

SUPRASPINAL INVOLVEMENT IN ACUPUNCTURE ANALGESIA

by

K.C.YEE

M.D., Zuzhen Medical School, 1954
M.S., The University of Washington, 1983
Ph.D., Columbia University, 1986

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
DIVISION OF NEUROLOGICAL SCIENCES,
DEPARTMENT OF PSYCHIATRY

We accept this thesis as conforming
to the required standard

THE ~~UNIVERSITY~~ OF BRITISH COLUMBIA

May 1992

© K.C.Yee, 1992

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Neurosciences

The University of British Columbia
Vancouver, Canada

Date 7/21, 1992

ABSTRACT

Electroacupuncture (EA) on Zusanli (st. 36) and Shangjuxu (st. 38) (10 Hz, 1.0 ms) was found to produce a long-lasting inhibition of wide dynamic range neurones in the dorsal horn of the spinal cord and to prolong the latency of the tail-flick reflex in the lightly anaesthetized rat. This inhibition was effectively produced at a stimulation intensity which excited only AB fibres. The effects of EA were eliminated by cold-blocking the spinal cord rostral to the recording site suggesting a supraspinal involvement in the EA-induced inhibition of spinal cord nociceptive transmission. EA also facilitated the discharge of non-clock-like dorsal raphe neurones (NCL). Bilateral lesions of the ventrolateral tract (VLT), but not the dorsolateral funiculi (DLF), blocked this effect suggesting that the ascending arm of the loop is via the VLT. The descending arm is located in the DLF since bilateral lesions of the DLF blocked the effects of EA in the spinal cord.

Evidence in the literature suggests that the dorsal raphe nucleus (DRN) may be involved in the above supraspinal loop as well as in an ascending inhibitory pathway to the nucleus parafascicularis (NPF). Examination of the DRN revealed three types of neurones: clock-like (CL), NCL and non-clock-like non-responding neurones (NCLN). The NCL neurones were excited by noxious and non-noxious natural peripheral stimuli as well as EA. The other neurones were non-responsive to these stimuli. NCL neurones of the DRN were also antidromically activated by

Abstract

NPF stimulation indicating that the projection from the DRN to the NPF is direct. Stimulation of the DRN produced an inhibition of NPF neurones with sudden onset and offset and a duration correlated with the length of stimulation. EA also produced long-lasting inhibition of these cells. The inhibitory pathway from the DRN to the NPF, which is activated by EA and presumably mediated by NCL neurones, would appear to be serotonergic. The evidence for this is that the inhibition evoked by DRN stimulation or EA is enhanced by alaproclate, a 5-HT uptake blocker, and blocked by 5,7-DHT, a 5-HT neurotoxic agent, or cyproheptadine, a serotonin antagonist.

J.G.Sinclair, Ph.D.

Supervisor

TABLE OF CONTENTS

ABSTRACT.....	ii
TABLE OF CONTENTS.....	iv
LIST OF ABBREVIATIONS.....	vii
LIST OF FIGURES.....	x
LIST OF TABLES.....	xiv
ACKNOWLEDGEMENTS.....	xv
INTRODUCTION.....	1
I. Rationale.....	5
II. Specific aims.....	6
BACKGROUND.....	8
I. History of acupuncture.....	8
II. Basic concepts of acupuncture meridians and points.....	10
III. Clinic application of acupuncture anaesthesia.....	12
IV. Acupuncture study in Western countries.....	13
V. Peripheral receptors and afferent fibres.....	15
VI. Spinal cord interactions.....	15
VII. Ascending-tract cells.....	17
(a) Spinothalamic tract (STT).....	17
(b) Spinoreticular tract (SRT).....	19
(c) Spinomesencephalic tract (SMT).....	20
(d) Spinocervical tract (SCT).....	21
(e) Dorsal column pathway (DCP).....	22
(f) Postsynaptic dorsal column pathway (PSDC).....	23
(g) Spinohypothalamic pathway (SHP).....	23
VIII. Descending modulation of nociceptive transmission.....	24
IX. Ascending modulation of nociceptive transmission.....	28

TABLE OF CONTENT

X. Drugs used to modify 5-HT transmission.....	30
(a) 5,7-DHT.....	30
(b) cyproheptadine.....	31
(c) Alaproclate.....	32
METHODS AND MATERIALS.....	35
I. Electrode preparation.....	35
(a) Carbon-fibre electrodes.....	35
(b) Multibarrelled microiontophoretic electrodes.....	35
(c) EA electrodes.....	36
(d) TF-EMG electrodes.....	37
(e) Peripheral nerve recording electrodes.....	37
(f) Spinal cord lesion electrodes.....	37
II. Equipment.....	37
III. Software and statistical analysis.....	38
IV. Experimental protocols.....	39
Experiment 1. The effects of EA on the spinal cord WDR dorsal horn neurones and the tail-flick reflex latency (TF-EMG).....	39
Experiment 2. The effects of stimulating acupuncture vs non-acupuncture points on the inhibition of the spinal cord WDR dorsal horn neurones.....	41
Experiment 3. The types of afferent fibres activated by EA.....	42
Experiment 4. Tests for a supraspinal involvement in EA-evoked inhibition of spinal cord nociceptive transmission.....	43
Experiment 5. Ascending and descending tracts in EA-evoked inhibition of spinal cord nociceptive transmission.....	43
Experiment 6. Characteristics of the DRN neurones in response to natural noxious and non-noxious peripheral stimuli and EA.....	44
Experiment 7. Characteristics of the NPF neurones in response to natural noxious and non-noxious peripheral stimuli and EA.....	45
Experiment 8. Test of a direct projection from the DRN to the NPF.....	47
Experiment 9. Effects of the DRN stimulation on NPF neuronal activity.....	48
Experiment 10. Tests for a serotonergic projection involvement in the ascending pathway from the DRN to the NPF.....	49
1) Serotonin neurotoxin study.....	49
2) Iontophoretic studies.....	51
V. Histology.....	55

TABLE OF CONTENT

RESULTS.....	57
EA on spinal cord WDR dorsal horn neurones and the tail-flick latency (experiment 1).....	57
The effects of stimulating acupuncture vs non-acupuncture points on the inhibition of the spinal cord WDR dorsal horn neurones (experiment 2).....	60
Types of afferent fibres activated by EA (experiment 3).....	62
A supraspinal involvement in EA-evoked inhibition of the spinal cord nociceptive transmission (experiment 4).....	65
Ascending and descending tracts in EA-evoked inhibition of spinal cord nociceptive transmission (experiment 5).....	69
The characteristics of the DRN neurones in response to natural peripheral stimuli and EA (experiment 6).....	71
Test of a direct projection from the DRN to the NPF (experiment 7).....	76
The characteristics of the NPF neurones in response to natural peripheral stimuli and EA (experiment 8).....	81
The effects of DRN stimulation on NPF neurones and the TF-EMG (experiment 9).....	88
Serotonergic involvement in the inhibitory projection from the DRN to the NPF (experiment 10).....	91
1) Studies on 5,7-DHT pretreated animals.....	91
2) Iontophoretic studies.....	97
DISCUSSION.....	118
CONCLUSION AND SUMMARY.....	137
BIBLIOGRAPHY.....	143
APPENDIX.....	167

LIST OF ABBREVIATIONS

AA	acupuncture analgesia
β	beta
C	centigrade
CL	clock-like neurones in the DRN
cm	centimeter
DCP	dorsal column pathway
EA	electroacupuncture
5,7-DHT	5,7-dihydroxytryptamine
DLF	dorsolateral funiculus
DRN	dorsal raphe nucleus
δ	delta
Fig	figure
g	gram(s)
GABA	gamma-aminobutyric acid
HRP	horseradish peroxidase
Hz	hertz
i.p.	intraperitoneal
kg	kilogram
KHz	kilohertz
M	molar
m	meter
mA	milliamperes
mg	milligram

LIST OF ABBREVIATIONS

min	minute
μ m	micrometer
mM	millimolar
mm	millimeter
ms	millisecond
nA	nanoampere
NaCl	sodium chloride
NCL	non-clock-like neurones in the DRN
NCLN	non-clock-like-non-responding neurones in the DRN
NCM	centromedian nucleus
NGC	nucleus gigantocellularis
NLA	nucleus lateral anterioris
NRM	nucleus raphe magnus
NPF	nucleus parafascicularis
PAG	periaqueductal gray
PCA	p-chloroamphetamine
P-CPA	p-chlorophenylalanine
PSDC	postsynaptic dorsal column pathway
R-S	raphe-spinal
s	second
SCT	spinocervical tract
SE	standard error of the mean
SHP	spinothalamic pathway

LIST OF ABBREVIATIONS

SMT	spinomesencephalic tract
SPA	stimulation-produced antinociception
SRT	spinoreticular tract
STT	spinothalamic tract
TF-EMG	tail-flick-electromyogram
VLT	ventrolateral funiculus
WDR	wide dynamic range

LIST OF FIGURES

- Fig. 1. EA inhibition of a spinal cord WDR neurone and TF-EMG
- Fig. 2. EA reproducibly inhibits WDR dorsal horn neurones and prolongs the tail-flick reflex.
- Fig. 3. The inhibition of WDR neurones is specific to acupuncture points.
- Fig. 4. Compound action potentials recorded from the tibial nerve upon EA stimulation.
- Fig. 5. The effects of EA and cold-block on the responses of a spinal cord WDR neurone to noxious radiant heat applied to the tail.
- Fig. 6. Cold-blocking the spinal cord eliminates the effects of EA.
- Fig. 7. Cold-blocking the spinal cord eliminates the effects of EA even after baseline adjustment.
- Fig. 8. Bilateral lesions of the VLT block the effects of EA on dorsal raphe neuronal nociceptor-evoked activity and the tail-flick reflex.
- Fig. 9. Bilateral lesions of the DLF block the effect of EA on the tail-flick reflex but do not alter the enhancement by EA on nociceptor-evoked activity in dorsal raphe neurones.
- Fig. 10. The extent of lesions noted in individual animals are shown for the DLF and VLT.
- Fig. 11. Oscilloscope traces illustrating the effect of noxious heat (50°C) applied to the tail on a clock-like and non-clock-like neurone.

List of Figures

- Fig. 12. Three dimensional representation of DRN CL, NCL and NCLN neurones.
- Fig. 13. EA enhances the nociceptor-evoked activity of a DRN neurone.
- Fig. 14. The effect of EA on the noxious radiant heat-evoked activity in non-clock-like DRN neurones.
- Fig. 15. The effect of EA on the noxious radiant heat-evoked and spontaneous activity in clock-like DRN neurones.
- Fig. 16. An example of a NCL DRN neurone exhibiting high frequency following and collision.
- Fig. 17. Oscilloscope trace illustrating the spontaneous and nociceptor-evoked activity of an NPF neurone.
- Fig. 18. EA inhibits the nociceptor-evoked activity of a NPF neurone.
- Fig. 19. The effect of EA on the noxious radiant-heat evoked activity in NPF neurones.
- Fig. 20. The effect of EA on the activity of a NPF and DRN neurone recorded simultaneously.
- Fig. 21. The effect of the DRN stimulus on the noxious radiant heat-evoked activity in NPF neurones and the TF-EMG latency.
- Fig. 22. Peristimulus histograms of 6 glutamate-driven NPF neurones tested by single pulse stimulation (1.0 s Hz, 0.2 ms, 0.6 mA) in the DRN.
- Fig. 23. An illustration of serotonergic immunofluorescence reaction in the DRN region of a control rat and a 5,7-DHT treated animal.

List of Figures

- Fig. 24. EA fails to inhibit the evoked activity of NPF neurones in response to noxious radiant heat in 5,7-DHT treated animals.
- Fig. 25. Stimulation of the DRN fails to inhibit the noxious radiant heat-evoked activity in NPF neurones or increase the tail-flick latency in 5,7-DHT treated animals.
- Fig. 26. An example of the effect of iontophoretic application of alaproclate at 30 and 45 nA on a NPF neurone.
- Fig. 27. The effects of iontophoresis of alaproclate on NPF neurones activated by glutamate.
- Fig. 28. Iontophoretic application of alaproclate enhances the inhibition produced by 5-HT but not GABA on NPF neurones.
- Fig. 29. An example of the effect of iontophoretic application of 5-HT, alaproclate and GABA on a NPF neurone.
- Fig. 30. The effect of iontophoretically applied alaproclate on noxious radiant heat-evoked activation of NPF neurones and inhibition by DRN stimulation.
- Fig. 31. An example of the effect of iontophoretically applied alaproclate and DRN stimulation on noxious radiant heat-evoked activation of a NPF neurone.

List of Figures

- Fig. 32. The effects of alaproclate on DRN- and EA evoked-inhibition of a glutamate-driven NPF neurone.
- Fig. 33. The duration of the NPF inhibition produced by DRN stimulation of 1, 2, 4 and 30 s.
- Fig. 34. The magnitude of the NPF inhibition produced by DRN stimulation.
- Fig. 35. Alaproclate iontophoretically applied at 30 nA enhances the magnitude but not the duration of EA-evoked inhibition of NPF neurones.
- Fig. 36. Cyproheptadine blocks the DRN-evoked inhibition of a glutamate-driven NPF neurone.
- Fig. 37. Cyproheptadine blocks the DRN-evoked inhibition of glutamate-driven NPF neurones.
- Fig. 38. Cyproheptadine blocks EA-evoked inhibition of a glutamate-driven NPF neurone.
- Fig. 39. Schematic arrangement of neurones activated by EA and a noxious stimulus.

LIST OF TABLES

- Table 1. Latencies and types of compound action potentials recorded from two sets of electrodes at various current intensities.
- Table 2. Characteristics of DRN neurones.
- Table 3. Changes in weight, behavioural activity and indirect immunofluorescence reaction in 5,7-DHT treated rats.
- Table 4. Iontophoretic application of alaproclate and Na⁺ on NPF neurones activated by glutamate.
- Table 5. The effect of DRN stimulation on NPF neurones driven by glutamate.
- Table 6. The effect of alaproclate on EA-induced inhibition of glutamate-driven NPF neurones.
- Table 7. Cyproheptadine blocks the DRN-evoked inhibition of glutamate-driven NPF neurones.

ACKNOWLEDGEMENTS

I am deeply grateful to Dr. J. G. Sinclair for his guidance, support and patience throughout this research.

I also wish to thank the other members of my research committee, Drs. J. A. Pearson and H. McLennan, for their help and advice. I also wish to extend my thanks to Dr. Steven Vincent, Mr. Roland Burton and Jeff Hockings for their valuable assistance.

INTRODUCTION

Although acupuncture has been practiced for centuries and the neuronal mechanisms of acupuncture have been investigated over the past forty years, the ascending pathways of electroacupuncture (EA) are not well established. The focus of this study is to re-examine the validity of the proposed supraspinal loop on the spinal effects of EA in the rat, to characterize neurones in the dorsal raphe nucleus (DRN) and nucleus parafascicularis (NPF) in response to natural peripheral stimuli and EA, to determine whether there is a direct projection from the DRN to the NPF and to determine whether this ascending pathway is serotonergic.

EA has been shown to inhibit wide dynamic range (WDR) neurones of the spinal cord in cats (Pomeranz et al., 1977; Pomeranz and Cheng, 1979; Wu et al., 1986). There were, however, several discrepancies between these studies. Pomeranz and Cheng (1979) elicited a relatively low magnitude, delayed onset but prolonged inhibition, whereas Wu et al. (1986), using a higher stimulus intensity, observed a greater inhibition with immediate onset but which was abolished almost immediately with the offset of the EA stimulus. The finding that the EA-induced inhibition disappeared immediately after discontinuing EA is not consistent with the prolonged time course of acupuncture analgesia in humans (Yee, 1973). Further Pomeranz et al. (1977) implicated supraspinal structures in the long-lasting inhibition of the WDR neurones produced by EA. They found that the inhibition was abolished by spinal cord transection, midcollicular decerebration or hypophysectomy and suggested that the

effect was mediated by the release of a pituitary hormone. Wu et al. (1986) found the results were inconsistent on two neurones tested for a supraspinal involvement in EA. The inhibition on one cell was blocked after cold-blocking the spinal cord while the inhibition on another cell was not altered after transection of the dorsal half of the spinal cord. The inconsistencies noted above as well as the lack of information available on the rat encouraged me to re-examine the supraspinal involvement of EA in attenuating spinal cord nociceptive transmission and the time course of this inhibition by EA.

In other studies on the cat, Du and Chao (1976) and Shen (1975) clearly showed that EA inhibited the viscerosomatic reflex through a supraspinal loop. In addition, Shen et al. (1975), based on spinal cord lesion studies, concluded that the ascending arm of the loop was located in the ventrolateral tract (VLT) and the descending arm in the dorsolateral funiculus (DLF).

Anatomical and physiological studies have shown that the periaqueductal gray (PAG) neurones project directly to the nucleus raphe magnus (NRM) in both the cat (Holstege, 1991) and rat (Pomeroy and Behbehani, 1979). Further, Liu et al. (1986) compared the effects of EA and PAG stimulation on NRM neuronal activity in the rat. They found most neurones to be responsive to both types of stimuli with an increase in activity and an inhibition of nociceptor-evoked responses. Bilateral destruction of the PAG markedly reduced the effect of EA indicating that EA activates NRM neurones partly through the PAG. Du and Chao (1976)

reported that a lesion in the NRM resulted in a significant decrease of the inhibitory effect of the viscerosomatic reflex during acupuncture. Their evidence indicates that the NRM is involved in the descending inhibitory effects in acupuncture analgesia. A lesion of NRM also reduced the inhibitory effects of viscerosomatic reflexes produced by the stimulation of the PAG (Du and Zhao, 1986).

Cellular origins of projecting fibres in the DLF are concentrated in the NRM and in the adjacent nucleus reticularis paragigantocellularis in the rat (Basbaum and Fields, 1979; Pomeroy and Behbehani, 1974, 1979). Xiang et al. (1986) found that the great majority of raphe-spinal (R-S) neurones did respond to noxious or EA stimuli. The receptive fields of the R-S neurones were very wide, covering almost all of the body. The effect of EA on these R-S neurones was mainly to increase their firing rates and to inhibit their nociceptive-evoked responses. After transection of DLF, the R-S neurones could still be activated by EA indicating that the ascending projection of EA is not in the DLF of the spinal cord (Xiang et al., 1986).

Aghajanian et al. (1978) reported that the spontaneously firing neurones in the DRN of the rat typically have a regular or irregular rhythm with a slow firing rate. However a stimulus to the sciatic nerve (constant current pulses of 50 μ A, 0.5 ms, 1 Hz) was shown to produce an inhibitory response in the regular firing neurones. The mean duration of total suppression was 316 ms. There was no suppression found in irregular firing neurones. These results would appear to be in conflict

with the report of Shima et al. (1987) although different species were used. It should be mentioned that most investigators did not find regular firing neurones to respond to a noxious natural stimulus such as pinching (Aghajanian, et al., 1977a,b, 1978; Haigler, 1976; Wang and Aghajanian, 1977a,b).

