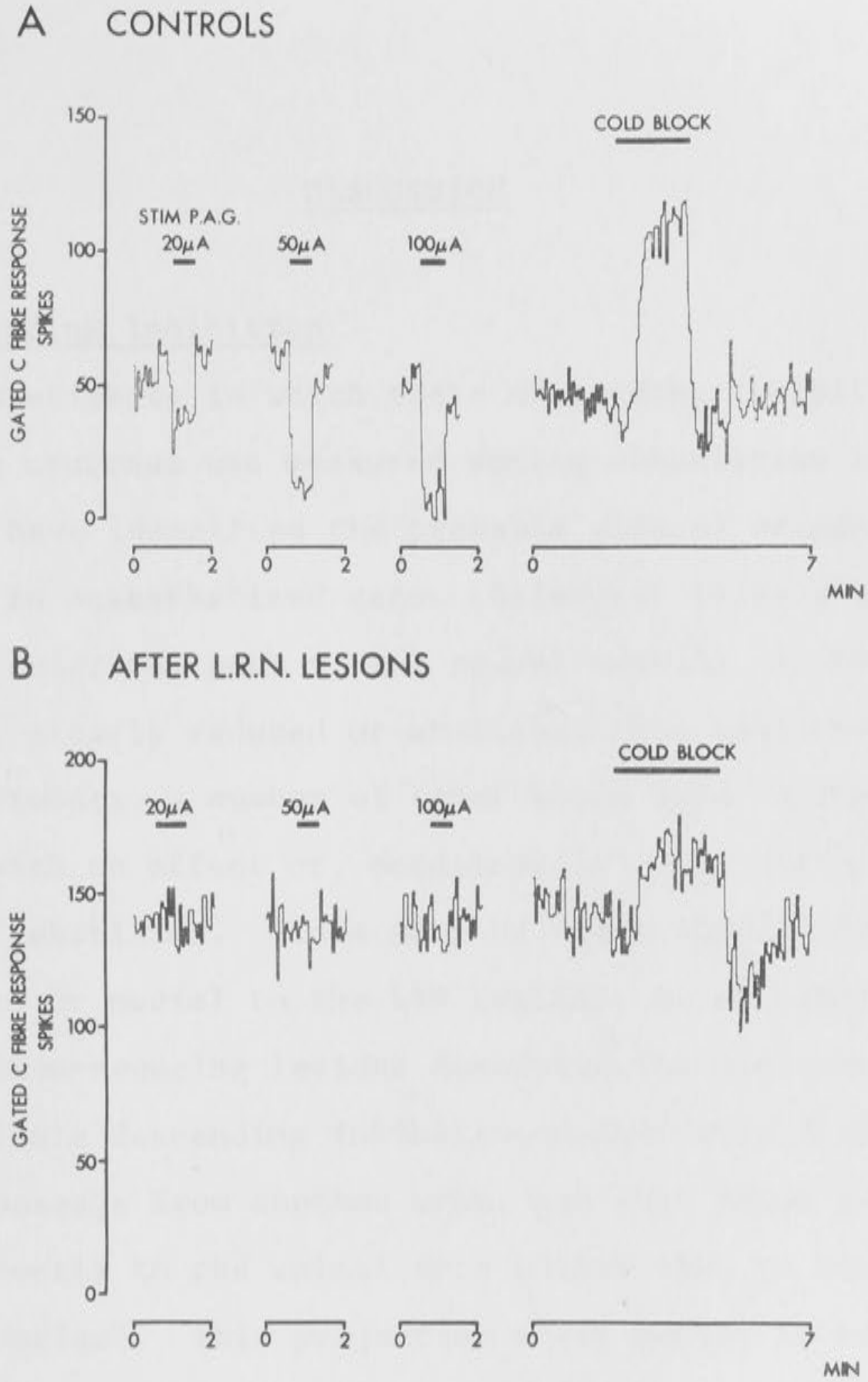


Fig. 40. The effect of bilateral coagulation in the LRN regions of the medulla on the inhibition of a lamina IV neurone by electrical stimulation in the PAG. As in Figures 38 and 39, the recordings show the gated C responses of the dorsal horn neurone to electrical stimulation (0.28 Hz) of the ipsilateral tibial nerve at a strength suprathreshold for C fibres. The black bars indicate the periods of PAG stimulation (AP +1, V +1.5) by a tetanic train (thirty-one 0.5 msec pulses at 310 Hz, 0.28 Hz) at 3 stimulus currents, and the periods of spinal cold block (L_1).

A. Inhibition of C responses by PAG stimulation and enhancement of C responses during cold block of spinal conduction.

B. Lack of effect of PAG stimulation, and the remaining tonic inhibition, following bilateral coagulation in the LRN regions.