Shima and his colleagues (1987) classified DRN neurones into two groups with different patterns of firing in the anaesthetized cat: regularly firing (clock-like) and irregularly firing (non-clock-like) neurones. Clock-like neurones (CL) did not change their firing rate to nociceptive and non-nociceptive stimulation. On the other hand, many non-clock-like neurones (NCL) responded to nociceptive and non-nociceptive stimulation. Also, NCL neurones were activated by the administration of morphine which was dose-related and reversed by the narcotic antagonist, naloxone. In the report of Shima et al. (1987) there was no information on whether NCL neurones of the DRN are serotonergic.

In addition to the involvement of DRN neurones in descending effects, electrophysiological, autoradiographic and degeneration studies show an ascending projection from the DRN to the NPF of the thalamus (Bobillier et al, 1975; Conrad et al., 1974; Pierce et al., 1976) and the cortex (Olpe, 1981). Electrophysiological evidence in the rat and the cat shows the NPF receiving nociceptive input that arises from spinothalamic pathways (Albe-Fessard et al., 1962; Chang, 1986).

Thus the DRN may modulate nociceptive transmission via ascending as well as descending projections. Qiao et al. (1983), Zhang et al. (1986) and Qiao and Dafny (1988) reported that electrical stimulation of the DRN had a marked inhibitory effect upon nociceptive neurones in the NPF. This inhibitory effect persisted, although reduced, after transection of the dorsal half of the spinal cord at T3-4, suggesting that the inhibitory effect is not totally due to a reduction of the ascending input from the spinal cord. The nociceptive discharges of the NPF can also be inhibited by activation of acupuncture point "Zusanli" in the rat (Chang, 1986) or "Hegu" in the rabbit (Zhang et al., 1986). Therefore it is of interest to determine whether EA activates DRN neurones which project to the NPF. There is a suggestion by investigators at the Beijing Medical College (1986) that about half of the CL DRN neurones are facilitated by EA.

Zhang et al. (1986) reported that the nociceptive discharges of the NPF were inhibited after intraventricular injection of 100 μ g of serotonin (5-hydroxytryptamine, 5-HT). Andersen and Dafny (1983a) reported that NPF neurones in animals treated with 5,7-dihydroxytryptamine (5,7-DHT), a serotonin neurotoxic agent, were not inhibited by DRN stimulation. They therefore proposed that the pathway from the DRN to the NPF is serotonergic.

I. Rationale:

The inconsistencies noted in the time course of inhibition of WDR dorsal horn neurones of the spinal cord produced by EA and the lack of information available on the rat encouraged us to re-examine the supraspinal involvement of EA in attenuating spinal cord nociceptive transmission.

There are conflicts in the reports studying CL and NCL neurones of the DRN as to which type respond to noxious or non-noxious stimuli. There is no information showing what kind of neurones in the DRN project to the NPF, whether a direct projection from the DRN to the NPF occurs, whether EA activates this pathway and, if so, whether serotonin is involved in this ascending inhibitory pathway. Therefore, it is necessary to characterize the DRN neurones in response to natural noxious and non-noxious as well as EA stimulation, to examine the projection from the DRN to the NPF and to test for a serotonergic involvement.

II. Specific Aims of This Work Are to Determine:

1. The effect of EA on WDR dorsal horn neurones of the spinal cord and the tail-flick reflex latency.
2. The types of afferent fibres activated by EA.
3. The effects of stimulating acupuncture vs non-acupuncture points on the inhibition of the spinal cord WDR dorsal horn neurones.
4. If there is a supraspinal loop involved in EA-evoked inhibition of the spinal cord nociception transmission.

5. Whether the ascending and descending EA neuronal activity travels via the VLT or the DLF if there is a supraspinal loop.
6. The characteristics of neurones in the DRN responding to natural noxious and non-noxious peripheral stimuli and EA.
7. The effects of DRN stimulation on NPF neuronal activity.
8. The effects of EA on NPF neuronal activity.
9. Whether there is a direct projection from the DRN to the NPF.
10. Whether the ascending pathway from the DRN to the NPF is serotonergic. To this end, responses of NPF neurones to DRN stimulation or EA will be tested using the following agents: 5-HT, a 5-HT uptake inhibitor (alaproclate); a 5-HT neurotoxin (5,7-dihydroxytryptamine) and a 5-HT antagonist (cyproheptadine).

BACKGROUND

I. History of Acupuncture

The information regarding acupuncture history in this background was gleaned from following books: "Acupuncture Manual, a Western Approach" (Chu, et al., 1979) "Acupuncture, Textbook and Atlas" (Stux and Pomeranz, 1987) and "Academy of Traditional Chinese Medicine" (Shanghai Traditional Chinese Medical College, 1975).

Acupuncture is one of the oldest medical treatments in the world. Early in primitive society stone needles were used and can be traced as far back as the New Stone Age, about 4,000-10,000 years ago. Flint needles dating from 7000 to 5000 B.C. have been found which may suggest that Neolithic humans used these needles to relieve pain and sickness.

The emperors, Fu Hsi (accession 2852 B.C.) and Shen Nung (accession 2737 B.C.), were responsible for the development of Chinese civilization, agriculture and medicine. Huang Ti, "the Yellow Emperor" (accession 2697 B.C., translated by H.C. Lu, 1973), conducted many wide-ranging dialogues on the subject with his minister, Chi Po, which were later gathered into the celebrated classic known as the Nei Ching [The Yellow Emperor's Treatise on Internal Medicine] which includes the Su Wen and the Ling Shu. The Su Wen describes the entire field of medicine; the Ling Shu is a supplement and includes the first-known discussion of acupuncture therapy.

Between 700-221 B.C. the theory of Jingluo (meridians) was established. Hua To (A.D. 110-207), the first-known Chinese surgeon, used acupuncture for headache and herb anesthesia for minor surgery.

The "Zhen-jiu Jiayijing", the first classic book on acupuncture, was written between 256-260 A.D. In the Tan dynasty (A.D. 617-907), the "Chai Kin Yao Fan" and "Chai Kin Yi Fan" were written by Sun Szu Mo in which all essential acupuncture methods, points and contraindications were discussed. During this period, an Acupuncture Institute was established at the Imperial Medical College in Peking. This is the earliest medical school in China.

One of the most important achievements in acupuncture practice took place in the eleventh century during the Sung dynasty (A.D. 960-1206) when Wang Wei wrote the Tun Jen Ching in which he standardized acupuncture points and suggested using a bronze model of the human form to demonstrate the location of the meridian and acupuncture points. This hollow bronze figure soon became a highly effective teaching device (Ornstein, 1976).

In Europe, physicians first learned of acupuncture from the German botanist, physician and traveler, Eugene Kamper (1651-1716). In London, the surgeon, Croley (1802), reported some success using acupuncture for headache, backache and rheumatism. John Elliotson (1791-1868) used the acupuncture technique at Saint Thomas Hospital in London. Sir William Osler (1849-1919) recommended acupuncture therapy

for Tumbago in his classic work, *The Principles and Practice of Medicine* (1892).

II. Basic Concepts of Acupuncture Meridians and Points

Health depends upon the flow of chi, meaning "life-force" along invisible pathways called meridians. Chi can be understood collectively as the vital energetic or functional components necessary for organic life. Disease or pain is the result of a blockage of chi at one or more meridians. By inserting needles into various acupoints, the acupuncturist dissipates the excess or replenishes the undersupply of chi to the vital organs. Thus acupuncture restores health or dynamic equilibrium by regulating or rebalancing chi.

The routes of energy circulation are known as the meridians. The meridian is the simple name for channel (vertical paths of circulation) and collateral (horizontal paths of circulation). There are 12 main meridians and branch meridians. Internally, the meridians connect to the zang-fu (organs) and superficial circulation throughout the body. The 12 meridians directly connected with the organs are as follows: lung, large intestine, stomach, spleen, heart, small intestine, bladder, kidney, pericardium, liver, gallbladder and triple warmer. The triple warmer, although named as an organ, is not a organ by Western definition. The energy circulation is said to start in the lung meridian and successively flow through each of the meridians and their

associated organs to complete the cycle by going through the liver meridian.

Acupuncture points are key points along the energy pathways of the body known as meridians. The size of the meridians do not remain consistent throughout whole body. In some places the meridians widen and in other places they become narrow. The places on the meridian that are wider and where reactions appear on the skin surface are known as acupuncture points. Acupuncture points are regarded as "gates" or openings by which the meridians communicate with the external environment. Treating the meridians by stimulating the affected acupuncture points serves to alleviate the dysfunction. Therefore the acupuncture points, in addition to being points at which one's condition can be diagnosed, are points at which treatment can be applied. In the case of abdominal pain, for example, the acupuncture points used to diagnose and to treat the problem will vary according to the location of the pain and which meridians are in the vicinity as well as whether it is a stomach condition or a liver condition.

There are more than 360 acupuncture points on the body. These are most commonly located in depressed spaces in the joints, creases in skin at joints, clefts between muscle and bone, in places where nerve trunks come close to the surface or in places where cutaneous nerves reach the skin and the muscles. In a sense these are all places in the body that are structurally more sensitive to a physical stimulus than other areas.

III. Clinic Application of Acupuncture Analgesia (AA)

Acupuncture has a comparatively good analgesic effect. This has long been known and used to treat a variety of inflammations and pains. More than 2,000,000 operations have been conducted in China under AA. These include more than 100 different types of minor and major operations, such as abdominal tubal ligation, cesarean section, subtotal gastrectomy, splenectomy and open heart surgery. AA has the following characteristics: patients remain in a conscious state throughout the operation; all mental, sensory and motor functions are normal except for a dull pain sensation during the operation. Little or no anaesthetic is needed, precluding the possibility of postoperative drug side-effects apart from anaesthetic accidents. Postoperative pain is mild, and generally there are no such reactions as nausea and vomiting.

Problems remaining to be solved in AA are incomplete analgesia and control of visceral reaction. EA has not yet been improved to such a level that it can ensure complete painlessness. Therefore the judicious use of certain adjuvants to increase its analgesic action is justifiable. The adjuvants most frequently employed in electroacupuncture (EA) are central nervous system sedatives and analgesics. Local anaesthetics may also be used. It is considered important to estimate the effectiveness of AA before the operation, in order to select suitable cases. Such patients respond to EA with an increased pain threshold. Generally, the results of analgesia are good

in patients in whom the pain threshold is high or can be elevated by needling.

IV. Acupuncture Studies in Western Countries

Acupuncture has been practiced for centuries in China, but only recently it has begun to be accepted in the Western countries. Several hospitals have been using EA for surgical procedures in the United States (Gaary, 1975; Ledergerber, 1976). Gaary (1975) reported that 56 surgical procedures such as hip pinning, multiple dental extractions and removal of 2 tibial staples were performed using EA. All patients were alert, cooperative and responsive throughout the procedures. Ledergerber (1976) used EA (0.2 ms, 0.93 mA, 0.25 - 30 Hz) for the induction of labor and delivery. Of the 15 cases, 6 were completely successful requiring no medication in labor and no local or regional block for forceps or episiotomy, 3 were partially successful and 6 were total failures. Richter et al. (1975) reported on 125 patients undergoing open heart surgery using EA in Germany. They indicated that one of the important advantages was a considerable reduction of the use of analgesics in the postoperative stage.

Acupuncture has also been reported to be effective therapy for the treatment of substance abuse and AIDS (Smith, 1990) and chronic severe depression (Jacob, 1990).

Acupuncture has been accepted slowly in North America perhaps because of insufficient scientific explanations to describe its mechanisms of action. Some studies on acupuncture mechanisms have been conducted at a number of laboratories in the U.S.A. (Brockhaus and Elger, 1990; Chapman et al., 1977, 1980; Davis, 1973; El-Etr and Pesch, 1973; Gaw et al., 1975; Greguss, 1973; Hynynen et al., 1981; Kitahata, 1975, 1977; Lee et al., 1975; Linzer and Atta, 1973; Looney 1973a, b, 1975; Matsumoto et al., 1973; Mayer and Liebeskind, 1974; Mayer et al., 1976; Numoto and Donaghy, 1973; Oleson and Kroening, 1983; Oleson et al., 1978; Philips and Rusy, 1973; Wagman 1973), Canada (Cheng and Pomeranz, 1979, 1980; McLennan et al., 1977; Pomeranz, 1973, 1977; Pomeranz, et al., 1977; Pomeranz and Cheng, 1979; Pomeranz and Paley, 1979), Japan (Takeshige, 1981, 1985; Takeshige et al., 1976, 1980, 1981), Finland (Duggan 1978) and Sweden (Anderson and Holmgren, 1975). Three national symposia on acupuncture in China in the last 25 years have illustrated that acupuncture analgesia involves the central nervous system. The proceedings of two of these have been published (Advances in Acupuncture and Acupuncture Anaesthesia, 1979; Research on Acupuncture, Moxibustion and Acupuncture Anesthesia, 1986). The structures and background most pertinent to the present study are described below.

V. Peripheral Receptors and Afferent Fibres

Cutaneous neurones have large ($A\alpha\beta$) and small ($A\delta$) myelinated fibres as well as unmyelinated C-fibres. The conduction velocity of these fibres are 30-100 m/s, 4-30 m/s and less than 2.5 m/s, respectively (Boivie and Perl, 1975; Gasser, 1950). Muscle nerves are described as groups I, II, III and IV (Lloyd and Chang, 1948). Groups III and IV fibres are similar to $A\delta$ and C-fibres.

Several types of sensory receptors are located at the peripheral ends of the above nerves. These are extensively described by Willis and Coggeshall (1991). Mechanoreceptors which are activated by individual hairs in mammals are coded by two general classes of receptors: rapidly adapting and slowly adapting (Brown and Iggo, 1967; Burgess et al., 1968). A number of these receptors respond to non-noxious stimuli.

Nociceptors signalling the presence of damaging stimuli may be subdivided into mechanical, thermal and polymodal.

VI. Spinal Cord Interactions

Primary afferent fibres enter the spinal cord for the most part by the dorsal roots. Upon entering the spinal cord the small fibres bifurcate sending axonal trajectories rostrally and caudally through the tract of Lissauer. In a transverse section, Lissauer's tract corresponds to a columnar area of fibres located between the dorsal root entry zone and the superficial boundary of the dorsal horn gray matter.

The large fibres project more medially and also bifurcate sending rostral and caudal projections in the dorsal columns. In each case collaterals from the main axon branch off, enter the gray matter of the spinal cord and make synaptic connections (Brown, 1981).

The scheme used by Rexed (1952) is a useful one and widely used to indicate the location of neurones in the spinal cord. He organized the spinal cord gray matter into 10 laminae: the first 6 are located in the dorsal horn, 7-9 in the ventral horn and lamina 10 surrounds the central canal. Although certain types of cells tend to be more prominent in one lamina than another, there is much overlap and the position of a cell in a lamina cannot be used to indicate its function.

Collaterals from small fibres terminate mainly in the superficial laminae of the dorsal horn, lamina I (the marginal layer) and II (the substantia gelatinosa). The A6 fibres primarily terminate in lamina I while C-fibres end mainly in laminae II (Light and Perl, 1977, 1979). In contrast, the large-diameter fibres terminate mainly in lamina III and deeper laminae of the dorsal horn (Brown, 1981).

Sensory processing in the spinal cord results from interactions among primary afferent fibres, interneurones, ascending-tract cells and descending-tract cells which modulate spinal cord neurones and afferent terminals. The majority of neurones in the spinal cord are interneurones; it is estimated that 1% are ascending-tract cells and 2% are motoneurones (K. Chung et al., 1984).

Spinal cord neurones responding to a noxious stimulus are generally of two types: WDR and nociceptive specific (NS). WDR cells, also referred to as lamina V-type, multireceptive or convergent neurones, are numerous and respond to both noxious and non-noxious natural stimuli. They can be found in most laminae of the spinal cord but tend to be concentrated in lamina V. NS cells as the name implies, respond only to a noxious peripheral stimulus and are found mainly in lamina I (Christensen and Perl., 1970).

VII. Ascending-Tract Cells

Perception and behavioural response to sensory information requires that the sensory signals be transmitted in ascending pathways to supraspinal levels. It seems evident that the various tracts are involved in particular functions, but neurones in several tracts have been shown experimentally to respond to a noxious stimulus. There are species differences and due to the bulk of the literature most of the following review will be restricted to the rat.

(a) Spinothalamic Tract (STT)

The STT cells are primarily located in the dorsal horn of the spinal cord although some cells are in the intermediate zone and ventral horn (Willis and Coggeshall, 1991). Using retrograde tracing methods of injecting horseradish peroxidase (HRP) into various regions of the

thalamus, one finds the largest concentration of cells in the cervical segments of the spinal cord. In lower spinal cord segments cells are found mainly in the lumbo-sacral enlargement. Almost all the labeled cells at these levels are found contralateral to the injection site (Giesler et al., 1979). Cells projecting to the lateral thalamus are found in the marginal layer, nucleus proprius and medial intermediate groups.

STT cells projecting to the medial thalamus are in the medial base of the dorsal horn and the intermediate gray. Axons to the lateral thalamus ascend more laterally than those to the medial thalamus (Giesler et al., 1981). STT axons pass dorsolateral to the inferior olivary nucleus in the medulla and via the medial lemniscus to the thalamus.

Target cells in the thalamus for STT axons include the VLP nucleus, the centrolateral thalamic nucleus of the intralaminar complex [as well as adjacent parts of the medial dorsal and parafascicular nuclei] and the posterior complex (Lund and Webster, 1967; Peschanski et al., 1983).

STT cells, as determined by conduction velocities, have a wide range of axon diameters including large and small myelinated axons and unmyelinated axons (Trevino et al., 1973).

STT cells in rats usually respond to both noxious and non-noxious natural stimuli (Giesler et al., 1976) and would be classified as WDR

neurones. However relatively little work has been done on these cells in the rat but they have been studied extensively in the primate (Willis and Coggeshall, 1991). Of particular importance to the present study are the inputs to the medial thalamus. Some STT cells in laminae IV-VI send collaterals to the medial thalamus and some in the deeper layers of the spinal cord gray matter project only to the medial thalamus. Since many of these neurones are of the high-threshold type and thus unlikely to provide sensory discrimination information, it is speculated that they may instead trigger a motivational-affective response (Willis and Coggeshall, 1991).

(b) Spinoreticular Tract (SRT)

Neurones in this tract, as the name suggests, originate in the spinal cord and terminate in the reticular formation. The axons project in the ventrolateral white matter. Like the STT, the majority (85%, Chaouch, 1983; 74%, Kevetter and Willis, 1983) of SRT cells originate in the spinal cord contralateral to their termination in the brain. Most cells are located in laminae V, VII and VIII.

One part of the SRT projects to the lateral reticular nucleus, a precerebellar nucleus. The medial part of the SRT innervates neurones in the caudal brainstem, which may be involved in descending sensory-motor control systems, or project to higher levels including the midbrain and diencephalon. Using 2 different tracers, Kevetter and

Willis (1982, 1983) found cells projecting to the reticular formation and the thalamus with about 10% of the cells projecting to both sites. The projections to the thalamus are primarily in the medial thalamus (Peschanski and Besson, 1984).

The majority axons of almost all SRT cells are myelinated with conduction velocities ranging from 2-96 m/s (Fields et al., 1977b; Maunz et al., 1978). Again, most SRT neurones are of the WDR variety.

(c) Spinomesencephalic Tract (SMT)

Using HRP injections to the midbrain tegmentum in the rat, Menetrey et al. (1980) found SMT cells, mostly contralateral, in lamina I, the lateral spinal nucleus, lamina V and laminae at all levels of the spinal cord. R. P. Liu, (1983) found that the PAG projections were largely in the ventrolateral PAG (including the DRN). Interestingly, many of the lamina I neurones projected to the PAG (Swett et al., 1985). Pechura and Liu (1986) have shown that some SMT neurones have projections both to the PAG and medullary reticular formation. Yezierski et al. (1991) found that 74% of SMT cells had a contralateral projection and 26 % an ipsilateral projection.

SMT axons ascend in the white matter of the ventral part of the spinal cord along with STT and SRT axons. However some SMT lamina I neurones project in the DLF. The PAG is innervated by SMT cells at all rostro-caudal levels (Zemlan et al., 1978; Swett et al., 1985). In the

rat, SMT cells include the low-threshold, WDR and high-threshold variety (Menetrey et al., 1980).

In general, the main difference between these tract cells is their point of termination. STT, SRT and SMT cells have a somewhat similar distribution, the ascending projection for all is predominantly contralateral in the VLT and they seem to be subject to similar peripheral inputs.

(d) Spinocervical Tract (SCT)

SCT cells project in the DLF of the spinal cord to synapse with neurones in the lateral cervical nucleus (LCN) which is located in the cervical region of the spinal cord, just ventrolateral to the dorsal horn. The axons of LCN neurones decussate and ascend into the brainstem joining the medial lemniscus on their way to the thalamus.

The SCT does not seem to be well developed in the rat and probably for that reason has not received much attention in this species. It has been studied much more extensively in the cat.

Injections of HRP into the LCN results in labeled neurones concentrated in the ipsilateral nucleus proprius, particularly lamina IV (Giesler et al., 1978; Craig, 1976; Brown et al., 1980). A few neurones were found in various laminae contralaterally.

Axons of SCT cells terminate in the LCN but some fibres are noted to be collaterals of large fibres that continue to ascend in the DLF

(Enevoldson and Gordon, 1989). The targets of these continuing fibres are not known but some appear to synapse in the dorsal column nuclei (Enevoldson and Gordon, 1989).

Brown and Franz (1969) characterized SCT neurones in the cat. They found that 30% of the cells were excited just by hair movement, 48% were excited by hair movement and pressure while 21% were excited by pressure and pinch. Consistent with this finding, Brown et al. (1975) reported that 29% of SCT cells were activated by A fibres while 71% were excited by both A and C-fibres. Cervero et al. (1977) found almost all SCT cells were responsive to non-noxious stimuli but most were also excited (61%) or excited and inhibited by noxious stimuli.

The SCT is thought to be an important tactile pathway due to the vigorous activity of cells to small movements of hair. However, the fact that many also respond to a noxious stimulus and indirectly project to the thalamus suggests that they may also be involved in nociception.

(e) Dorsal Column Pathway (DCP)

This pathway consists of branches of primary afferent fibres which ascend in the dorsal column. The dorsal column may be divided into the fasciculus cuneatus (containing fibres from the midthoracic to upper cervical levels) and the fasciculus gracilis (where fibres originate below the midthoracic level). These fibres terminate in nuclei with the same name in the caudal medulla. Together they are called the dorsal

column nuclei. Only a portion of the primary afferents in the DCP reach the DC nuclei, the majority terminating in the gray matter of the spinal cord. The DC also contain axons from propriospinal neurones and postsynaptic DC neurones. Fibres from the DC nuclei project to the contralateral thalamus via the medial lemniscus.

The majority of cutaneous fibres in the DC contain rapidly adapting hair follicle receptors at their peripheral terminals (Brown, 1968).

(f) Postsynaptic Dorsal Column Pathway (PSDC)

Neurones forming this pathway in the DC are spinal cord neurones. HRP injections into the DC nuclei in the rat show that the cell bodies are located in the nucleus proprius below the substantia gelatinosa (Giesler et al., 1984).

In the rat, Giesler and Cliffer (1985) found that the majority of these cells responded exclusively to non-noxious stimuli. The majority responded to non-noxious stimuli and strong mechanical stimuli. Almost none responded to noxious heat and thus, this pathway is not considered to play a major role for nociception in the rat.

(g) Spinothalamic Pathway (SHP)

Recent studies in Giesler's laboratory (Burstein, et al., 1987, 1990) have revealed a bilateral projection to the medial and lateral

hypothalamus in the rat. The cells of origin are in the deep dorsal horn and lateral spinal nucleus and in laminae I and VII. Some STT cells in the rat have collaterals to the hypothalamus. The termination of these ascending SHP fibres has not yet been reported. SHP neurones respond to noxious heat. It has been speculated that these cells, are involved in autonomic and endocrine integration including viscerosomatic responses to noxious stimuli. However it is also of interest that the hypothalamus supplies a major afferent input to the PAG (Beitz, 1982). Electrical stimulation of certain regions of the hypothalamus also produces analgesia so it is possible that this loop is involved in the descending modulating system for nociceptive transmission.

VIII. Descending Modulation of Nociceptive Transmission

The PAG has received much attention in analgesia studies since Reynolds (1969) showed that electrical stimulation can produce a marked analgesia without apparently modifying other sensory modalities. This effect has since been referred to as "stimulation-produced analgesia" (SPA). Shortly thereafter, Guilbaud et al. (1973) showed that stimulation of the PAG produced a selective inhibition of nociceptive cells in the dorsal horn of the spinal cord. Further, discrete lesions of the DLF blocked the SPA (Basbaum et al., 1976). The ventrolateral region of the PAG, including the DRN, was generally found to be the most effective site to produce SPA (Guilbaud et al., 1973). Mayer et al.,

(1974) found that there was no correlation between SPA and self-stimulation behaviour.

Midbrain stimulation in the vicinity of DRN in chronically implanted, awake cats and rats evoked profound analgesia to peripherally applied noxious stimuli (Aimone, et al., 1987; Hung, C. et al., 1982; Liebeskind et al., 1973, 1983; Oliver et al., 1975, 1979). Du et al. (1978) reported that stimulation of the DRN also produced inhibitory effects on viscerosomatic reflexes in the cat. The peripheral field of analgesia sometimes includes the entire body.

DRN neurones in the PAG, however, do not project to the spinal cord (Kneisley, et al., 1976; Kuypers, and Maisky, 1975). Rather, DRN neurones have been shown to project to and excite neurones in the ventromedial medulla that contain the NRM and the adjacent reticular formation, the nucleus paragigantocellularis (Pomeroy and Behbehani, 1979; Vanegas et al., 1984; Gallagher and Pert, 1978). These neurones, in turn, project to various regions of the spinal cord via the DLF (Basbaum, et al., 1978). Many of these descending neurones contain 5-HT. The termination is most dense in the superficial regions of the dorsal horn which is, of course, the site of termination of the small nociceptive primary afferent fibres (Basbaum et al., 1978).

Consistent with their role in the descending inhibitory pathway for nociceptive transmission, stimulation of the NRM produces antinociception (Oleson et al., 1978; Proudfit and Anderson, 1975).

Also, lesions of the NRM and adjacent reticular formation antagonizes the antinociception produced by glutamate injections into the PAG (Behbehani and Fields, 1979).

The neurotransmitters released from PAG neurones to excite NRM cells are not known. However, one candidate is neurotensin which is present in a number of the projecting neurones (Beitz, 1982). In addition, this substance produces a dose-dependent antinociception when injected into the ventromedial medulla (Fang et al., 1987). The excitatory amino acids, glutamate and aspartate, are other candidates (Aimone and Gebhart, 1986).

Bennett and Mayer (1979) and Mayer and Liebeskind (1974) reported that the analgesia produce by the simulation of the PAG was equal to or greater than that produced by 10 mg/kg morphine.

The DRN, which is located in the ventromedial region of the PAG, possesses the largest clusters of 5-HT neurones in the brain (Dahlstrom and Fuxe, 1964; Descarries, et al., 1982). Numerous electrophysiological studies have been performed on neurones contained in this nucleus. Aghajanian and his colleagues, working on chloral hydrate anaesthetized rats, have concentrated on the slow, regular firing neurones and have provided the following evidence that these are serotonergic: they are located in the vicinity of clusters of 5-HT neurones demonstrated histochemically (Aghajanian and Haigler, 1974), they are antidromically activated by stimulating the 5-HT ascending

pathway in the ventromedial tegmentum (VMT, Wang and Aghajanian, 1977b) and these cells cannot be located after treatment with the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (PCPA, Sheard et al., 1972) or the 5-HT neurotoxic agent, 5,7-dihydroxytryptamine (5,7-DHT, Aghajanian et al., 1978). These neurones are reliably inhibited by drugs that would be expected to enhance 5-HT neurotransmission (Sheard et al., 1978; Bradshaw et al., 1983; Bramwell and Gonye, 1976). The conduction velocity of their axons can be calculated to be in the range of 0.3-1.5 m/s with the majority below 1 m/sec in the rat (Wang and Aghajanian, 1977 b). Sanders et al. (1980), however, found that antidromic activation of DRN neurones by VMT stimulation was not a valid criterion for identifying 5-HT neurones.

The great majority of these cells were reported to be resistant to natural influences such as light flashes or noxious stimuli (Aghajanian and Wang, 1978; Haigler, 1976; Mosko and Jacobs, 1974). However Aghajanian et al. (1978), in a poststimulus histogram analysis, found these neurones to exhibit a transient inhibition following a low frequency and low intensity stimulus to the sciatic nerve.

Aghajanian et al. (1978) also reported on two other types of neurones located in the DRN. They referred to Type 2 neurones as quiescent or having a very slow discharge rate and Type 3 as having a relatively higher discharge rate, and irregular in rhythm. Neither of these types of cells were considered to be serotonergic in that they

could not be activated by VMT stimulation nor were they eliminated by 5,7-DHT pretreatment. Type 2 cells could be activated by peripheral stimulation and Type 3 cells were either unaffected or showed an overall enhanced response which was not time-locked to the stimulus.

In both the anaesthetized (Nakahama et al., 1981) and unanaesthetized (Shima et al., 1986) cat, regularly firing CL neurones were located in the DRN. Interestingly, almost no CL neurones were activated by noxious natural stimuli whereas about half of the NCL neurones were excited by noxious stimuli. In addition, Shima et al. (1987) found that CL neurones were not activated by intravenous morphine while the majority of the nociceptive NCL neurones were responsive. Thus only the NCL neurones activated by noxious stimuli were responsive to morphine. These investigators did not carry out studies to determine which type of DRN neurone was serotonergic.

IX. Ascending Modulation of Nociceptive Transmission

Evidence indicates that the DRN is involved in ascending as well as descending projections to modulate nociceptor-driven transmission. Oleson and Liebeskind (1976) recorded evoked potential and multiple-unit responses in the medial thalamus as well as behavioural responses in awake, partially restrained rats. A noxious stimulus evoked nocifensive behaviour, an evoked potential and increased multiple unit activity in the medial thalamus. In the great majority of rats, stimulation in the

midbrain PAG markedly reduced the behavioural responses as well as the evoked neurophysiological responses.

More recently, several papers have been published from Dafny's laboratory on the inhibition of NPF neurones by DRN stimulation (Andersen and Dafny, 1982, 1983a,b,). They have presented some evidence that this is a 5-HT mediated inhibition. As mentioned earlier, some NPF neurones are activated by a noxious stimulus.

Numerous investigators have shown that the NPF in the medial thalamus receives nociceptive information (Albe-Fessard and Kruger, 1962; Andersen and Dafny, 1983b; Benabid et al., 1983; Dong et al., 1978). Several reports indicate that neurones in this nucleus have large receptive fields (Albe-Fessard and Kruger, 1962; Dong et al., 1978; Nyquist and Greenhoot, 1974 and Peschanski et al., 1981). This, plus reports that these cells are not intensity coded and respond to high intensity stimulation has lead to the suggestion that they are not important in sensory discrimination but rather they may be involved in identifying novel sensory stimuli, especially harmful ones (Peschanski et al., 1981). This is controversial, however, since Dong et al. (1978) did find the neurones in this region of the cat to be intensity coded. Reports on rats are consistent in that at least 75% of the cells respond to a noxious stimulus (Benabid et al., 1983; Peschanski, et al., 1981).

Consistent with this work is the study by Conrad et al. (1974). They performed an autoradiographic and degeneration study to map the

projections from the DRN. The major ascending projections were found to sweep ventrally from the nucleus, course rostrally through the ventral tegmentum and into the medial forebrain bundle. Projections to the NPF were noted in this study.

X. Drugs Used to Modify 5-HT Transmission

(a) 5,7-DHT

Bjorklund et al. (1974) have reviewed the properties of 5,7-DHT as a 5-HT neurotoxin. It appears that 5,7-DHT uses the neuronal uptake system and thus concentrates within the neurones and produces its neurotoxicity. Although 5,7-DHT is most effective on 5-HT neurones it also produces a lower toxicity to noradrenaline neurones. However experimentally these neurones can be protected by pretreatment with desimipramine, a relatively specific noradrenaline uptake blocker. Surprisingly dopamine neurones do not seem to be affected by 5,7-DHT. This neurotoxic agent would produce its maximal effect within one day after a 200 μ g intraventricular injection and maintain the 5-HT depletion (70% - 90%) at almost the maximal level for 30 days (Bjorklund, et al., 1974).

(b) Cyproheptadine

Stone et al. (1961) showed cyproheptadine was effective in blocking 5-HT actions in organ systems. They found it to be 165 times

more potent against 5-HT than noradrenaline. It failed to block the actions of acetylcholine but did have an antihistaminic effect.

Segal (1975) examined the inhibition produced by DRN stimulation on rat hippocampal pyramidal cells. He presented evidence that the pathway was serotonergic including the finding that in 4 of 5 cases cyproheptadine blocked the effect. It is however not clear what dose level was used. Segal (1976) also found cyproheptadine to block the inhibition in these cells when iontophoretically released.

Wang and Aghajanian (1977a) presented evidence for a direct inhibitory DRN-amygdala serotonergic pathway in the rat. However, cyproheptadine (2-12 mg/kg, i.v.) or iontophoretically applied did not block the inhibition.

Olpe (1981) found DRN stimulation to produce an inhibition of rostral and posterior cingulate cortical neurones in chloral hydrate anaesthetized rats. Cyproheptadine (10 mg/kg, i.p.) was required to consistently block the effects. Doses of 1.0 and 3.0 mg/kg affected only 1 of 4 cells tested. Iontophoretically applied cyproheptadine blocked the 5HT-induced depression on the same cells while being ineffective on GABA-induced inhibition.

Interestingly, McLennan et al. (1977) found cyproheptadine (1.0 mg/kg, i.v.) completely blocked the effects of EA in the rabbit.

The type of 5-HT receptors blocked by cyproheptadine is not clear. There has been a lot of confusion concerning the nomenclature of 5-HT-

receptors (Glennon, 1986) but the classification proposed by Bradley et al. (1986) seems now to be largely accepted (Bonate, 1991). Under this system there are four main 5-HT receptors, 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ with the 5-HT₁ receptors further subdivided into 5-HT_{1a}, 5-HT_{1b}, 5-HT_{1c} and 5-HT_{1d}. Cyproheptadine is generally regarded as a 5-HT₂ antagonist (Bonate, 1991). However, cyproheptadine and mianserin share a similar binding profile and also have a high affinity for the 5-HT_{1c} site (Asarch et al, 1985; Glennon, 1986). On the other hand, Peroutka (1986) reports a cyproheptadine affinity for 5-HT_{1a} > 5-HT_{1b} > 5-HT_{1c}. Thus, cyproheptadine would appear to be a 5-HT₂ antagonist, the effect on 5-HT₁ receptor subtypes is not presently clear. Cyproheptadine cannot be regarded as a specific 5-HT antagonist since it can block other receptors as well, particularly histamine H₁ receptors.

(c) Alaproclate

The major method of inactivation of 5-HT neurotransmission is through a neuronal uptake mechanism and thus drugs which block this uptake would be expected to enhance 5-HT synaptic activity. It is useful to use a drug which has a high specificity for the uptake system because other neurotransmitters are also inactivated through uptake systems.

Alaproclate, a monocyclic compound [2-(4-chlorophenyl)-1, 1-dimethylethyl-2-aminopropanoate] was first reported by Lindberg et al.

(1978) to be a specific 5-HT uptake blocker. They found alaproclate to have an IC_{50} of 1.4×10^{-7} M for the inhibition of 5-HT accumulation in synaptosomes. It was found to be 100 times more potent in blocking the uptake of 5-HT than noradrenaline.

Ogren et al. (1984), in an *in vivo* study, reported that a dose of 40-60 mg/kg completely blocked the depletion induced by 4-methyl- α -ethyl-m-tyramine (H 75/12) in rats. Clomipramine, another 5-HT uptake blocker, even at high doses failed to completely block the depletion. The same dose of alaproclate as mentioned above had no effect on the depletion of noradrenaline or dopamine induced by 3-hydroxy-4-methyl- α -ethyl-phenethylamine HCl (H 77/77). Alaproclate showed a regional selectivity in blocking 5-HT uptake. Alaproclate was found to be most potent in the hippocampus (ED_{50} = 4 mg/kg) and hypothalamus (ED_{50} = 8 mg/kg) followed by the striatum (ED_{50} = 12 mg/kg) and cerebral cortex (ED_{50} = 18 mg/kg). It exhibited a low potency in the spinal cord (ED_{50} > 30 mg/kg). Alaproclate failed to have an effect at concentrations of $< 10 \mu M$ on the following receptors examined: 5-HT, histamine H_1 ; α_1 , α_2 -adrenergic; dopamine D_2 and muscarinic. Ogren et al. (1985) reported that the apparent potentiation of the muscarinic response was via a serotonergic mechanism.

In another study, Ogren and Holm (1980) found alaproclate to be effective in the hot-plate test but not the tail-flick test. Later Eide and Hole (1988) confirmed that alaproclate was ineffective in the tail-

flick test when administered in a single dose but was effective when administered chronically. Ogren and Berge (1985) found that p-chloroamphetamine (PCA), a 5-HT releasing compound, produced analgesia in the hot plate test in rats which was blocked by alaproclate (20 mg/kg) while desipramine was ineffective. Interestingly, cyproheptadine (1.0 mg/kg) was also ineffective in blocking the response of PCA but the dose may have been too low.

METHODS AND MATERIALS

I. Electrode Preparation

(a) Carbon-fibre electrodes

All single unit recordings and DRN stimulation in this study were made through carbon-fibre electrodes. They were prepared according to the method of Armstrong-James and Millar (1979). A carbon fibre (8 μm) of suitable length (>10 cm) was inserted into a glass capillary filled with acetone (KIMAX-51 capillaries of 10 cm length and 0.8 mm (o.d.); Kimble Products) and pulled on a vertical microelectrode puller (Narashigi). The electrode formed had several cm of carbon fibre protruding from the tip which was then cut with scissors to a few mm from the glass tip. Under a light microscope, the fibre was positioned into a silver loop containing a drop of 1 M chromic acid. The fibre was etched to a point by passing a current of 0.12 - 0.30 mA (AC) so that not more than 15 μm of carbon fibre protruded from the microelectrode. The electrodes had an impedance which ranged from 200 k Ω to 2 M Ω .