DISCUSSION

Tonic Descending Inhibition

The experiments in which tonic descending inhibition of dorsal horn neurones was measured during coagulation in the brain stem have identified the probable site of origin of this inhibition in anaesthetized cats. Bilateral lesions confined to the ventrolateral part of the caudal medulla, in the region of the LRN, clearly reduced or abolished this inhibition. In these experiments, a number of other brain stem regions were destroyed with no effect or, occasionally, even increased descending inhibition. Since many of these ineffective regions were rostral or medial to the LRN regions, it is likely that the inhibition-reducing lesions destroyed the neurones of origin of tonic descending inhibition rather than descending fibres of passage from another area, and that these cells project directly to the spinal cord rather than to another medullary nucleus. This projection would appear to be both crossed and uncrossed since tonic inhibition was not abolished until both ipsilateral and contralateral LRN regions were coagulated.

As discussed earlier, electrical stimulation of several brain stem regions produces inhibition of dorsal horn neurones and behavioural analgesia, but the present results indicate that the integrity of these areas is not required for the inhibition which is tonically present on dorsal horn neurones in the anaesthetized cat. These areas include the PAG, DRN, NRM, NGC, NMC and the locus coeruleus. The tonic descending

inhibition studied in these experiments is relatively selective for the nociceptive responses of dorsal horn neurones and hence is a possible tonic control of nociception in the conscious animal. The ineffectiveness of PAG lesions in the present experiments is therefore consistent with previous behavioural observations that such lesions did not enhance sensitivity to pain, both in cats (Melzack et al., 1958; Kelly and Glusman, 1968) and monkeys (Poirier et al., 1968).

Comparable neurophysiological experiments to determine the supraspinal source of similar inhibition of dorsal horn neurones in the rat have not been described, but in this species PAG lesions did produce hyperalgesia (Rhodes, 1979). In addition, the NRM has been implicated in tonic inhibition of nociception in this species, since hyperalgesia has been produced by NRM inactivation (Proudfit & Anderson, 1975; Proudfit, 1980, 1981) and by intrathecal (Proudfit & Hammond, 1981) and systemic (Berge, 1982) administration of pharmacological antagonists of 5-HT, which is present in many raphé-spinal fibres. By contrast in the cat, tonic descending inhibition was not reduced by administration of methysergide (Griersmith et al., 1981), pCPA or fluoxetine (Soja & Sinclair, 1980). Although these observations in the cat do not exclude the possibility that another substance released by descending raphé-spinal fibres may mediate tonic inhibition, the results of the present experiments provide clear evidence against the involvement of <u>any</u> neurotransmitter from the medullary raphé in this inhibition in the anaesthetized cat. Collectively the data indicate that there may be species differences in the

supraspinal source of tonic descending inhibition of the nociceptive excitation of dorsal horn neurones.

There have been no previous accounts of supraspinal regions which tonically inhibit the excitation of dorsal horn neurones by impulses in unmyelinated primary afferents. In the context of tonic supraspinal inhibition of spinal neurone activity in the cat, the studies of Lundberg and colleagues would appear to be most relevant. This group found that tonic descending inhibition of the transmission of impulses in flexion reflex pathways was reduced but not abolished by a series of raphé lesions extending 10 mm rostrally from the obex (Engberg et al., 1968a). The classification of "flexion reflex afferents" by the Lundberg group, however, includes all cutaneous myelinated afferents and small myelinated muscle and joint afferents, but not visceral afferents nor unmyelinated fibres (Holmqvist & Lundberg, 1961). Thus these studies are not comparable since the tonic inhibition studied in the present experiments has relatively little effect on excitation by impulses in large myelinated afferents.

It is difficult to be certain which neurones were destroyed in these experiments. In the later experiments where tonic inhibition was reduced, the lesions observed histologically were mostly confined to the caudal LRN region. Clearly defined cell groups in this part of the medulla are the LRN itself and the adjacent nucleus ambiguus. Many neurones of the LRN project to the cerebellum as mossy fibres (Brodal, 1943; Clendenin, Ekerot, Oscarsson & Rosen, 1974a) and relay spinocerebellar afferents (Bruckmoser, Hepp-Reymond & Wiesendanger,

1970; Clendenin, Ekerot, Oscarsson & Rosen, 1974b), while the nucleus ambiguus contains laryngeal motoneurones. It seems unlikely that destruction of these neurones was responsible for the results of the present experiments. However, a spinal projection from neurones of this ventrolateral region has been demonstrated following injection of retrograde tracers into rostral and caudal levels of the spinal cord of cats (Kuypers & Maisky, 1975; Basbaum & Fields, 1979), rats (Hokfelt et al., 1979; Basbaum & Fields, 1979; Ross, Ruggiero & Reis, 1981) and rabbits (Blessing, Goodchild, Dampney & Chalmers, 1981). In cats, injection of HRP into the lumbar dorsal cord produced some, but not dense, retrograde labelling in the ventrolateral reticular area (Kuypers & Maisky, 1975; Basbaum & Fields, 1979).