(b) Multibarrelled microiontophoretic electrodes

Five-barrel microelectrodes were constructed from glass capillaries with an outside diameter of 1.0 mm (Glass Company of America, Omega Dot Brand). These capillaries contained a single glass fibre strand to facilitate their filling by capillary action. Four

Methods and Materials

individual capillaries were bent to an obtuse angle at about 0.5 cm from one end. The length of these capillaries from the bend to the opposite end was about 4.0 cm. Four of these capillaries were glued together (cold cure denture material) with a 10 cm (0.8 mm, o.d.) centre glass capillary which contained a carbon-fibre. This centre capillary protruded about 3.0 cm beyond the bent end of the outer barrels. The lower end of this assembly was also held together by glue. After 24 hrs of curing at room temperature, the capillary assembly was pulled into a multibarrelled microelectrode using the Narashigi vertical puller. Here, the unit was held at each end by the central capillary tube, heated via a coil midway between the glued ends and gently twisting 180° while being allowed to fall 0.5 - 1 cm by gravity as the glass melted. The heat source was turned off and the glass allowed to cool. It was then pulled in the normal fashion as single electrode. Under a light microscope the carbon-fibre of the central barrel was trimmed and etched to a point as described in (a). The surrounding barrels were filled with appropriate drug solutions.

(c) EA electrodes

Stainless steel uninsulated needles (China National Chemicals Import and Export Corporation) 1.0 - 2.0 cm long, 34 gauge (G).

(d) Tail-Flick-Electromyogram (TF-EMG) electrodes

Stainless steel needles 2-4 cm long, 32 G and insulated except for the tip.

(e) Peripheral nerve recording electrodes

Silver chlorided hook electrodes, 24 G.

(f) Spinal cord lesion electrodes

Concentric bipolar platinum electrodes (David Kopf Instruments, Model NE-100) were used to lesion the DLF or the VLT of the spinal cord.

II. Equipment

The recording microelectrode was secured to a hydraulic microdrive holder (David Kopf Instruments) which, in turn, was mounted to a fine adjustable electrode carrier. The signal picked up by the recording microelectrode was fed into a high impedance preamplifier (WPI, Microprobe system, M-707A), bandpass filtered at 3 KHz, amplified and subsequently displayed on a oscilloscope (Tektronix, M-D11). The output of the oscilloscope was usually fed to an four-channel tape recorder (Teac, A-3440), a window discriminator (Digitimer D130) set for period 2.5 s and range 0.25 s, a D.C driver amplifier of a polygraph (Grass M-79D) and a IBM computer via an A/D interface.

III. Software and Statistical Analysis

Extensive software was written in Turbo Pascal 5.5, which allowed the microcomputer system to be used for data collection, display, analysis and real time control of equipment (see Appendix 1).

Various versions of peristimulus and latency histogram programs were written to allow analysis of the neuronal responses to different treatments. In most cases the histograms consisted of 200 - 300 bins with a bin width of 0.5 ms - 10 s. Data collected by these programs were then stored onto floppy diskettes. Programs provided the stored data to display and calculated the required statistical measures.

Other programs controlled the application of drugs by iontophoresis while simultaneously recording and displaying the neuronal firing rate visually, both as a time versus rate display and numerically. Again, the collected data were stored on floppy diskettes for recall by other programs for further display and analysis. These programs allowed a number of drug application trials to be averaged. Graphs were plotted on a Sun (3/60) computer system. All software programs are listed in Appendix 1.

The data were analyzed statistically using the one way ANOVA test and the computed F ratio was used to determine significant difference among group means (Devore, 1982). The significant difference between pairs of means was then determined using Fisher's Least Significant

Difference multiple range test. A significant difference between compared values was accepted at $p < 0.05$.

IV. Experimental Protocols

Experiment 1. The Effect of EA on the Spinal Cord WDR Dorsal Horn Neurones and the Tail-Flick Reflex Latency (TF-EMG).

Male Wistar rats (200-300g) were anaesthetized with urethane (1.0 g/kg, i.p.) and supplemented during surgery with the addition of halothane. A laminectomy was performed in the T10 - L6 region of the spinal cord. The animals were then rigidly placed in a stereotaxic headholder and spinal frame (Narishige M-11A). A cold-block device, described by Sinclair et al. (1980), was positioned on the spinal cord immediately rostral to the thoraco-lumbar junction. Two stainless steel electrodes were placed bilaterally in the abductor caudal dorsalis muscles at the base of the tail to record the tail-flick reflex latency (TF-EMG; Peets and Pomeranz, 1987). EA electrodes were inserted about 0.4 cm apart bilaterally into the m. tibialis anterior 3.0 - 5.0 mm deep. The sites correspond approximately to the classical human acupuncture points "Zusanli" (st. 36) and "Shangjuxu" (st.38). Rectal temperature was monitored by an electronic thermometer and automatically maintained within physiological limits by a feedback-controlled direct current heating pad. The animals exhibited no signs of discomfort

Methods and Materials

following removal of the halothane but they did respond with reflex movements to a noxious stimulus.

A tail-flick reflex was produced by focusing a halogen projector lamp on a thermocouple placed on the tail 3.0 cm from the tip. The intensity of the lamp was feedback-controlled to maintain a temperature of 50°C. The TF-EMG latency, measured at 2 min intervals, was the time between the onset of the lamp and tail-flick. A latency histogram computer program (first channel) with bin width 0.5 s was used in these experiments (S4, see Appendix 1). The cut-off time was 8.0 s and the average latency of the TF-EMG was between 4 and 5 s in control animals.

In the same animals, a carbon-fibre microelectrode was lowered into the dorsal horn at a depth of 0.3 mm to 0.7 mm below the dorsal surface of the lumbar spinal cord to record single unit activity from WDR neurones. As the electrode was advanced, mechanical stimuli (finger pressure) were applied to the body or the tail. Neurones exhibiting WDR characteristics to mechanical stimuli were selected for further study if they also responded to noxious radiant heat of the tail. Peristimulus histograms (second channel) with bin width 0.5 s were computed from 4 responses to noxious radiant heat applied to the tail at 2 min intervals (S4, see Appendix 1). The spontaneous activity was averaged in the 20 s prior to the radiant heat and the mean evoked activity was averaged in the 10 s following the onset of the heat pulse.

Methods and Materials

Once reproducible TF-EMG latency and dorsal horn neurone control readings had been obtained, EA, consisting of pulses at 10 Hz, 1.0 ms and an intensity which produced a slight toe twitch, was applied for 10 min. These parameters were also used whenever EA was applied in subsequent experiments. The TF-EMG latency and dorsal horn neuronal activity were monitored for 20 min following the cessation of EA. In the initial group of animals the above protocol was presented a second time to ensure the reproducibility of EA effects.

A graph was constructed for each animal by averaging the spontaneous and evoked activity of 4 consecutive responses and plotting the control value at time 0 and subsequent averaged values in the middle of the collection period. Similarly, the TF-EMG latencies were averaged and plotted at the same time points. The graphs illustrated depict the averaged responses from six animals. This method was used in constructing graphs of evoked activity in other experiments as well.

Experiment 2. The Effects of Stimulating Acupuncture vs Non-Acupuncture Points on the Inhibition of the Spinal Cord WDR Dorsal Horn Neurones.

To determine whether EA-induced inhibition of WDR dorsal horn neurones was restricted to acupuncture points, EA electrodes were positioned bilaterally in acupuncture points described above and about 0.4 cm apart in non-acupuncture points in the gastrocnemius muscles. In each animal of this group the effects of EA applied to acupuncture

points were determined on the nociceptor-evoked WDR dorsal horn neuronal activity as described in Experiment 1. Following recovery, the experiment was repeated except that an identical stimulus was bilaterally applied to the non-acupuncture points.

Experiment 3. The Types of Afferent Fibres Activated by EA.

Following Experiment 2, the same animals were used with the EA electrodes left in place in an attempt to determine what type of fibres were activated by EA stimulation. Compound action potentials were recorded through two electrodes placed 20 mm apart on the tibial nerve. A tight ligature was tied around the tibial nerve as high as possible to ensure that the potentials recorded were travelling in the afferent direction. The intensity of the EA stimulus (1.0 ms at 1.0 Hz) was gradually adjusted upward. The stimulation current required to produce threshold responses for AB and A δ fibres was noted and the potentials averaged. The potentials produced at the EA stimulus intensity used previously in the animal were then averaged. Peristimulus histogram L. was used in this experiment (see Appendix 1). The conduction velocities of AB and A δ fibres were determined from the latency differences when the potentials were recorded from the two sets of electrodes on the tibial nerve.

Experiment 4. Tests for a Supraspinal Involvement in EA-Evoked Inhibition of Spinal Cord Nociceptive Transmission.

In another group, once the effects of EA were initially determined on TF-EMG and dorsal horn neuronal activity, the spinal cord was cold-blocked. When reproducible dorsal horn neurone and TF-EMG effects were attained with the spinal cord blocked, the effects of EA were again determined to check for a supraspinal involvement. Additional control experiments were necessary to compensate for the baseline changes produced by the cold-block. This involved conducting experiments as described above except the noxious radiant heat intensity was readjusted after the application of the cold block so that the evoked activity was comparable to the control levels. Then the effects of EA were again tested.

Experiment 5. Ascending and Descending Tracts in EA-Evoked Inhibition of Spinal Cord Nociceptive Transmission.

To determine whether the ascending and descending pathways of the EA supraspinal involvement were located in the DLF or VLT the following sets of experiments were performed. The animals were prepared for recording TF-EMG latency and from neurones in the DRN responding to a noxious peripheral stimulus applied at 2 min intervals. Neurones in the DRN were chosen for this study since the nucleus is located supraspinally and has been implicated in EA (G. Zhang, et al., 1986). A

Methods and Materials

search for neurones took place in the DRN as described in the next section. Neurones were selected for the study if they showed an increase in neuronal firing rate during noxious radiant heat applied to the tail. Neurones used in this study turned out to be of the NCL variety (see next section).

In one group of animals the effects of EA were determined on the TF-EMG latency and DRN neuronal activity. An electrolytic lesion was then made in the left VLT of the spinal cord by passing a DC current (0.9 mA) for 30 seconds through a concentric bipolar electrode. Once the effects of this lesion were determined on the EA-induced changes on the TF-EMG latency and DRN activity, a similar lesion was made in the VLT of the contralateral side. Again the effects of EA on the TF-EMG latency and the DRN neurone activity were evaluated.

In another group of animals, the same procedure was used except that an electrolytic lesion was applied unilaterally and then bilaterally to the DLF of the spinal cord.

Experiment 6. Characteristics of DRN Neurones in Response to Natural Noxious and Non-Noxious Peripheral Stimuli and EA.

Rats used in these experiments were anaesthetized and rigidly fixed in a stereotaxic headholder as described above. After making an incision in the scalp and retracting the skin, a hole (3 mm diameter) was drilled in

Methods and Materials

the midline 7.8 mm caudal to Bregma. Exposed tissue was covered with agar (4% in saline) to prevent drying and to dampen brain tissue motion. A carbon-fibre microelectrode was then directed into the DRN (Bregma - 7.3 to -8.3 mm, -0.5 to + 0.5 mm of the midline, 6.0 to 7.0 mm ventral to cortical surface) according to the atlas of Paxinos and Watson (1982) to record single unit extracellular activity. As the electrode was advanced a silent period occurred which represented penetration of the aqueduct. Neurones recorded 0.1 - 0.3 mm below the silent area were characterized as DRN cells. DRN neurones were found to exhibit a slow regular CL or irregular NCL neuronal discharge pattern as reported by Shima et al. (1986) in the cat. In each case they were characterized according to their responses to natural non-noxious (touch or light pressure on the extremities and the body) and noxious (pinching of the tail) stimuli. NCL neurones which did not response to peripheral stimuli were designated as NCLN neurones. Thus a survey of DRN neurones within the DRN was made by inserting the microelectrode in a grid of 200 μm intervals within the 1 mm² above the DRN. EA was tested on several neurones belonging to each cell category in the DRN.

Experiment 7. Test of a Direct Projection from the DRN to the NPF.

The animals in this group were prepared as described in Experiments 6. Two microelectrodes were directed into the DRN and the

Methods and Materials

NPF to record single unit extracellular activity of DRN and NPF neurones (as described in Experiment 8). Once an NPF and a CL, NCL or NCLN DRN neurone had been isolated, the NPF was electrically stimulated (0.2 ms, 0.3 mA) through the recording electrode. If the DRN neurone projects directly to the NPF it should be antidromically activated. The stimulator was triggered by a spontaneous action potential in the DRN neurone. The stimulus output was delivered to the electrode in the NPF after a preselected delay. The delay was varied to demonstrate collision in an antidromically activated neurone. Computer program L. was used in this experiment.

Units were classified as being antidromically activated if they displayed constant latency at threshold, followed a three pulse stimulation of the NPF at a frequency greater than 100 Hz and demonstrated collision between the NPF stimulus-evoked action potential and a spontaneous action potential. Collision was considered to have occurred if the potential failed when an orthodromic action potential preceded it by less than twice the propagation time between the stimulating and recording electrodes plus the absolute refractory period of the unit at the site of stimulation.

Experiment 8. Characteristics of NPF Neurones Responding to Natural Noxious and Non-Noxious Peripheral Stimuli and EA.

Rats were prepared for these experiments in a similar manner to that described for Experiment 6 except that a hole was drilled in the midline 4.3 mm caudal to Bregma. A microelectrode was then directed to the NPF (Bregma -3.8 - 4.8 mm, lateral 0.5 - 1.5 mm, 5.5 - 6.5 mm ventral to cortical surface) to record single unit extracellular activity. Electrophysiologically, when the NPF was entered at the depth of 5.5 mm-6.0 mm there was generally a high incidence of spontaneous activity. A single cell was isolated and its discharge pattern was noted. If the cell responded to a tail pinch it was monitored for 10 min to verify stability and then it was subjected to further study.

Control data were obtained on the responses of the cell to noxious radiant heat applied to the tail at 2 min interval. EA was then applied for 10 min. The spontaneous and noxious radiant heat-evoked activity were monitored for 20 min following the cessation of EA. This protocol was presented a second time to ensure the reproducibility of EA effects.

Another group of 4 rats was prepared as described in Experiment 7. A multibarrelled microiontophoretic electrode containing glutamate and 0.95 % NaCl was directed into the NPF and a single microelectrode was also directed into the DRN. Single unit extracellular glutamate-driven activity was recorded in a NPF neurone. Spontaneous activity of a DRN neurone was recorded. EA was then administered while simultaneously

recording the activity of these cells. Computer program S41 was used in this experiment (Appendix 1).

Experiment 9. Effects of DRN Stimulation on NPF Neuronal Activity and TF-EMG.

This group of animals was prepared for recording the TF-EMG and single units in the NPF and in the DRN as previously described in Experiments 1, 6 and 7. The DRN electrode was then switched from a recording to a stimulating electrode. Control responses were obtained from a NPF neurone in response to noxious radiant heat applied to the tail at 2 min intervals. After at least 8 responses, the DRN was electrically stimulated for 30 s (square wave pulses, 10 Hz, 0.2 ms, 0.3 mA) through the carbon-fibre microelectrode. This stimulus occurred 10 s following the end of the last control noxious radiant heat pulse. The activity of the NPF neurone and the TF-EMG were monitored until recovery was seen. The above protocol was presented a second time to ensure the reproducibility of the DRN stimulus effects. Computer program S4 was used in this experiment.

Another group of animals was tested for the effect of single pulse DRN stimulation on NPF neurones. Here a five-barrel microelectrode containing two barrels of NaCl and two barrels of glutamate was used to record the activity of an NPF neurone whose background activity was substantially increased by the iontophoretic release of glutamate (45 -

50 nA). The DRN was stimulated with a single pulse delivered to the DRN (0.2 ms, 0.6 mA) at 1.0 Hz. Peristimulus histograms were constructed (bin width 1 ms) comprised of 400 sweeps (S46, see Appendix 1).

Additional experiments involving DRN stimulation are described in Experiment 10.

Experiment 10. Tests for a Serotonergic Involvement in the Ascending Pathway from the DRN to the NPF.

The responses of NPF neurones to DRN stimulation or EA were tested using the following agents: a 5-HT neurotoxin, 5,7-dihydroxytryptamine creatinine sulfate, (5,7-DHT; Sigma); 5-hydroxytryptamine creatinine sulfate (5-HT; Sigma); a 5-HT uptake inhibitor, alaproclate HCl (Astra) and a 5-HT antagonist, cyproheptadine HCl (Merck Sharp and Dohme).

1) Serotonin neurotoxin study

This group of rats was treated with 5,7-DHT to destroy serotonergic neurones in the brain (Bjorklund et al., 1974). Since catecholaminergic neurones can also be affected by 5,7-DHT, animals were pretreated with 25 mg/kg (i.p.) of desimipramine hydrochloride (Sigma) before administration of 5,7-DHT to block uptake into catecholaminergic neurones (Bjorklund et al., 1974). The rats were anaesthetized with halothane and placed in a stereotaxic instrument. Two small holes were drilled in the skull (0.9 mm posterior to Bregma and 2.0 mm lateral to

Methods and Materials

the midline) and a 31-gauge cannula was lowered 3.5 mm from the surface of the cortex into the left lateral ventricle. Then an infusion pump was used to deliver 100 μ g of 5,7-DHT in 200 μ l saline containing 0.1% ascorbic acid (to prevent oxidation) over a 10 min period. The cannula was slowly removed and the hole was plugged with dental cement. The same procedure was repeated at the right lateral ventricle. The animals were monitored for the behavioral signs of hyperaggressiveness, hyperactivity as well as weight loss which occur within 24 hours and is always indicative of serotonergic neuronal destruction in the brain (Bjorklund et al., 1974). Dr. Steven Vincent (Department of Psychiatry, University of British Columbia), using the immunofluorescence method (Appendix 2), kindly examined the PAG region from two of the treated animals after 2 weeks of treatment and compared them to two of the control animals. The animals were allowed 2 weeks for the neurotoxic action to occur before the start of the electrophysiological experiments.

In one set of experiments, 2 - 3 weeks following 5,7-DHT treatment, 6 animals were used for recording the activity of DRN and NPF neurones as described in Experiments 6 and 7, respectively. They were characterized in response to natural noxious and non-noxious stimuli and to EA.

A separate group of six 5,7-DHT treated animals was prepared as in Experiments 8 and 9 to examine the effect of DRN stimulation on the

activity of the NPF neurones and to test DRN neurones for antidromic activation from the NPF.

2) Iontophoretic Studies

The initial study was designed to examine the effect of alaproclate on NPF neurones. The rats were surgically prepared for recording of NPF neurones as previously described. A five-barrel microelectrode consisting of a central recording carbon-fibre electrode and outer barrels filled with glutamate (0.1 M, pH 4.5, Sigma), alaproclate (0.1 M, pH 4.5) and two barrels filled with 0.95 NaCl was directed into the NPF. This electrode was connected to a Dagan 6400 Six Channel Micro-Iontophoresis Current Generator with automatic current balancing capabilities. One NaCl barrel was used for current balancing. A retaining current of 10 nA was applied to all drug barrels. A single NPF neurone was isolated which responded to a noxious pinch of the tail.