As discussed previously, pharmacological experiments have not provided any clear indication as to the identity of the neurotransmitter(s) involved in tonic descending inhibition of dorsal horn neurones. The localization of the cells of origin of a neuronal pathway, however, can provide information in this direction. Histochemical studies have repeatedly drawn attention to the ventrolateral medulla in a number of species, in association with catecholamine-containing neurones. This region contains a group of catecholamine-fluorescent cells, extending antero-posteriorly from the level of the pyramidal decussation to the rostral inferior olive, originally described in the rat as the A1 group by Dahlstrom and Fuxe (1964). The presence of an analogous cell group in other species was

subsequently reported (Nobin & Bjorklund, 1973; Felton, Laties & Carpenter, 1974; Garver & Sladek, 1975; Crutcher & Humbertson, 1978; Blessing, Chalmers & Howe, 1978; Blessing, Frost & Furness, 1980). In these studies, the catecholamine-containing neurones were observed adjacent to, and sometimes within (e.g. Nobin & Bjorklund, 1973; Garver & Sladek, 1975), the LRN. In the cat, the fluorescent neurones were found in close proximity to, rather than within, this nucleus (Blessing et al., 1980; Wiklund, Léger & Persson, 1981). These ventro-lateral catecholaminergic neurones in the rat showed enhanced fluorescence following cervical cord transection (Dahlstrom & Fuxe, 1965), suggesting a spinal projection of their axons. In the rabbit, however, Blessing et al. (1981) found few of these medullary catecholamine-fluorescent cells retrogradely labelled following cord injections of HRP. A similar result has been obtained in the rat. Following spinal injections and retrograde transport of either HRP or antibody to dopamine-β-hydroxylase, the localization of immunoreactive cells in the brain stem has revealed that descending noradrenergic projections arise from the pons but not the medulla (Westlund, Bowker, Ziegler & Coulter, 1981, 1982). Thus although the coagulated regions in the present experiments in cats were not confined to the LRN, thereby not excluding the possibility that catecholaminergic cells of this region contribute to tonic descending inhibition, the anatomical studies in other species, through not finding spinal projections by the A1 cell group, do not support this hypothesis. It is of course possible that an as yet undescribed substance released by descending fibres from

lateral reticular neurones produces tonic inhibition.

The ventrolateral medulla has previously been subjected to considerable investigation in the context of cardiovascular regulation. The pressor effect elicited in cats by electrical stimulation in this region, in close proximity to the LRN, is well documented (Wang & Ranson, 1939; Alexander, 1946; Neumayr, Hare & Franz, 1974). In an investigation specifically directed at the LRN, Thomas, Ulrichsen and Calaresu (1977) found that electrical stimulation at many sites within this nucleus produced pressor and cardioacceleratory responses. The topical application of many drugs to the ventral surface of this brain stem region has caused cardiovascular changes, with inhibitory substances producing hypotension (Guertzenstein, 1973; reviewed by Feldberg, 1976). The LRN was found particularly sensitive to clonidine, with microinjection of low doses (75 ng/kg) producing hypotension and bradycardia (Bousquet, Feldman, Bloch & Schwartz, 1981). In a study by Guertzenstein and Silver (1974), the bilateral topical application of glycine to a circumscribed area of the medullary ventral surface in cats produced pronounced hypotension. Bilateral electrolytic lesions of the glycine-sensitive regions had a similar effect, and histological examination revealed destruction of part of the parvicellular portion of the LRN, at approximately AP -10 (co-ordinates of Berman, 1968). With both procedures, the hypotensive effect was seen only after bilateral interference. Such observations have implicated this region in the tonic maintenance of blood pressure, and are consistent with the results of the present experiments in which bilateral

coagulation reducing tonic descending inhibition was strongly associated with persistent hypotension. This effect may be species-specific, however, since in the rabbit bilateral lesions of the caudal LRN had little effect on blood pressure, the effective ventrolateral regions being more rostral (Dampney & Moon, 1980).

Since the selectivity of tonic descending inhibition for nociceptive excitation of dorsal horn neurones favours relating it to control of nociception, it is possible that control of circulation and of nociception are related through control mechanisms located in the lateral reticular regions of the medulla. An association between cardiovascular control and control of pain has been inferred from several lines of indirect evidence, including an anatomical similarity in the supraspinal regions implicated in these systems, and the effects of drugs and of electrical stimulation of supraspinal structures on both these systems (see Zamir, Simantov & Segal, 1980). For example, electrical stimulation in the locus coeruleus elicits a pressor response (Ward & Gunn, 1976) but also analgesia (Segal & Sandberg, 1977). In this context, it is interesting that several investigators have drawn attention to the co-occurrence of hypoalgesia and hypertension. Rats with spontaneous or experimentally-induced (renal artery stenosis) hypertension were relatively hypoalgesic compared with normotensive controls, and the reduced pain sensitivity was naloxone-reversible (Zamir & Segal, 1979; Wendel & Bennett, 1981; Saavedra, 1981). This hypertension was accompanied by

increased levels of opioid peptide activity in the spinal cord (Zamir et al., 1980) and by a reduction in [^{3}H]-naloxone binding in the dorsal horn (Zamir, Segal & Simantov, 1981). Essential hypertension in humans was also shown to be associated with diminished pain perception (Zamir & Shuber, 1980). Such findings suggest that nociception and cardiovascular control may have several linkages, underlining the need to study both systems simultaneously.