The experimental protocol was to excite the neurone under study by ejecting pulses of glutamate for 10 s with 20 s intervals. Alaproclate was then tested on the background activity by ejecting the drug at currents of 15, 30 and 45 nA for periods of 150 s. A current effect was tested by ejecting Na⁺ from the other NaCl barrel using a current of 45 nA.

In other experiments, the five-barrel electrode contained 5-HT (0.05 M, pH 5,) in addition to one barrel each of glutamate, alaproclate

Methods and Materials

and NaCl as described above. In preliminary tests for each neurone a current was established which when applied to the 5-HT barrel reduced the glutamate-evoked activity to about 50% of control. The same protocol was then used in testing a number of cells. The procedure included pulsing glutamate for 10 s with 20 s intervals, applying 5-HT for 260 s in which alaproclate was released concurrently for the final 150 s. Following recovery, alaproclate at the same current was tested alone on the glutamate-evoked responses.

In an attempt to obtain information on the specificity of alaproclate, similar experiments were performed examining the interaction between iontophoretically released GABA and alaproclate. Thus the five-barrel electrodes were the same as described above except that GABA (0.5 M, pH 4.5, Sigma) was substituted for 5-HT. GABA released with a current which decreased the glutamate-evoked responses to about 50% of control was applied for 150 s. After recovery, alaproclate was applied for 290 s with GABA again applied concurrently for the final 150 s.

In another set of experiments the effect of alaproclate was examined on the nociceptor-evoked activity of NPF neurones. In addition, the combined effects of alaproclate and DRN stimulation was tested on the nociceptor-evoked activity of these neurones. The animals were prepared for NPF neuronal recording but, in addition, a stimulating electrode was positioned in the DRN as described in Experiment 8. The

Methods and Materials

five-barrel electrode directed into the NPF contained two barrels of glutamate and one each of alaproclate and NaCl. A cell was located which responded to noxious radiant heat (50°C) applied to the tail. After having established that alaproclate ejected at 30 nA did not alter glutamate-evoked responses in the cell, the drug at this current was tested on the nociceptor-evoked activity which was elicited at 90 s intervals. Here alaproclate was ejected for 240 s beginning immediately after an evoked response. After recovery, the procedure was repeated to ensure reproducibility. Similarly, a 1.0 s DRN stimulation (10 Hz, 0.2 ms, 0.3 mA) was applied 1.0 s before the noxious radiant heat stimulus to the tail. Finally, DRN stimulation was tested during alaproclate release.

Experiments were also performed to test the effects of DRN stimulation or EA on NPF neurones whose background activity was maintained by the constant iontophoretic release of glutamate. The animals were prepared as described immediately above but, in addition, EA electrodes were positioned as described in Experiment 1. Once an NPF neurone which responded to a noxious peripheral stimulus was isolated, glutamate was continuously ejected to provide a rapid stable background for testing the effects of DRN stimulation. The data were collected in 10 s bins and visually displayed on a monitor during the course of an experiment. The effects of DRN stimulation at 1, 2, 4 and 30 s were examined using the constant parameters of 10 Hz, 0.2 ms and 0.3 mA. The

Methods and Materials

effect of alaproclate (30 nA), which by itself was ineffective, was tested on a 4 s stimulus.

If the recording condition was stable and the neurone was "held" sufficiently long, the effect of a 10 min application of EA was determined on the glutamate-driven cell. Following recovery the EA procedure was repeated with concurrent iontophoretic release of alaproclate (30 nA) to the cell.

Another group of animals was prepared much the same as that described above. However the five-barrel electrode contained glutamate, alaproclate, GABA and NaCl. After testing DRN stimulation and the effects of alaproclate on the 4 s stimulus, alaproclate was also tested on the inhibition produced by iontophoretic GABA. Cyproheptadine HCl, a 5-HT antagonist, was then slowly administered intravenously in a dose of 5.0 mg/kg. The procedure was repeated 5 min after the cyproheptadine was administered to determine effects of the drug on DRN stimulation and GABA-induced inhibition.

Finally, in two animals the above procedure was followed except that after testing DRN stimulation and alaproclate on the 4 s DRN stimulus, EA was applied for 10 min. Following recovery, cyproheptadine (5.0 mg/kg, i.v.) was administered and the procedure repeated.

Computer program S41 was used in these experiments.

V. Histology

In the above experiments the locations of the recording and stimulating sites as well as the magnitude of the spinal cord tract lesions were marked by passing a direct negative current through the recording, stimulating ($5\ \mu\text{A}$ for 10 min) or lesion electrodes ($0.9\ \text{mA}$, 30 s). The animal was killed with an overdose of anaesthetic, the chest opened and a needle, attached to a 50 ml syringe filled with normal saline, was introduced into the left ventricle of the heart. The right ventricle was then slit and the animal perfused with saline (30 ml over 2 min) followed by a 10% formaldehyde solution (100 ml over 15 min). The brain and spinal cord were removed and stored in 10% formaldehyde solution for at least three days. The brain and the spinal cord were cut into $10\times 8\times 5\ \text{mm}$ and $3\times 2\times 2\ \text{mm}$ sections, respectively, around the lesion and parallel to the tracks of the electrodes. The neuronal tissue was put on the centre of a holding plate, surrounded with water and frozen at -20°C . The tissue was then cut into $50\ \mu\text{m}$ slices with a freezing microtome (Damon/IEC Division). The slices were mounted onto glass slides using an ethanol-gelatin solution (2 g gelatin in 2 liters of 40% ethanol). The tissue was stained with cresyl violet using conventional procedures and viewed under magnification to determine the recording or stimulating sites as well as the extent of the spinal cord lesions. Micrographs were obtained by taking pictures with a camera attached to a light

Methods and Materials

microscope. Black and white as well as color variable contrast film (400 iso) was used.

RESULTS

EA on Spinal Cord Wide Dynamic Range (WDR) Dorsal Horn Neurones and the Tail-Flick Latency (Experiment 1).

Fig. 1 illustrates the inhibitory effect of EA on a WDR neurone recorded extracellularly and the tail-flick reflex. The control record (Fig. 1A) shows that noxious radiant heat to the tail produces a rapid increase in discharge rate which is arrested shortly after the tail-flick reflex which occurred after 4.5 s in this case. Immediately following EA the heat-evoked discharge is markedly decreased although the duration is prolonged as is the tail-flick latency (6.5 s; Fig. 1B). A gradual recovery occurred within 30 min (Fig. 1C, and D.). EA was found to reproducibly inhibit the nociceptor-driven activity of WDR dorsal horn neurones (Fig. 2A) and to produce a corresponding increase in the tail-flick latency (Fig. 2C). These effects outlasted the period of EA stimulation by several minutes. The spontaneous activity, which was low, was not affected (Fig. 2B). The anatomical locations of the acupuncture points (Zusanli and Shangjuxu) in the anterior tibial muscle are shown in Fig. 2D.

Since a change in the cutaneous temperature is known to alter the tail-flick latency (Berge, 1988; Tjolsen et al., 1988), the cutaneous tail temperature was monitored by taping a thermistor to the root of the tail

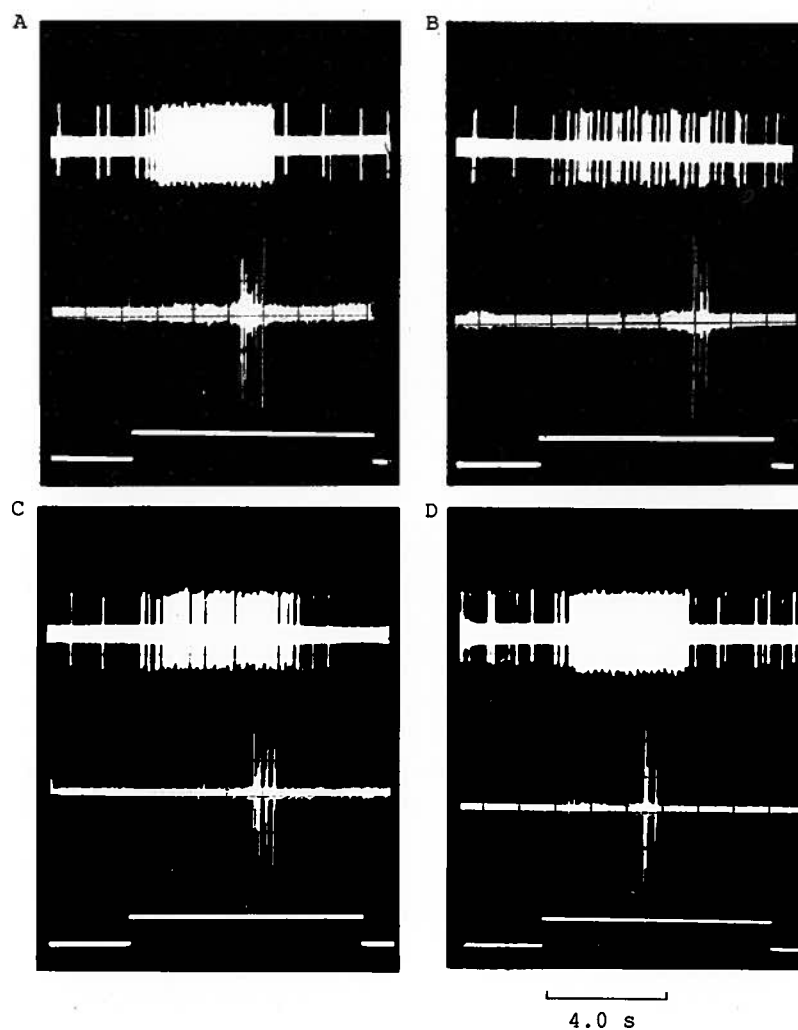


Fig. 1. EA inhibition of a spinal cord WDR neurone (top) and TF-EMG (middle). Each frame illustrates one sweep of the oscilloscope with the lower pulse representing the 8.0 s duration of the heat application, A: Control. B, C and D were collected 10, 20 and 30 min. after the onset of EA, respectively.

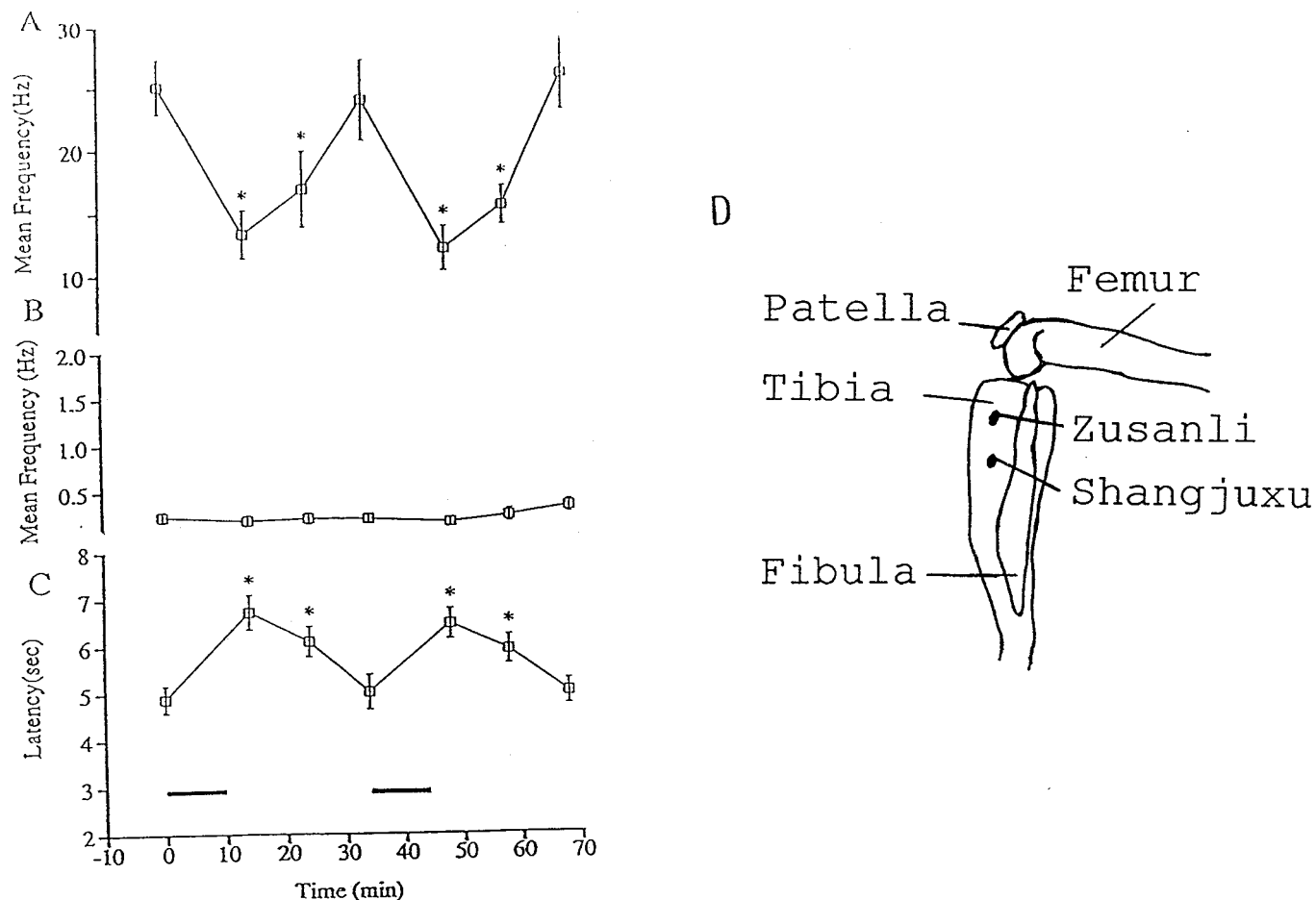


Fig. 2. EA reproducibly inhibits WDR dorsal horn neurones and prolongs the latency of the tail-flick reflex. A: Dorsal horn neurone nociceptor-evoked activity. B: Spontaneous activity of the same cells immediately prior to the noxious stimulus. C: Tail-flick reflex latency. EA was applied during the periods indicated by the bars. Each point in this and subsequent graphs represents the mean \pm SE and was compared to its corresponding control value (* $p < 0.05$, $n = 6$). D: Anatomical locations of the acupuncture points are shown on right limb.

in 4 animals. On no occasion did EA alter the cutaneous or core temperature of the animals.

The Effects of Stimulating Acupuncture vs Non-Acupuncture Points on the Inhibition of the Spinal Cord WDR Dorsal Horn Neurones (Experiment 2).

To determine whether EA-induced inhibition of WDR neurones in the spinal cord was restricted to acupuncture points, a group of 6 rats was treated with EA as described previously and then, following recovery, an identical stimulus was applied bilaterally to non-acupuncture points in the gastrocnemius muscles in the same animal. EA stimulation of non-acupuncture points failed to alter the evoked WDR activity suggesting that the effects of EA on WDR neurones are specific for acupuncture points (Fig. 3).

The above experiments show that EA applied bilaterally for 10 min in the rat produces a reproducible long-lasting inhibition of the spinal cord nociceptive transmission. This is reflected by an inhibition of WDR dorsal horn neurones. Thus the evoked activity in these dorsal horn neurones following EA was inhibited even though the receptive field was exposed to the noxious heat for a longer period of time. The same stimulation applied to non-acupuncture points failed to alter nociceptive responses.

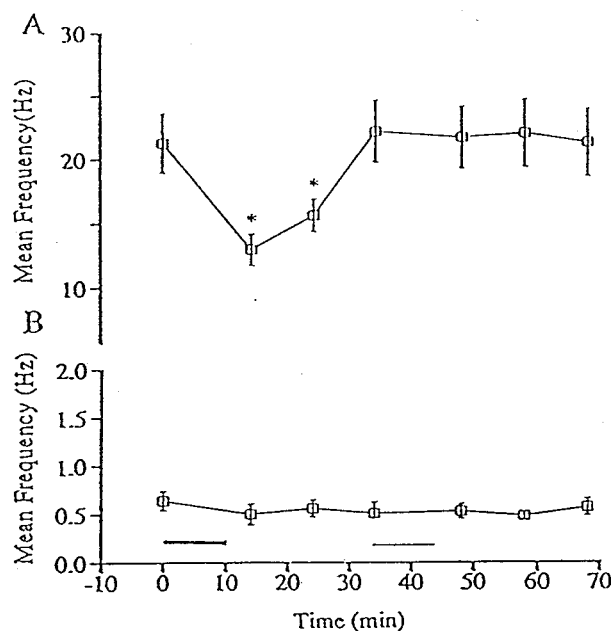


Fig. 3. The inhibition of WDR neurones is specific to acupuncture points. A: WDR nociceptor-evoked activity. B: Spontaneous activity in the same cells immediately prior to the noxious stimulus. The heavy bar represents EA stimulation on "Zusanli" and "Shangjuxu" points in the anterior tibial muscle. The thin bar denotes the same stimulation parameters applied to non-acupuncture points in the gastrocnemius muscle. Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 6$).

Types of Afferent Fibres Activated by EA (Experiment 3).

Following Experiment 2, the same animals with the EA needles still in place were used in an attempt to determine what type of fibres were activated by EA stimulation. The compound action potentials on the tibial nerve elicited by stimulation through the EA electrodes at the active sites are illustrated in Fig. 4. At intensities which effectively inhibited dorsal horn neurones, potentials representing in fibres smaller than A β were not noted (Fig. 4B). A much higher stimulation intensity was required to produce an A δ potential (Fig. 4C). In the six animals tested, the mean stimulation intensities (\pm SE) which elicited threshold A β responses, which were effective in producing EA and which elicited threshold A δ potentials were 0.88 (0.24), 0.92 (0.02) and 4.20 (0.10) mA, respectively. The mean conduction velocities (\pm SE) of the fibres producing the A β and A δ potentials in these experiments were 41.7 (2.0) m/s and 22.1 (1.7) m/s, respectively. Table 1 lists the latencies of compound action potentials recorded from the two sets of electrodes placed 20 mm apart on the tibial nerve. Table 1 also includes the required currents to produce A β and A δ potentials in each experiment. The set "a" electrodes were placed on the tibial nerve closer to the EA stimulation sites than set "b" electrodes. Accordingly, the latency recorded at set "a" electrodes was always smaller than the latency recorded at set "b" electrodes.