Stimulation-Produced Inhibition. I. Lateral Spinal Funiculi

The experiments in which the lateral spinal columns were electrically stimulated while recording intracellularly from lumbar dorsal horn neurones have provided some information relevant to possible mechanisms of the supraspinal control of these neurones. This study sought to examine the effects of activation of descending fibres which mediate descending inhibition of dorsal horn neurones. Selective destruction procedures have localized the appropriate descending fibres for tonic descending inhibition (Willis et al., 1977) and stimulation-produced inhibition (Fields et al., 1977) to the DLF, but it is very probable that the stimulation procedure used in the present experiments activated other descending fibres in addition to those relevant to these inhibitions. With the majority of neurones studied, such stimulation produced a somatic hyperpolarization, prefaced in some cases with a depolarization. The synaptic excitation of the neurones by tibial nerve stimulation was reduced when just preceded by

this hyperpolarization. These results are in general agreement with previously observed effects of DLF stimulation in cats. When recordings were obtained from spinocervical tract axons, spontaneous activity and that evoked by noxious cutaneous stimuli or electrical stimulation of peripheral nerve were reduced by electrical stimulation of descending fibres in the ipsilateral or contralateral DLF, dorsal columns or ventral funiculi (Brown, Kirk & Martin, 1973; Brown, Hamann & Martin, 1974). Similarly, the spontaneous and evoked firing of substantia gelatinosa neurones was inhibited by DLF stimulation (Cervero et al., 1979). On the other hand, Dubuisson and Wall (1980) reported excitation to be the predominant effect of DLF stimulation on neurones of laminae I to III. These results obtained with extracellular techniques, however, are not necessarily irreconcilable since with a significant number of the neurones studied in the present experiments, lateral column stimulation produced a depolarization-hyperpolarization sequence, often associated with firing of the neurone.

It is not possible to be absolutely certain that the observed effects were due to activation of descending fibres. Brown et al (1973) have pointed out that antidromic activation of ascending fibres may affect spinal transmission at more caudal levels since collateral branches of the ascending axons may be connected to the neuronal system being observed. Such a mechanism, however, has been described only for dorsal column axons.

The results obtained with potassium chloride-containing electrodes suggest that the inhibition of laminae IV, V and VI

neurones produced by lateral column stimulation is similar to postsynaptic inhibition of neurones in several areas of the central nervous system, namely it is associated with an increase in chloride conductance of postsynaptic membranes (Coombs et al., 1955; Ito & Yoshida, 1966; Kelly, Krnjevic, Morris & Yim, 1969).

It is probable that descending fibres from several supraspinal sites were excited by the stimuli used and hence it is not possible to relate the present results to a particular supraspinal region. Tonic descending inhibition is relatively selective for the excitation of dorsal horn neurones by noxious cutaneous stimuli, and such selectivity is unlikely to be produced by a somatic hyperpolarization. Thus activation of the descending fibres which normally convey this tonic inhibition is unlikely to be fully responsible for the effects observed in the present experiments. On the other hand, there are several reports (reviewed previously) of non-selective inhibition from supraspinal stimulation, an effect consistent with somatic hyperpolarization. In particular, the study by Giesler et al. (1981) found that electrical stimulation in the NRM produced IPSPs in monkey spinothalamic tract neurones.

The most important finding from this study is that an apparent selectivity in inhibition of responses can arise from a somatic hyperpolarization when neurones are excited by electrical stimulation of peripheral nerve. The greater reduction in excitation by impulses in C fibres when compared with A fibres almost certainly resulted from the differences in the amplitude and duration of EPSPs evoked by impulses in the

two types of fibre. Such a difference may not occur with natural stimuli and therefore it may be incorrect to infer that an inhibition is selective for a sensory modality from results obtained with electrical stimulation of peripheral nerve. This assumes that the hyperpolarizations observed did produce the inhibitions measured and that there was no concurrent inhibition of interneurones interposed between primary afferents and the cells studied nor presynaptic inhibition of primary afferents. A similar selectivity was observed by Brown et al. (1973), who noted that activation of descending spinal fibres produced similar inhibition of responses in spinocervical tract axons to electrical stimulation of small myelinated afferents and low intensity stimulation of large myelinated afferents, but did not affect responses produced by stimulation of the latter at many times threshold. The results of the present experiments may provide an explanation for the puzzling disparity observed with the selectivity of the inhibition of spinothalamic tract neurones produced by supraspinal stimulation in monkeys. Electrical stimulation in the NRM and lateral reticular formation of the medulla produced comparable inhibition of responses to impulses in C and Aδ cutaneous afferents and to both noxious and non-noxious cutaneous stimuli, but excitation by stimulation of large myelinated Aαβ fibres was less affected (Willis et al., 1977; Gerhart et al., 1981a). The present results suggest that the medullary stimulation in these experiments produced a somatic hyperpolarization, an effect subsequently observed by this group (Giesler et al., 1981). Such findings suggest the need for caution when

choosing the method of activation of neurones in studies of selective inhibition.

Although the bipolar stimulation technique used in these experiments may have activated descending systems producing selective inhibition in the dorsal horn, such effects would have been masked by concurrent activation of non-selective systems. In view of the later findings that non-selective inhibition could be obtained with bipolar stimulation in many regions of the brain stem such as the midbrain VT (Section C(b)) and the medulla (Section D(c)), it is not surprising that this effect is most likely to be observed when stimulating descending fibres in spinal funiculi.

II. Midbrain

The study of inhibition of dorsal horn neurones by electrical stimulation in the midbrain has provided new information about the nature of such inhibition elicited from midbrain sites. These results confirm previous findings that electrical stimulation in the PAG inhibits the nociceptive responses of dorsal horn neurones of the cat (Oliveras et al., 1974a; Carstens et al., 1979c, 1980a,b). Problems arise, however, when attempting to relate such neurophysiological observations to the phenomenon of stimulation-produced analgesia. In the study of midbrain stimulation in cats by Oliveras et al. (1974a), inhibition of nociceptive excitation of dorsal horn neurones was a far more ubiquitous observation than analgesia, for which the effective sites were mainly localized to the ventral PAG, in the vicinity of the DRN. The initial experiments of the

present series confirmed this puzzling observation of a widespread distribution of inhibition-producing sites. When excitation of the same cells by non-noxious cutaneous stimuli was examined, however, selective inhibition of responses to impulses in C fibre afferents was produced by stimulation in the PAG, whereas with more ventral sites in the VT, both nociceptive and non-nociceptive responses were reduced. This observation is important in relation to the hypothesis that selective inhibition of nociceptive responses is more readily related to behavioural analgesia in the conscious animal, for which PAG stimulation is well known. Thus the inhibition of nociceptive responses alone by supraspinal stimulation may not be an adequate basis for predicting the effectiveness of a given supraspinal region for stimulation-produced analgesia.

Unlike selective inhibition, it is not possible to speculate on the physiological significance of the non-selective inhibition produced by electrical stimulation at sites ventral to the PAG. There are previous reports of such stimulation producing non-selective inhibition of both nociceptive and non-nociceptive responses of spinocervical tract (Taub, 1964) and dorsal horn (Duggan & Griersmith, 1979a) neurones of the cat. It seems unlikely, however, that such inhibition is concerned with the spinal transmission of nociceptive information alone.

Several groups have previously described the effects of PAG stimulation on nociceptive and non-nociceptive excitation of multireceptive neurones. Electrical stimulation in the PAG selectively reduced nociceptive activity in ascending axons in

the rat (Jurna, 1980) and excitation of multireceptive spinothalamic neurones by noxious cutaneous stimuli in the monkey (Hayes et al., 1979). In the cat, stimulation in the PAG near the DRN selectively inhibited the responses of nine of seventeen multireceptive lamina V neurones to noxious cutaneous stimuli without affecting non-noxious responses (Oliveras et al., 1974a). In the studies of Carstens et al. (1980b, 1981a), however, the predominant effect of PAG stimulation was non-selective inhibition, although a partial selectivity for nociceptive excitation was observed with higher stimulus currents. In the present experiments, a partial selectivity for C responses was observed with 20% of the neurones studied during PAG stimulation. On the other hand, PAG stimulation in the cat has produced non-selective inhibition of neurones in nucleus reticularis dorsalis but selective inhibition of nociceptive responses of subnucleus caudalis neurones (Yokota & Hashimoto, 1976). Since stimulation at sites immediately ventral to the PAG has produced non-selective inhibition (Taub, 1964; Duggan & Griersmith, 1979a; the present experiments), some of these differing effects may be due to stimulus spread to ventral regions. It should be pointed out, however, that experiments may not be strictly comparable unless other factors such as exact electrode location, stimulation technique and parameters, and method of anaesthesia are taken into account.

Differences between the inhibitory effects of stimulation in the PAG compared with other midbrain sites have been described previously. Examinations of the temperature-response curves of dorsal horn neurones activated by noxious cutaneous

heat revealed that stimulation in the lateral reticular formation produced parallel rightward shifts of the curves, whereas PAG stimulation reduced their slope without changing the response threshold (Carstens et al., 1980a). This suggestion of different mechanisms was supported by the observations that pCPA pretreatment or systemic administration of methysergide differentially affected the inhibitions evoked by stimulation of these two regions (Carstens et al., 1981b). The present experiments also provide evidence that the underlying mechanisms of inhibition in the dorsal horn activated by PAG stimulation appear to differ from those activated by stimulation of other midbrain sites.