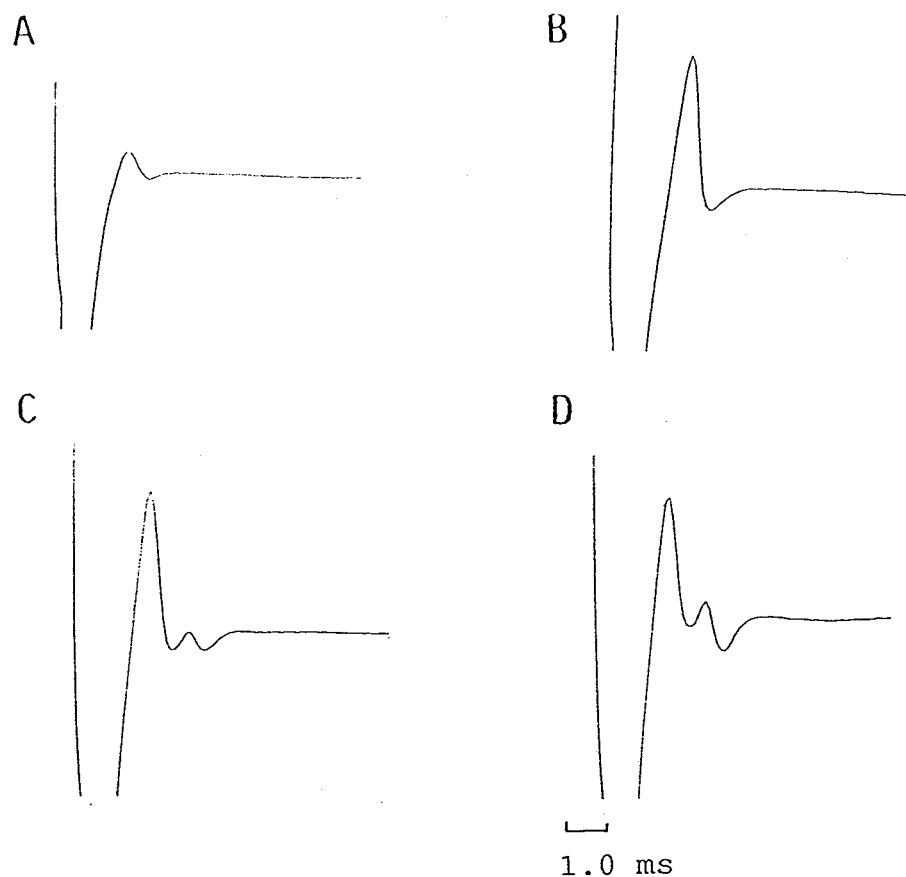


Fig. 4. Compound action potentials recorded from the tibial nerve upon EA stimulation. Each trace is an average of 40 sweeps. A: A response just above threshold at a stimulus intensity of 0.88 mA. B: The A β potential recorded at a intensity which was effective in producing EA (0.92 mA). C: The appearance of an A δ potential at a stimulus intensity of 4.2 mA. D: As in C but at a stimulus intensity of 4.6 mA. The square wave pulse indicates the EA pulse duration of 1.0 ms (illustration of potentials reported in file 3-26, set a in Table 1.)

Table 1. Latencies and Types of Compound Action Potentials
Recorded from Two Sets of Electrodes at Various Current Intensities.

File: 3-18		Latency (ms)	Current (mA)	Potential	File: 3-19		Latency (ms)	Current (mA)	Potential
Set a	3-18-1	2.60	0.88	A β _T	Set a	3-19-1	2.88	0.90	A β _T
	3-18-2		0.93	A β		3-19-2		0.92	A β
	3-18-3		3.60	A δ _T		3-19-3		3.80	A δ _T
	3-18-4	3.78	3.90	A δ		3-19-4	3.48	3.96	A δ
Set b	3-18-5	3.08		A β _T	Set b	3-19-5	3.38		A β _T
	3-18-6			A β		3-19-6			A β
	3-18-7			A δ _T		3-19-7			A δ _T
	3-18-8	4.70		A δ		3-19-8	4.60		A δ
A β = 20 mm/(3.08 ms - 2.60 ms) = 41.7 m/s A δ = 20 mm/(4.70 ms - 3.78 ms) = 21.7 m/s					A β = 20 mm/(3.38 ms - 2.88 ms) = 36.4 m/s A δ = 20 mm/(4.60 ms - 3.48 ms) = 17.9 m/s				
File: 3-25		Latency (ms)	Current (mA)	Potential	File: 3-26		Latency (ms)	Current (mA)	Potential
Set a	3-25-1	2.68	0.88	A β _T	Set a	3-26-1	2.78	0.96	A β _T
	3-25-2		0.92	A β		3-26-2		1.00	A β
	3-25-3		4.20	A δ _T		3-26-3		3.80	A δ _T
	3-25-4	3.60	4.60	A δ		3-26-4	3.78	4.20	A δ
Set b	3-25-5	3.20		A β _T	Set b	3-26-5	3.20		A β _T
	3-25-6			A β		3-26-6			A β
	3-25-7			A δ _T		3-26-7			A δ _T
	3-25-8	4.40		A δ		3-26-8	4.92		A δ
A β = 20 mm/(3.20 ms - 2.68 ms) = 38.5 m/s A δ = 20 mm/(4.40 ms - 3.60 ms) = 25.0 m/s					A β = 20 mm/(3.20 ms - 2.78 ms) = 47.6 m/s A δ = 20 mm/(4.92 ms - 3.78 ms) = 17.5 m/s				
File: 3-27		Latency (ms)	Current (mA)	Potential	File: 4-8		Latency (ms)	Current (mA)	Potential
Set a	3-27-1	2.08	0.75	A β _T	Set a	4-8-1	2.08	0.89	A β _T
	3-27-2		0.84	A β		4-8-2		0.94	A β
	3-27-3		3.80	A δ _T		4-8-3		4.20	A δ _T
	3-27-4	2.78	4.00	A δ		4-8-4	2.68	4.40	A δ
Set b	3-27-5	2.50		A β _T	Set b	4-8-5	2.60		A β _T
	3-27-6			A β		4-8-6			A β
	3-27-7			A δ _T		4-8-7			A δ _T
	3-27-8	3.70		A δ		4-8-8	3.38		A δ
A β = 20 mm/(2.50 ms - 2.08 ms) = 41.6 m/s A δ = 20 mm/(3.70 ms - 2.78 ms) = 21.7 m/s					A β = 20 mm/(2.60 ms - 2.08 ms) = 38.5 m/s A δ = 20 mm/(3.38 ms - 2.68 ms) = 28.6 m/s				

A β _T: A β potential recorded at a threshold current.

A β : A β potential recorded at intensity used to evoke EA effect.

A δ _T: A δ potential recorded at a threshold current.

A δ : A δ potential recorded at a suprathreshold current.

A Supraspinal Involvement in EA-Evoked Inhibition of the Spinal Cord Nociceptive Transmission (Experiment 4).

To determine whether there is a supraspinal involvement in the EA-induced attenuation of nociceptor-evoked responses, the effects of EA were determined during a cold-block of the spinal cord. Cumulative histograms of the responses of a spinal cord WDR neurone to EA before and after a cold-block of the spinal cord are shown in Fig. 5. Note that EA reduced the nociceptor-evoked activity of the cell (Fig. 5A-D) as was previously shown in Fig. 2. The application of a cold-block resulted in a marked increase in the spontaneous and evoked activity (Fig. 5E). EA applied during the cold block failed to alter these responses (Fig. 5F). The grouped data for six animals treated in this manner are plotted in Fig. 6. Note that there was a reciprocal relationship between the activity of the dorsal horn neurones and the tail-flick reflex latency (Fig. 6C).

Since the failure of EA to have an effect in the presence of the cold-block could have been due to the baseline changes produced by the cold-block, additional control experiments were performed as depicted in Fig. 7. Here the protocol was the same as that above except that once the effects on the dorsal horn neurone and tail-flick latency were determined in the presence of a cold-block, the temperature of the noxious radiant heat applied to the tail was reduced 2 - 3°C so that the

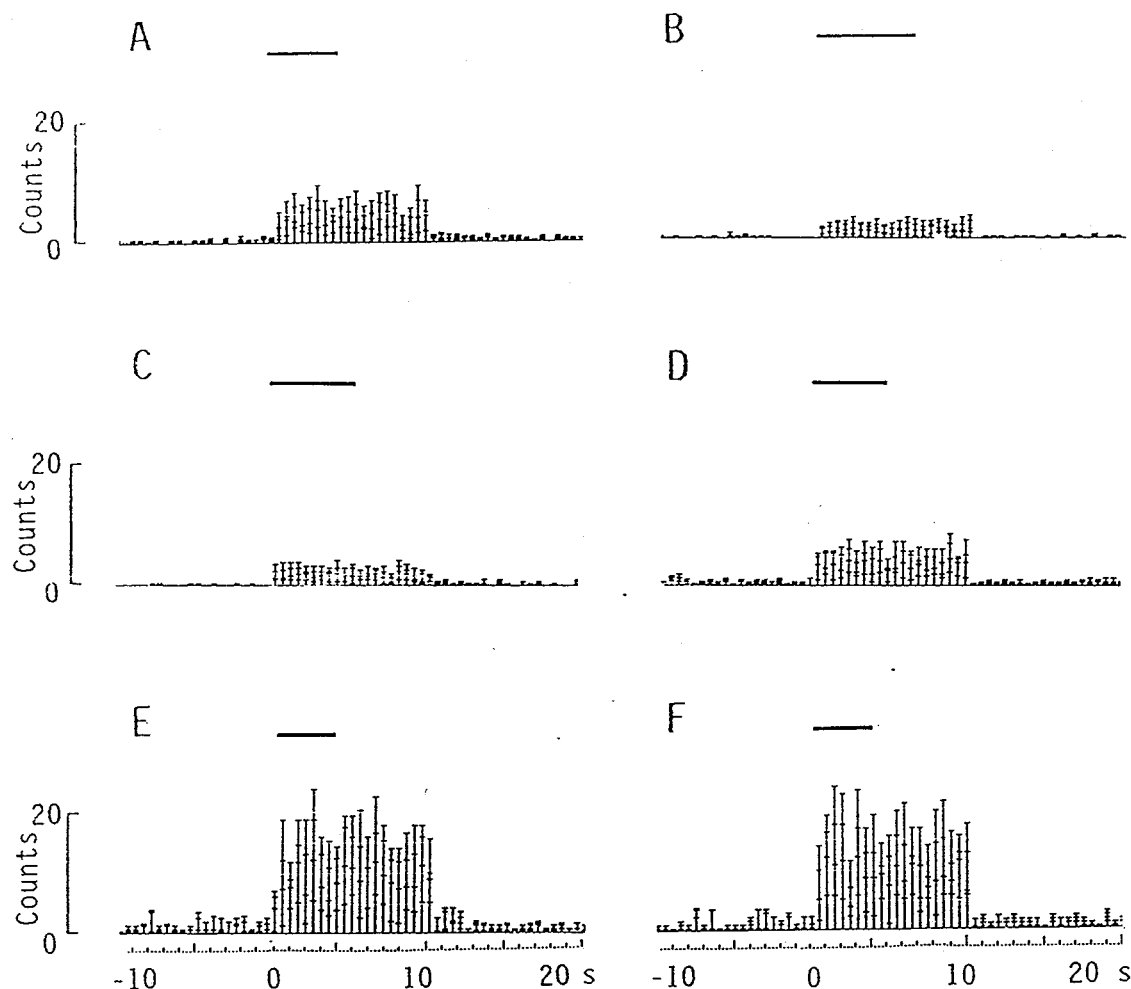


Fig. 5. The effects of EA and cold-block on the responses of a spinal cord WDR neurone to noxious radiant heat applied to the tail. Each histogram is comprised of 4 sweeps with each bin notch indicating the cumulative counts per sweep (bin width 0.5 s). The bars above the histograms begin with the onset of the heat pulse (50°C) and end with the tail-flick reflex. A: Control. B-D: 10, 20 and 30 min after initiating EA. E: During cold-block of the spinal cord. F: 10 min after initiating EA and during cold-block of the spinal cord.

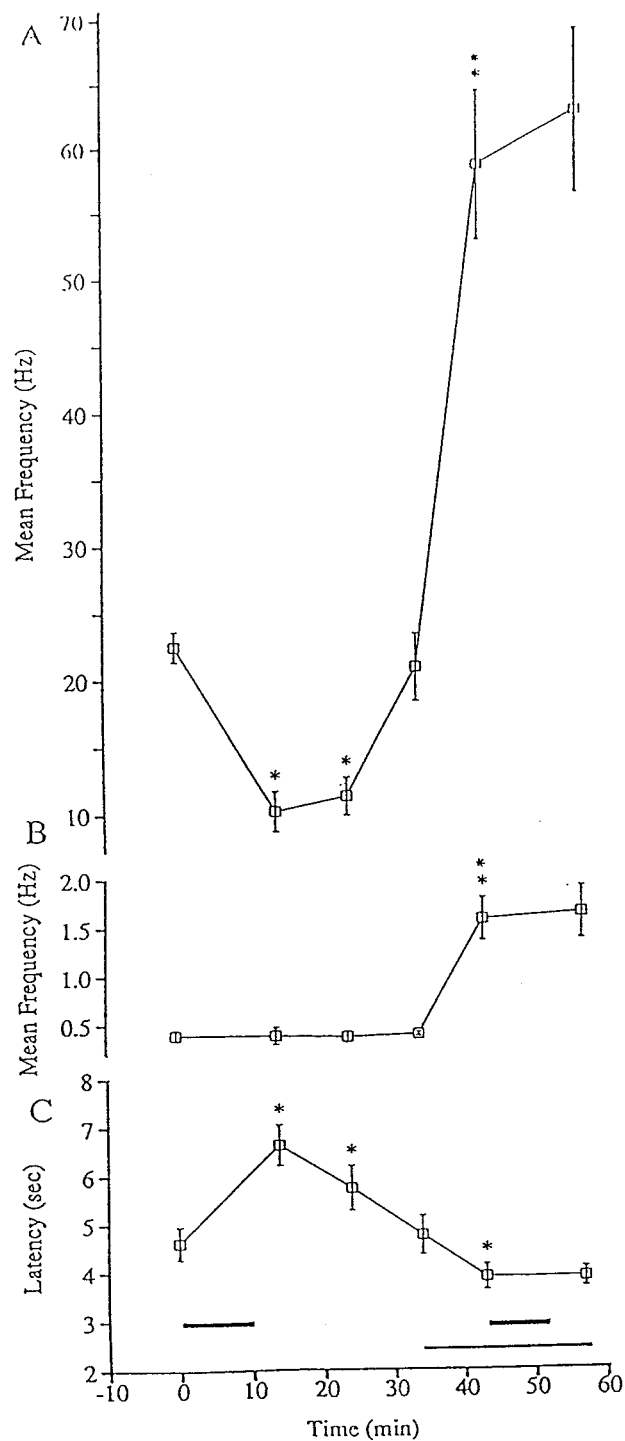


Fig. 6. Cold-blocking the spinal cord eliminates the effects of EA. The arrangement is the same as in Fig. 2. The period of cold-block is indicated by the thin bar above the time scale. Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, ** $p < 0.001$, $n = 6$).

Results

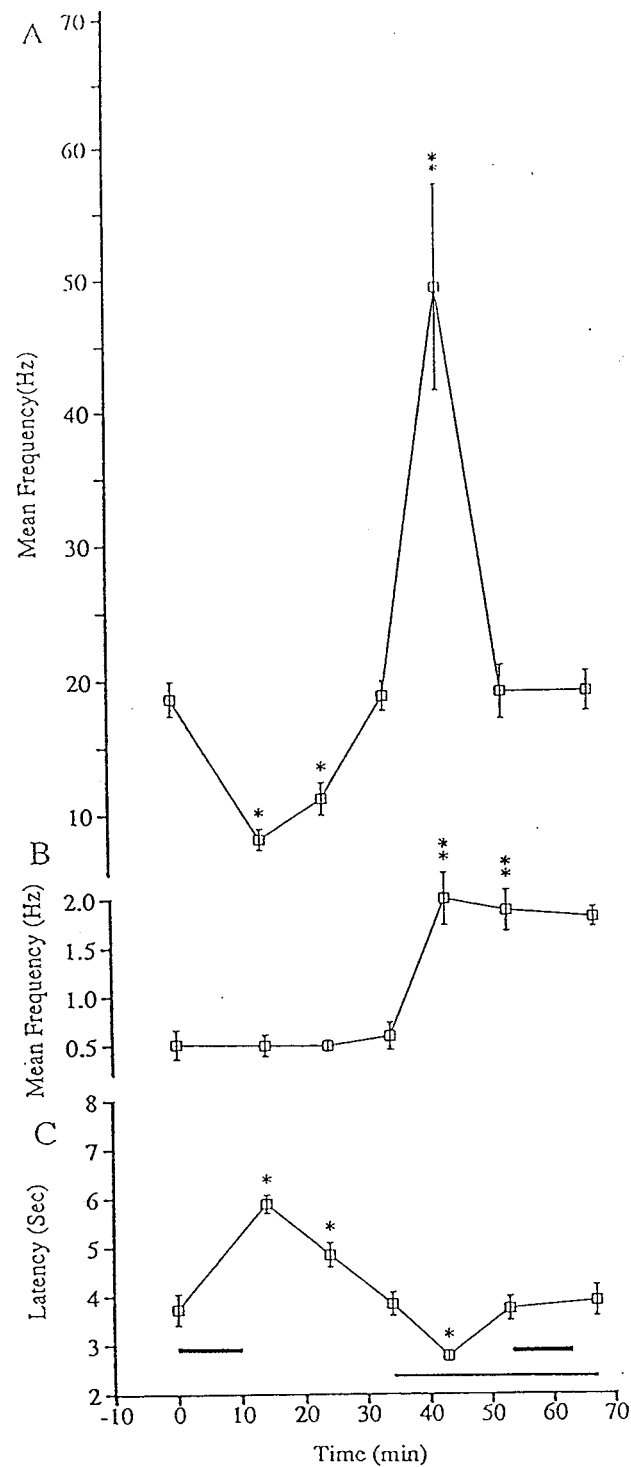


Fig. 7. Cold-blocking the spinal cord eliminates the effects of EA even after baseline adjustment. As in Fig. 6 except that the intensity of the heat lamp is reduced after determining the effects of cold-block in order to readjust the baseline to equal that in the absence of cold-block (* $p < 0.05$, ** $p < 0.001$, $n = 6$).

responses were comparable to control values in the absence of the cold-block. However EA applied at this point also failed to alter the dorsal horn neuronal activity or the tail-flick latency.

Thus, we found that the spinal cord effects of EA were eliminated if conduction in the spinal cord was blocked rostral to the recording site suggesting the involvement of a supraspinal loop in the mediation of the spinal cord inhibition.

Ascending and Descending Tracts in EA-Evoked Inhibition of Spinal Cord Nociceptive Transmission (Experiment 5).

The effects of unilateral and bilateral lesions of the spinal cord VLT on EA-induced changes in dorsal raphe neuronal activity and the tail-flick latency are illustrated in Fig. 8. EA reproducibly increased dorsal raphe NCL neurones nociceptor-evoked activity (Fig. 8A), the spontaneous activity of the same cells immediately prior to the noxious stimulus (Fig. 8B) and the tail-flick latency (Fig. 8C). A unilateral lesion of the VLT failed to alter the above effects of EA. A bilateral lesion, however, blocked all of these effects. Also, nociceptive responses of these neurones were eliminated after bilateral lesions of the VLT.

The results of using the same protocol but making unilateral and bilateral lesions of the DLF rather than the VLT are depicted in Fig. 9. Unilateral lesions failed to alter either the dorsal raphe neuronal facilitation or the increase in tail-flick latency following EA.