With electrical stimulation in any region of the brain, fibres of passage are almost certainly excited by the stimuli used and the contribution of this to the present results is not known. It was clear that stimulation in more rostral regions such as the hypothalamus could also selectively inhibit C responses of dorsal horn neurones, a finding consistent with that of Carstens (1982) using noxious heating of the skin. There is evidence (reviewed previously) that the hypothalamus projects directly to the spinal cord as well as to the mesencephalic PAG, which in turn has both a direct spinal projection and one to the medullary raphé nuclei. Concerning the VT, the red nucleus is located in this region and besides the well-documented rubrospinal tract (reviewed by Massion, 1967), an excitatory projection from the red nucleus to NRM neurones with spinal projections has been described in the cat

(West, Lovick & Wolstencroft, 1982). In the experiments of Behbehani and Fields (1979), microinjection of glutamate into the PAG of rats produced analgesia, presumably through selectively exciting the cell bodies of PAG neurones rather than descending axons. In the present experiments it is likely that both cell bodies and descending fibres of passage were activated by the stimulation technique, but a clear difference between the PAG and the VT was still observed.

There have been several previous reports of changes in peripheral circulation induced by electrical stimulation in the brain stem, including the PAG. In cats, stimulation at PAG sites produced pressor effects (Sachs, 1911; Kabat, Magoun & Ranson, 1935; Abrahams, Hilton & Zbrozyna, 1960) and also vasodilatation in skeletal muscle with vasoconstriction in the skin (Abrahams et al., 1960). (Such effects have also been observed with stimulation of more rostral regions (Eliasson, Folkow, Lindgren & Uvnas, 1951; Abrahams et al. 1960)). In rabbits, PAG stimulation produced vasoconstriction in the ear (Stitt, 1976). These reports are consistent with the results of the present experiments in which PAG stimulation produced muscle vasodilatation and cutaneous vasoconstriction. These investigations cited were primarily concerned with cardiovascular control rather than nociception. The circulatory concomitants of selective inhibition observed with PAG stimulation in the present experiments, however, may be another example of the association between the control of pain and cardiovascular systems. Some aspects of this association were discussed earlier, such as the role of the medullary LRN

regions in the maintenance of both tonic descending inhibition and blood pressure, and the diminished sensitivity to pain occurring with some forms of hypertension in rats and humans.

Selective inhibition of nociceptive responses of dorsal horn neurones accompanied by circulatory changes may be a complex and co-ordinated response of an animal to a particular stimulus. Several of the earlier reports of changes in peripheral circulation have emphasized that PAG stimulation is associated with a number of autonomic effects such as pupillary dilatation, piloerection, retraction of the nictitating membranes and respiratory effects (reviewed by Skultety, 1958). The nature of the response suggests that the stimulus may be a threatening or potentially injurious environment, since selective inhibition of nociceptive transmission would minimize disruption of motor performance by reflexes to impulses in nociceptive afferents due to injury to the animal. The increased perfusion of skeletal muscle at the expense of cutaneous blood flow would aid motor performance in an ensuing "fight-or-flight" reaction. This raises the possibility that the behavioural analgesia observed with PAG stimulation may be an integral part of this complex response, being invoked by the animal in anticipation of imminent pain rather than as a consequence of it.

There are many reports of aversive reactions resulting from electrical stimulation in the mesencephalon and diencephalon of conscious animals, including sites in the PAG. The various manifestations of this behavioural response (or "defence reaction") in cats include hissing, piloerection,

pupillary dilatation, attempts to escape, fear, rage and attack (Hunsperger, 1956; Bandler & Flynn, 1974; reviewed by Clemente & Chase, 1973). Such effects have prevented testing at some midbrain sites for behavioural analgesia (Liebeskind et al., 1973; Oliveras et al., 1974a). Stimulation-produced analgesia in the absence of aversive reactions was often observed, however, suggesting that it was not dependent upon a generalized arousal of the animal. The close association between these two phenomena, particularly in terms of the areas producing them, suggest a relationship between the defence reaction and a precautionary inhibition of the perception of pain.

Thus, although the peripheral circulatory effects detected in the present experiments were relatively small, the close correlation between these effects and selective inhibition of dorsal horn neurones suggests that analgesia from activation of the PAG system does not occur in isolation. Instead, it may be one component of a complex response to a particular environment, and intense activation of this system may culminate in the aversive "defence" reaction.

III. Medulla

The localization of the brain stem source of tonic descending inhibition of dorsal horn neurones has drawn attention to the role of lateral reticular regions of the medulla in the supraspinal control of these neurones. The study of electrical stimulation of these and adjacent regions has confirmed the

probable importance of the LRN regions in controlling the responses of dorsal horn neurones to impulses in unmyelinated primary afferent fibres. The tonic descending inhibition which emanates bilaterally from these regions is predominantly selective for the excitation of dorsal horn neurones by noxious cutaneous stimuli. Since similar selectivity was observed when the region of the LRN was electrically stimulated, and destruction of the areas stimulated almost abolished tonic inhibition, it is probable that the same neurones were responsible for both the tonic and stimulation-produced inhibition observed in these experiments. It appears that these neurones are not maximally active in the anaesthetized cat since the effect of tonic inhibition in the dorsal horn could be increased by direct electrical stimulation of these cells.

The preceding experiments on stimulation in the midbrain have argued in favour of studying excitation of multireceptive dorsal horn neurones by both nociceptive and non-nociceptive inputs when attempting to relate the neurophysiological effects of stimulation in a supraspinal region to behavioural analgesia. In those experiments, the sites eliciting selective inhibition (PAG) correlated well with those known to be effective for stimulation-produced analgesia. The finding that stimulation in the region of the LRN was also selective in its effects is further evidence in favour of relating this area to the control of pain perception.

The LRN region of the medulla has not been mentioned in accounts of brain stem regions which, when electrically stimulated, produce analgesia or inhibition of dorsal horn neurones.

The results of the present experiments suggest that this omission is not due to lack of effect but rather from lack of examination. As discussed previously, the medullary raphé has figured prominently in many studies of stimulation-produced inhibition of dorsal horn neurones but the results of the lesion experiments (Section A(a)) argue against a contribution by the raphé to the tonic descending inhibition observed in these experiments on anaesthetized cats. When mapping the extent of effective sites in the brain stem in the present experiments, inhibition was more readily produced from stimulation in the ventral lateral reticular regions than from midline structures. Indeed, when using monopolar stimulation with small stimulus currents, the sites eliciting inhibition were essentially restricted to these two regions.

The question of activation of fibres of passage was addressed earlier and should also be considered in relation to these experiments. In the lesion experiments (Section A), the failure of extensive coagulation rostral, medial and dorsal to the caudal LRN region to reduce tonic descending inhibition suggested its derivation from neurones of that area. In the present experiments moreover, electrical stimulation adjacent to this lateral reticular region both medially and dorsally and, to a limited extent, rostrally, also had little effect, again suggesting that the inhibition comes from neurones of this region. Such findings, however, do not fully exclude a possible contribution to the observed inhibition from activation of fibres of passage.

The influence of stimulation technique in these experiments also requires comment. When monopolar stimulation was used, the usual finding was a selective reduction of C responses from stimulation in a limited ventrolateral area near the LRN, but not in other areas. On the other hand, bipolar stimulation between a pair of electrodes (1.5 to 3.0 mm tip separation) often produced non-selective inhibition from widely dispersed sites. Differences between monopolar and bipolar stimulation in the brain in behavioural experiments have been reported (Valenstein & Beer, 1961; Stark et al., 1962), and analyses of current density around these electrode configurations have suggested that bipolar stimulation may be effective over a large egg-shaped volume of tissue (Stark et al., 1962). With low stimulus currents close to the appropriate structure, however, the exact location of the electrode tips in relation to the structure determines which technique has the more effective current spread (Stark et al., 1962). The present results suggest that bipolar stimulation, although apparently appropriate for stimulation of relatively large areas such as the PAG, is unsuitable for discrete regions of the medulla.

In these experiments, electrical stimulation in the LRN region produced changes, usually increases, in blood pressure. This confirms the findings of other authors (Neumayr et al., 1974; Thomas et al., 1977), but further investigation of these effects was not undertaken in this study.

Pathways for Inhibition from PAG Stimulation

The results of the experiments in which midline and lateral regions of the medulla were coagulated while observing inhibition of dorsal horn neurones produced by electrical stimulation in the midbrain PAG are significant in relation to current thinking on the role of medullary regions in PAG-induced inhibition. There is considerable evidence (reviewed previously) for an anatomical and functional projection from the midbrain PAG to the medullary NRM. This has led to the proposal that the analgesia and inhibition of spinal neurone activity produced by PAG stimulation is mediated by synaptic excitation of NRM neurones which project to the dorsal horn of the spinal cord. The present results, however, suggest that the medullary raphé is relatively unimportant compared with lateral reticular regions for the inhibition of dorsal horn neurones by PAG stimulation. This inhibition was reduced by about one-quarter by extensive coagulation in the medullary raphé and histologically, the lesions were considered to be functionally complete. In contrast, limited bilateral destruction in the LRN regions produced a much greater reduction of this inhibition. When this procedure followed raphé lesions, the remaining inhibition was abolished. These results suggest that separate pathways from both raphé and ventrolateral medullary areas can relay the inhibitory effects of PAG stimulation to the spinal cord.

There is evidence for a substantial projection from the PAG to the ventrolateral medulla. An autoradiographic study

in the squirrel monkey found that PAG efferents projected caudally and bilaterally to the region of the nucleus ambiguus (Jurgens & Pratt, 1979), while in the cat, HRP injection into the caudal medulla near this nucleus retrogradely labelled many PAG neurones (Rose, 1981). Electrical stimulation in the PAG produced short-latency responses in neurones of the ventrolateral medulla, including the LRN (Rose & Sutin, 1973). Neurones in the LRN region in the cat were retrogradely labelled following HRP injection into the lumbar dorsal cord (Kuypers & Maisky, 1975; Basbaum & Fields, 1979), indicating a spinal projection.

In the present experiments, bilateral destruction in the LRN regions reduced tonic descending inhibition of dorsal horn neurones as well as that produced by PAG stimulation. This raises the possibility that the effects of PAG stimulation are mediated by synaptic excitation of the same lateral reticular neurones which exert tonic inhibition on dorsal horn neurones. The preceding experiments have demonstrated that the effect of tonic inhibition could be enhanced by direct electrical stimulation in the LRN regions, and these inhibitions resemble that produced by PAG stimulation in being selective. It is equally plausible, however, that the lateral lesions merely interrupted the spinally-projecting axons of PAG neurones concurrently with destruction of the tonically active cells. The possibility remains that NRM neurones, synaptically driven from the PAG, inhibit spinal neurones by relaying through the LRN region also.