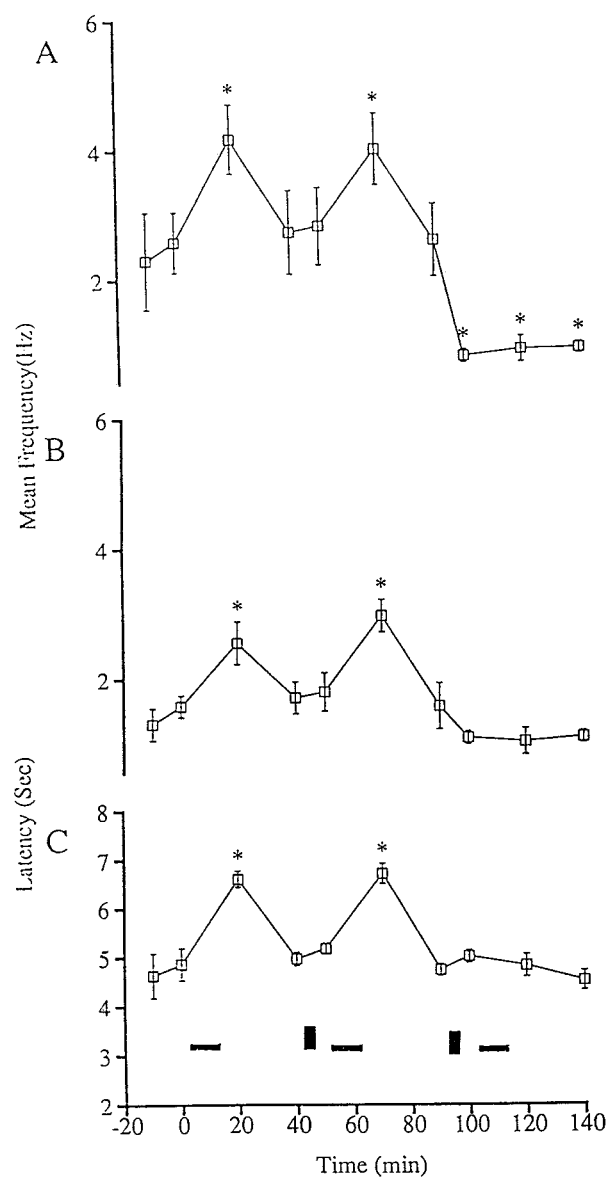


Fig. 8. Bilateral lesions of the VLT block the effects of EA on dorsal raphe neuronal nociceptor-evoked activity and the tail-flick reflex. A: Nociceptor-evoked activity of DRN cells. B: Spontaneous activity of the same cells. C: The tail-flick reflex. EA was applied during times indicated by the horizontal bars. The first and second vertical bars indicate unilateral and bilateral lesions of the VLT, respectively. Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 6$).

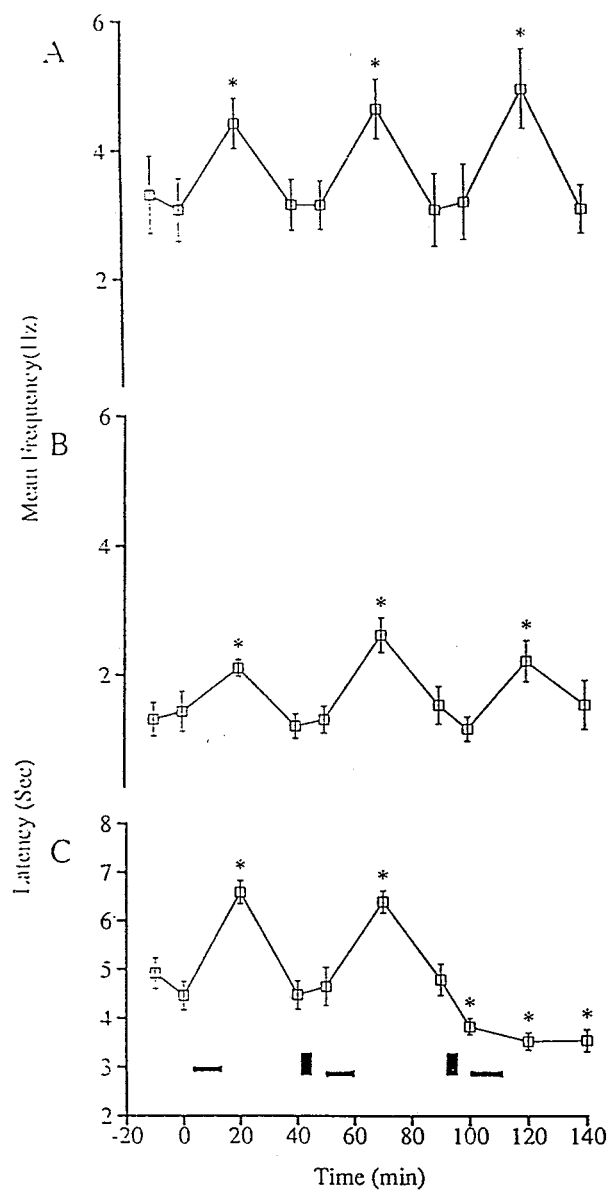


Fig. 9. Bilateral lesions of the DLF block the effect of EA on the tail-flick reflex (C) but do not alter the enhancement by EA on nociceptor-evoked activity in dorsal raphe neurones (A). The arrangements is the same as in Fig. 8 except that the lesions were made in the DLF (* $p < 0.05$, $n = 6$).

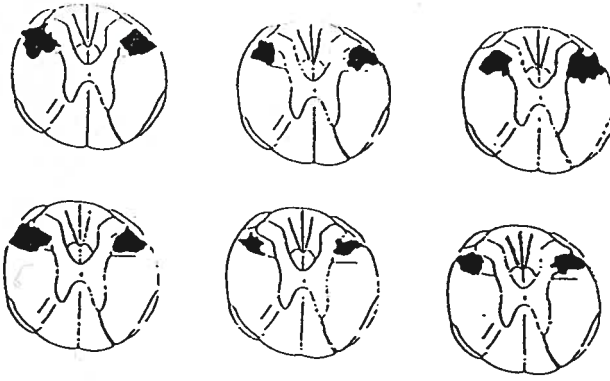
Bilateral lesions did not alter the effects of EA on nociceptor-evoked and spontaneous activity of dorsal raphe neurones (Fig. 9A and B) but resulted in a decrease in the tail-flick reflex latency (Fig. 9C). EA did not further influence the tail-flick latency.

The size of the lesions in individual animals are sketched in Fig. 10A and B. Fig. 10C shows a micrograph of a lesion in the spinal cord VLT.

The Characteristics of the DRN Neurones in Response to Natural Peripheral Stimuli and EA (Experiment 6).

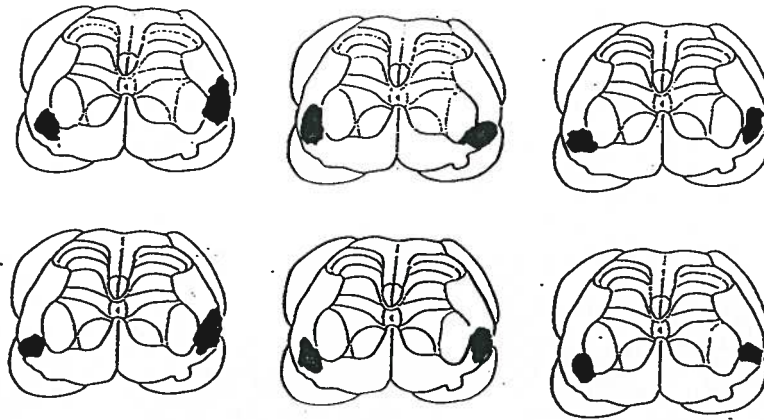
Neurones encountered in the DRN tended to have a slow firing rate and either a very regular or irregular firing pattern. These were initially designated as CL or NCL neurones. It soon became apparent that CL neurones never responded to natural peripheral stimuli but some NCL neurones could be activated (Fig. 11). The neurones were then classified as CL, NCL if they responded to peripheral stimuli and NCLN if they failed to respond. A total of 118 neurones were examined in 24 animals. These were subdivided into 35 CL, 32 NCL and 51 NCLN neurones. The distribution of the three different types of neurones in the DRN are shown in the three dimensional graphs (Fig. 12). They appear to be randomly distributed. The mean (\pm SE) spontaneous discharge rates of CL, NCL and NCLN neurones were found to be 1.7 (0.02), 1.4 (0.06) and 1.6 (0.05) Hz, respectively.

A



Results

B



C



Fig. 10. The extent of lesions noted in individual animals are shown for the DLF (A) and VLT (B). C: An example of lesions in the spinal cord VLT.

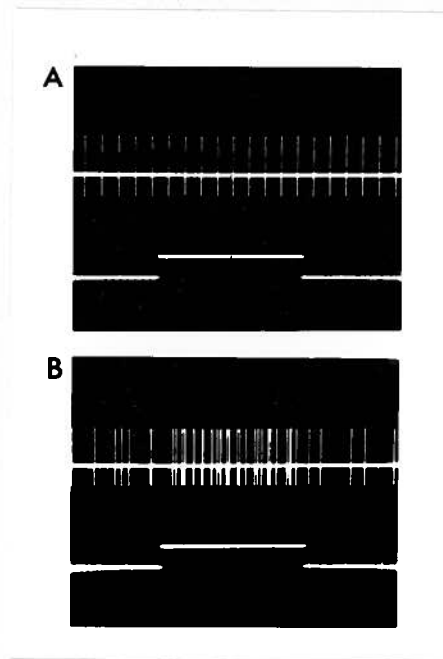


Fig. 11. Oscilloscope traces illustrating the effect of noxious heat on a clock-like (A) and non-clock-like (B) neurone. The pulse in the lower trace of each pair represents the 8.0 s duration of the heat pulse (50°C) applied to the tail. The tail-flick reflex latency occurred at 4.5 s in A and 4.7 s in B.

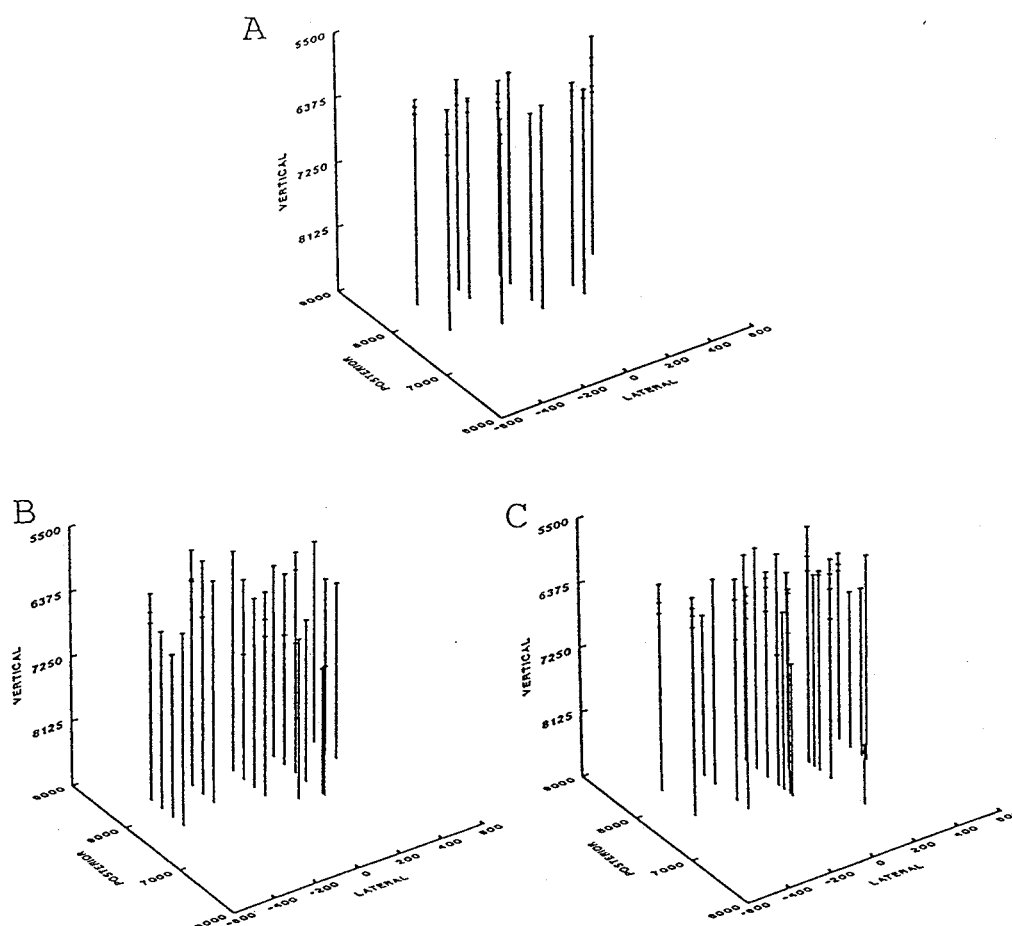


Fig. 12. Three dimensional representation of DRN CL (A), NCL (B) and NCLN neurones (C). The axes labelled vertical, posterior, and lateral show the three stereotaxic planes of the brain in μm . The posterior axis indicates distance caudal to Bregma, 0 on the lateral axis represents the midline, and the vertical axis indicates the depth from the surface of the cortex.

The noxious radiant heat applied to the tail produced a relatively small but consistent increase in the discharge rate of NCL neurones. Fig. 13 is an example of one such cell. Comparing the evoked activity to spontaneous activity in Fig. 14, one can see that the noxious stimulus almost doubled the discharge rate. This increase began shortly after initiating the noxious stimulus, was maintained during the stimulus and continued for several seconds after completion of the stimulus. This point is not obvious in Fig. 13. Here a tail-flick, although not monitored, would have occurred prior to the end of the heat pulse thus removing the receptive field from the heat source. CL neurones were not activated by noxious radiant heat to the tail as shown in Fig. 15. NCLN neurones, by definition, also did not respond.

The majority of the 15 NCL neurones tested were facilitated by EA with an approximate increase of 1 Hz. Only three cells showed no response to EA. None of the eleven CL and only 2 of the NCLN neurones tested responded to EA (Table 2). One NCLN was facilitated and one was inhibited of the 13 tested. The effects of EA on NCL and CL DRN neurones are illustrated in Figs. 14 and 15. Note the facilitation of NCL neurones lasted 10 - 20 min.

Test of a Direct Projection from the DRN to the NPF (Experiment 7).

Twenty-one NCL neurones of the DRN were tested for antidromic activation upon NPF stimulation. They all were activated and displayed

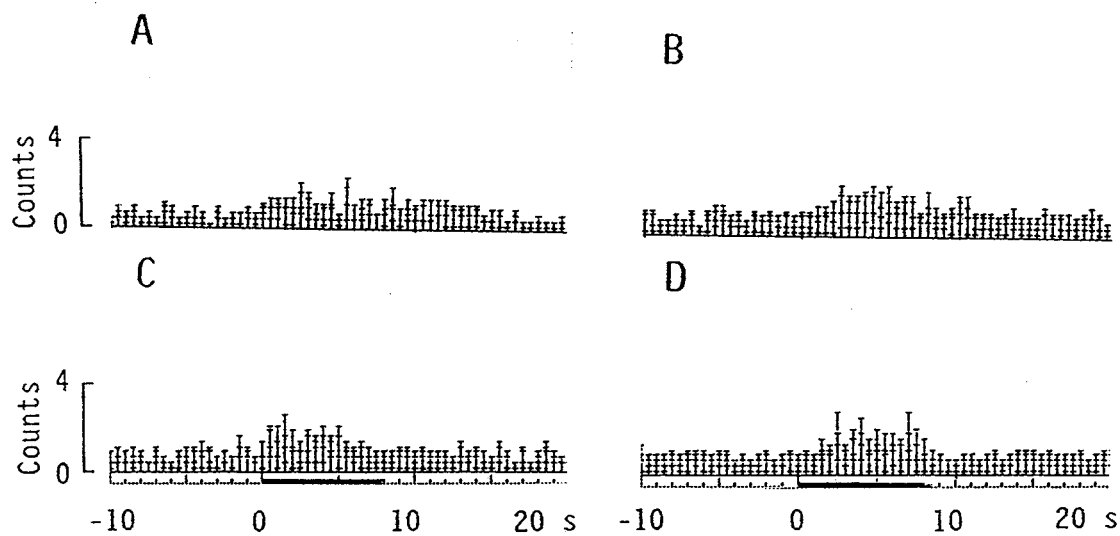


Fig. 13. EA enhances the nociceptor-evoked activity of a DRN neurone. Noxious radiant heat (500°C) indicated by the bar on the time scale was applied to the tail. Each histogram is comprised of 4 sweeps with each bin notch indicating the cumulative counts per sweep (bin width 0.5 s). A: Control. B-D: 10, 20 and 30 min after initiating EA.

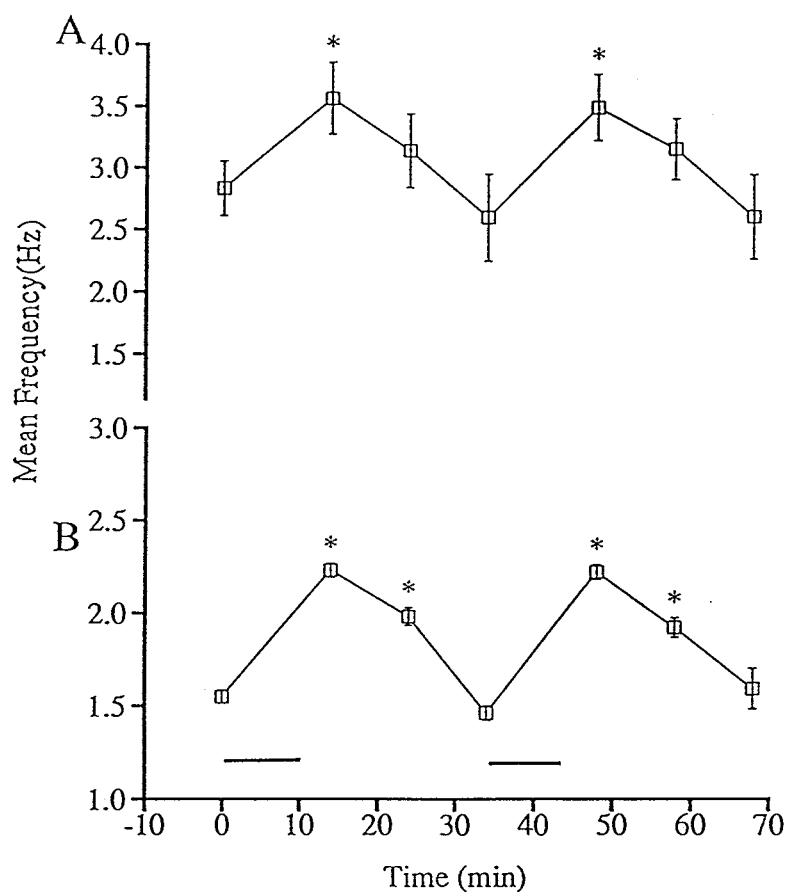


Fig. 14. A: The effect of EA (bars) on the noxious radiant heat-evoked activity in non-clock-like DRN neurones. B: Spontaneous activity of the same neurones immediately before the applications of noxious radiant heat. Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 15$).