The findings of the present experiments are consistent with a recent study which used microinjections of local anaesthetic to inactivate medullary regions. Inhibition of noxious cutaneous heat-evoked responses of dorsal horn neurones by electrical stimulation in the PAG or the mesencephalic lateral reticular formation was not affected by NRM injections, but was reduced by medullary reticular injections (2.5 mm lateral) and abolished by injection of both NRM and the medullary reticular formation (Sandkuhler, Thalhammer, Gebhart & Zimmermann, 1982). Although the reticular areas in these experiments were rostral and medial to those coagulated in the present work (Gebhart, personal communication), both studies serve to emphasize the relative lack of importance of the medullary raphé compared with lateral reticular regions for PAG-induced inhibition of dorsal horn neurones.

The importance of the medullary raphé for PAG-induced inhibition of nociception may be species-dependent since in rats, NRM lesions abolished the increased threshold for a flexion reflex to noxious cutaneous heat produced by microinjection of glutamate into the PAG (Behbehani & Fields, 1979). The effect of NRM lesions may also depend upon the stimulus site within the PAG. In rats, NRM destruction elevated the threshold for stimulation-produced analgesia from ventral but not dorsal PAG sites (Prieto et al., 1983). Previous studies had reported a differential susceptibility of analgesia from dorsal and ventral PAG sites to pharmacological procedures such as administration of pCPA (Akil & Mayer, 1972) or naloxone (Cannon, Prieto, Lee & Liebeskind, 1982). In the

present experiments in cats, however, stimulus sites were located in dorsal, ventral or intervening PAG regions, with no apparent correlation between site and effect of raphé lesions.

Several of the studies described in this thesis have emphasized the importance of lateral reticular regions of the medulla in the control of the nociceptive excitation of dorsal horn neurones. There are also reports which give cause for speculation that this lateral medullary system may be activated by peripheral stimulation. Du and Chao (1976) have reported that the inhibitory effect of acupuncture on a viscero-somatic reflex in cats was greatly reduced by medial lesions in the rostral and middle medulla. This inhibition was also reduced or nearly abolished by bilateral transection of the lateral lower medulla but only when the sections included the LRN. This region also appears in studies of analgesia produced in animals by physical stimulation of the vagina. There is a considerable literature on this phenomenon, especially in rats (reviewed by Komisaruk, 1982), and it may be relevant that vaginal probing in cats facilitates the firing of many neurones located in the LRN and near the nucleus ambiguus (Rose & Sutin, 1973). Since many reticular neurones have a sensory input, however, this hypothetical association will require substantial additional evidence.

CHAPTER V - CONCLUDING REMARKS

This thesis has presented data on several types of inhibition in the spinal cord. It is the purpose of these concluding remarks to propose inter-relations between these inhibitions as parts of an integrated behavioural response to actual or potential injury. It is believed that physiological events related to pain are better described in this context rather than pain be considered in isolation.

The response of an animal to injury may be divisible into distinct stages (Wall, 1979), each associated with a type of spinal inhibition. The early or anticipatory phase may be invoked even prior to injury, in response to a potentially injurious environment, and be marked by selective inhibition in the dorsal horn resulting in reduced perception of, and reflex responses to, noxious stimuli. This inhibition could originate from the PAG, probably does not depend upon opioid peptides, and is accompanied by circulatory changes appropriate for the defence reaction. Although this descending inhibitory system has been considered a link in a negative feedback loop activated by noxious input (Basbaum & Fields, 1978), it is equally plausible that anticipation of injury is the activating stimulus, rendering an animal hypoalgesic and prepared for "fight-or-flight".

The delayed response to injury may involve inhibition of motoneurones resulting in reduced movement of the animal. This inhibition appears to result in part from release of opioid peptides in the spinal cord by continuous nociceptive input.

This phase is thus marked by pain and a relative immobility appropriate for healing of injured areas.

Therefore, although it is likely that the opioid peptides function as inhibitory neurotransmitters in the spinal cord, some of the current concepts of their physiological roles may need revision. These roles may only be fully revealed by considering the overall response to injury rather than by separately studying individual components such as pain.

The presence of tonic descending inhibition of the nociceptive excitation of dorsal horn neurones observed in anaesthetized cats may indicate that normally, the spinal transmission of nociceptive information to higher centres is inhibited. It is conceivable that inhibition from phasic descending control systems such as the PAG is produced partly by potentiation of tonic descending inhibition. The pharmacological manipulation of this tonically active system may prove a useful future means of pain control.

There are obvious difficulties in this type of extrapolation from neurophysiological and neuropharmacological studies of single neurones to whole animal behaviour. Such speculation, however, emphasizes that in the study of pain, a systemic perspective of the participation of pain in integrated behavioural responses may be the most rewarding approach.

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