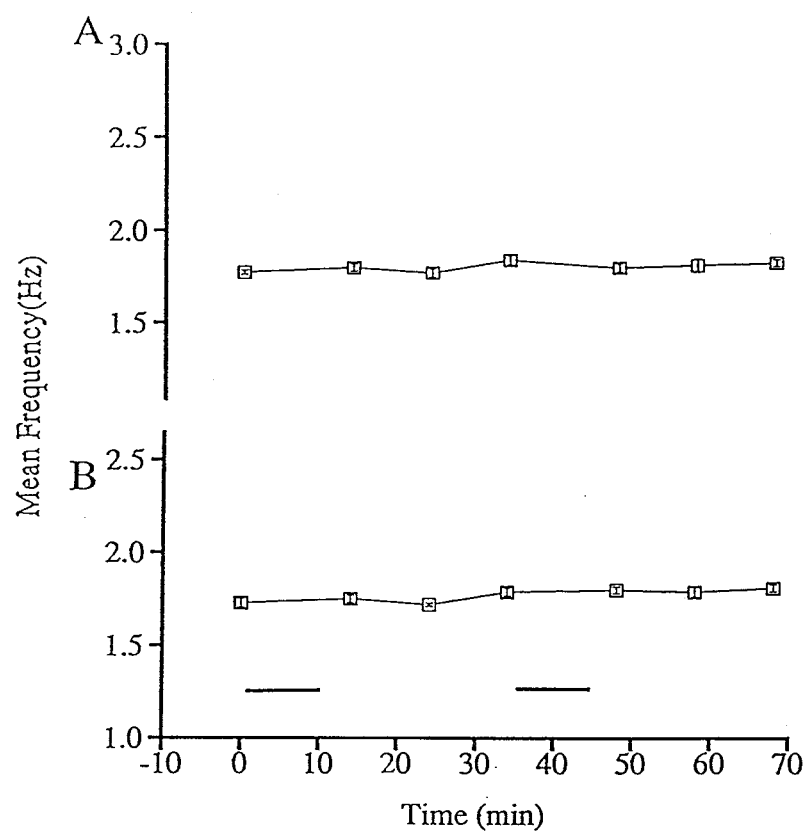


Fig. 15. A: The effect of EA (bars) on the noxious radiant heat-evoked activity in clock-like DRN neurones. B: Spontaneous activity of the same neurones immediately before the applications of noxious radiant heat. Each point representing the mean \pm SE (n=11).

Table 2. Characteristics of DRN Neurones*

	Clock-Like-Neurones (CL)	Non-Clock-Like-Neurone (NCL)	Non-Clock-Like-Neurone Not Responding to Noxious or Non-noxious Stimuli (NCLN)
Facilitated by EA	0/11	12/15	1/13
Inhibited by EA	0/11	0/15	1/13
No Response to EA	11/11	3/15	11/13
NPF Antidromic Activation	0/12	21/21	0/16

* Number of neurones responding / number of neurones tested.

a constant but wide range latency (0.6 ms to 9.0 ms), followed a three pulse train greater than 100 Hz and demonstrated collision between spontaneous and evoked action potentials (Fig. 16, Table 2). The conduction distance between the DRN and the NPF was calculated to be 3.5 mm and the conduction velocity of these neurones was found to range from 0.40 - 5.8 m/s with a mean of 1.85 m/s. None of the CL or NCLN neurones could be antidromically driven (Table 2).

Thus it seems that only the NCL neurones of the DRN play a role in EA. These neurones are activated by peripheral natural stimuli, facilitated by EA and project to the NPF.

The Characteristics of NPF Neurones in Response to Natural Peripheral Stimuli and EA (Experiment 8).

When the electrode entered the NPF at a depth of 6 mm below the surface of the cortex there was generally a high incidence of spontaneous activity. The discharge pattern could be categorized into three types: slow firing at a constant rate which varied from 0.8 to 8 Hz, bursting neurones (2 - 3 spike bursts every 1 - 2 s) with an overall discharge rate of 3-10 Hz and, finally, fast firing neurones. These neurones were not studied in detail but they seemed to be essentially the same as described by Andersen and Dafny (1983a). Most of the slow type cells as well as a small percentage of the bursting cells could be activated by a noxious stimulus to the tail. The fast firing

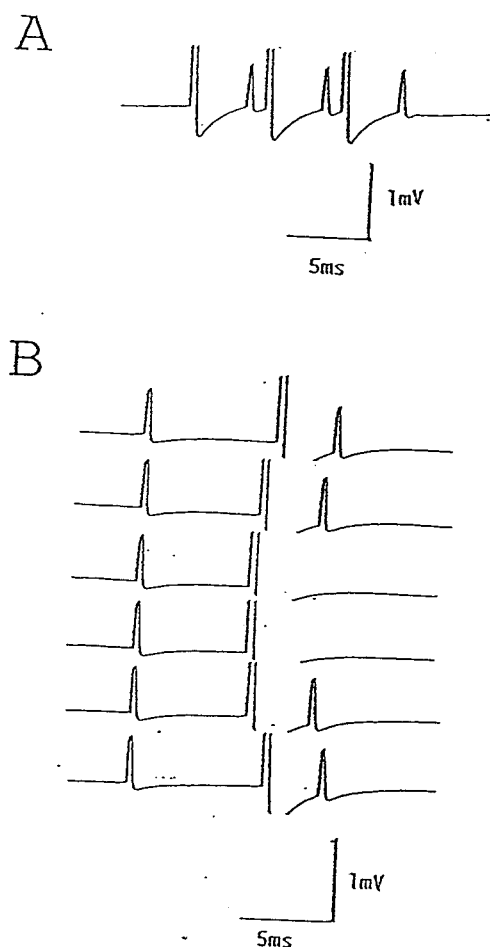


Fig. 16. An example of a non-clock-like DRN neurone exhibiting high frequency following and collision. A: The neurone follows a three-pulse train applied in the NPF at 200 Hz. The large deflections are stimulus artifacts. B: An example of collision in the same neurone. The stimulator was triggered by spontaneous action potentials (left) in a DRN neurone. The stimulus output (middle large artifact) was delivered to the electrode in the NPF after a preselected delay. Varying the delay demonstrates collision in third and fourth traces.

variety, however, never responded to a noxious peripheral stimulus. A few slow firing neurones in the NPF responded to non-noxious mechanical stimuli but the majority did not. The neurones in the NPF were selected for further study only if they responded to noxious radiant heat applied to the tail.

An example of a slow active NPF neurone responding to noxious radiant heat applied to the tail is shown in Fig. 17. The spontaneous activity of this neurone was 1.0 Hz. The increased discharge started shortly after the onset of the heat pulse, continued throughout the stimulation and for several seconds after the completion of the stimulus. The activation of these neurones by noxious radiant heat to the tail was much greater than that which occurred in DRN neurones (compare the spontaneous to evoked activity in Fig. 19 to that in Fig. 14).

Fig. 18 shows an example of the EA inhibition of the nociceptor-evoked activity of a NPF neurone. Each histogram is comprised of 4 sweeps with each bin notch indicating the cumulative counts per sweep. EA was found to inhibit this evoked activity in NPF neurones (Fig. 19) with a duration of action similar to that for the facilitation of NCL DRN neurones (Fig. 14). The spontaneous activity of the same NPF neurones was also inhibited by EA (Fig. 19B).

Fig. 20 shows that EA produces a reciprocal increase in activity of a NCL DRN neurone and a decrease in the firing rate of a glutamate-driven NPF neurone. The average latency for the onset of these effects

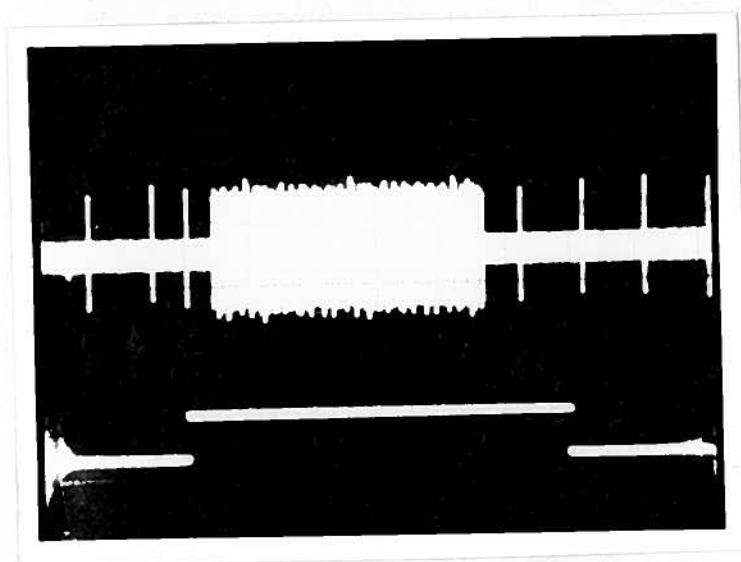


Fig. 17. Oscilloscope traces illustrating the spontaneous and nociceptor-evoked activity of an NPF neurone. Lower pulse representing the 8.0 s duration of the heat (50°C) applied to the tail. The tail-flick reflex occurred at 5.2 s.

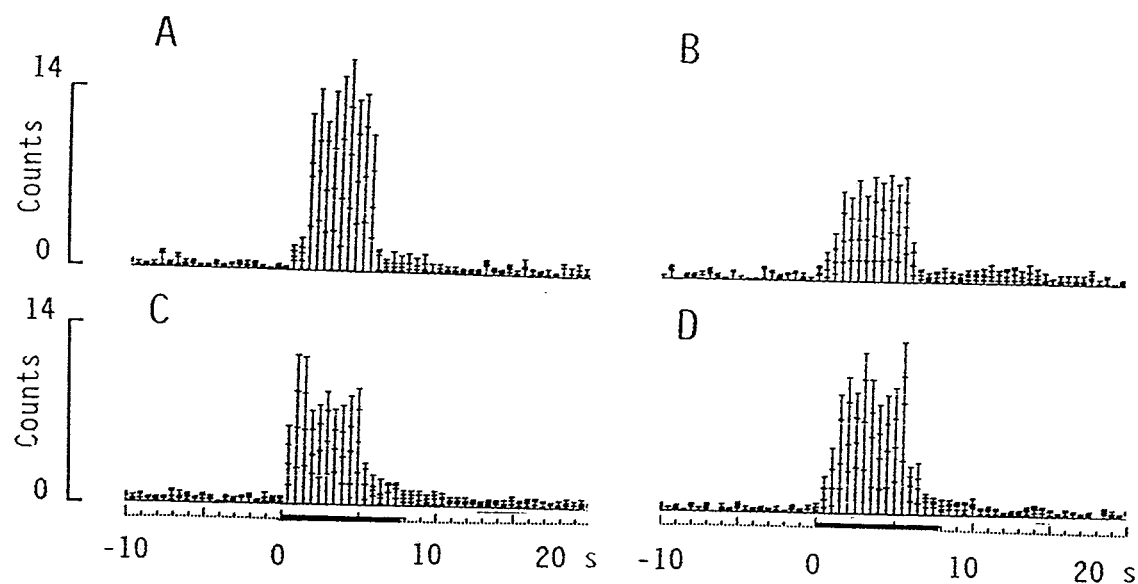


Fig. 18. EA inhibits the nociceptor-evoked activity of a NPF neurone. Noxious radiant heat (50°C) indicated by the bar on the time scale was applied to the tail. Each histogram is comprised of 4 sweeps with each bin notch indicating the cumulative counts per sweep (bin width 0.5 s). A: Control. B-D: 10, 20 and 30 min after initiating EA.

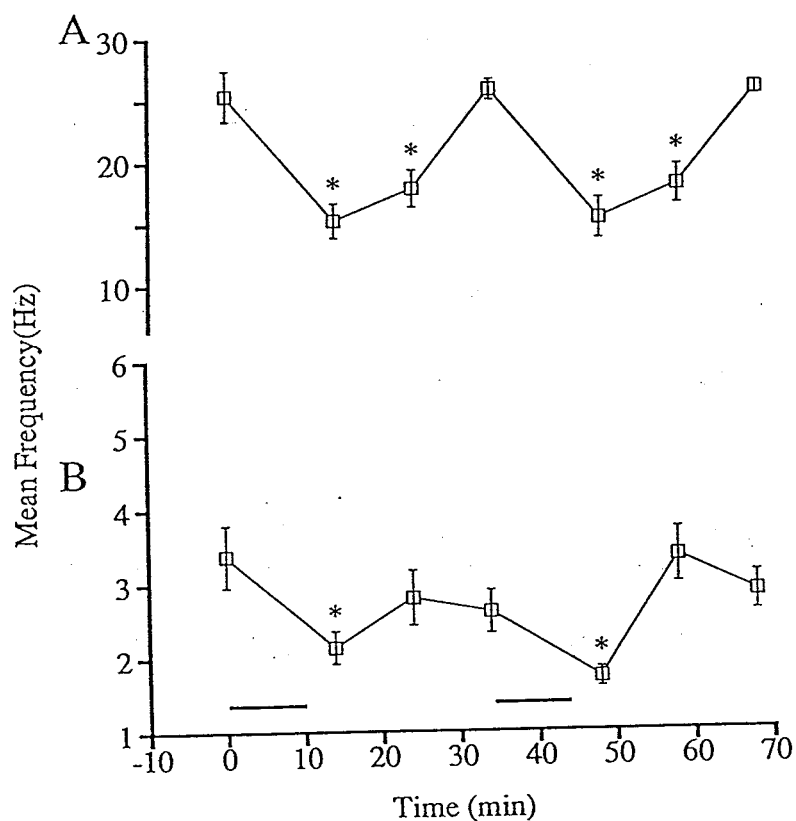


Fig. 19. A: The effects of EA (bars) on the noxious radiant heat-evoked activity in NPF neurones. B: Spontaneous activity of the same neurones immediately before the application of noxious radiant heat. Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 19$).

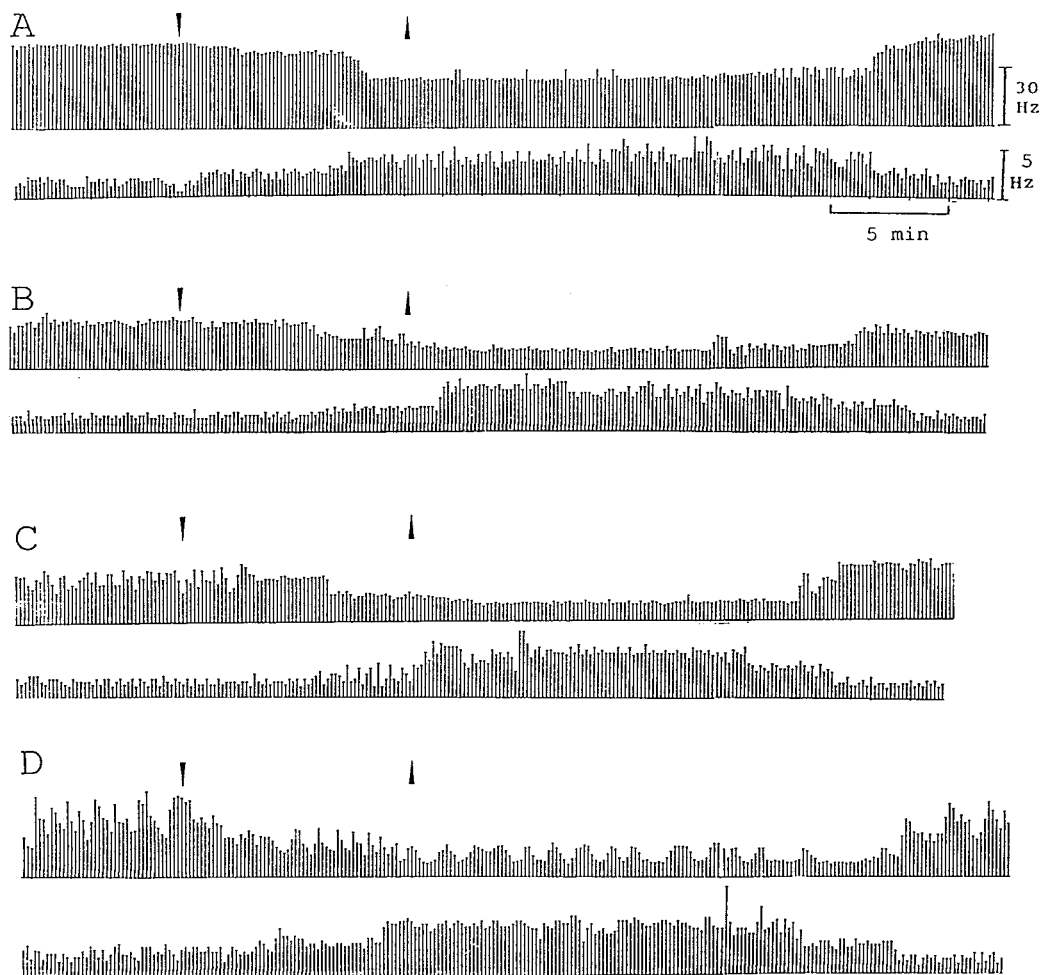


Fig. 20. The effect of EA on the activity of a NPF and DRN neurone recorded simultaneously. Each pair of traces (A-D) are from a separate experiment. The upper trace in each experiment is a glutamate-driven NPF neurone and the lower trace is a spontaneously active NCL cell in the DRN. The bin width is 10 s in each case. EA was applied for 10 min with the onset at the downward arrow and the offset at the upward arrow.

was 6.2 min (n=4). Interestingly, the onset and offset of these changes was usually quite sudden and sometimes the changes appeared to occur in steps. A recovery occurred 20 - 30 min after initiating EA.

The Effects of DRN Stimulation on NPF Neurones and the TF-EMG (Experiment 9).

Electrical stimulation (10 Hz, 0.2 ms, 0.3 mA, 30 s) of the DRN had a marked inhibitory effect upon nociceptive-evoked discharges and spontaneous activity on NPF neurones as well as concomitantly elevating the latency of the tail-flick reflex (Fig. 21). The DRN stimulation decreased the nociceptive responses on NPF neurones to about 50 % of control. The latency of TF-EMG increased from 5.0 (± 0.5) s to 6.8 (± 0.6) s. The duration of inhibition was approximately 20 min.

Stimulation of the DRN using trains of 1, 2 and 4 s durations were also studied on glutamate-driven NPF neurones. The data for these experiments are described under "Iontophoretic Studies".

The effect of single pulse DRN stimulation (1.0 Hz, 0.2 ms, 0.6 mA) on glutamate-driven NPF neurones also showed an inhibitory response in four of the six neurones tested. The latencies of the inhibition ranged from 6 to 12 ms (Fig. 22) with a mean latency of 8 ms. Thus, with a calculated conduction distance of 3.5 mm the range of conduction velocities is 0.3 to 5.8 m/s with a mean of 0.43 m/s. This was a similar range to that found by antidromic activation of DRN neurones

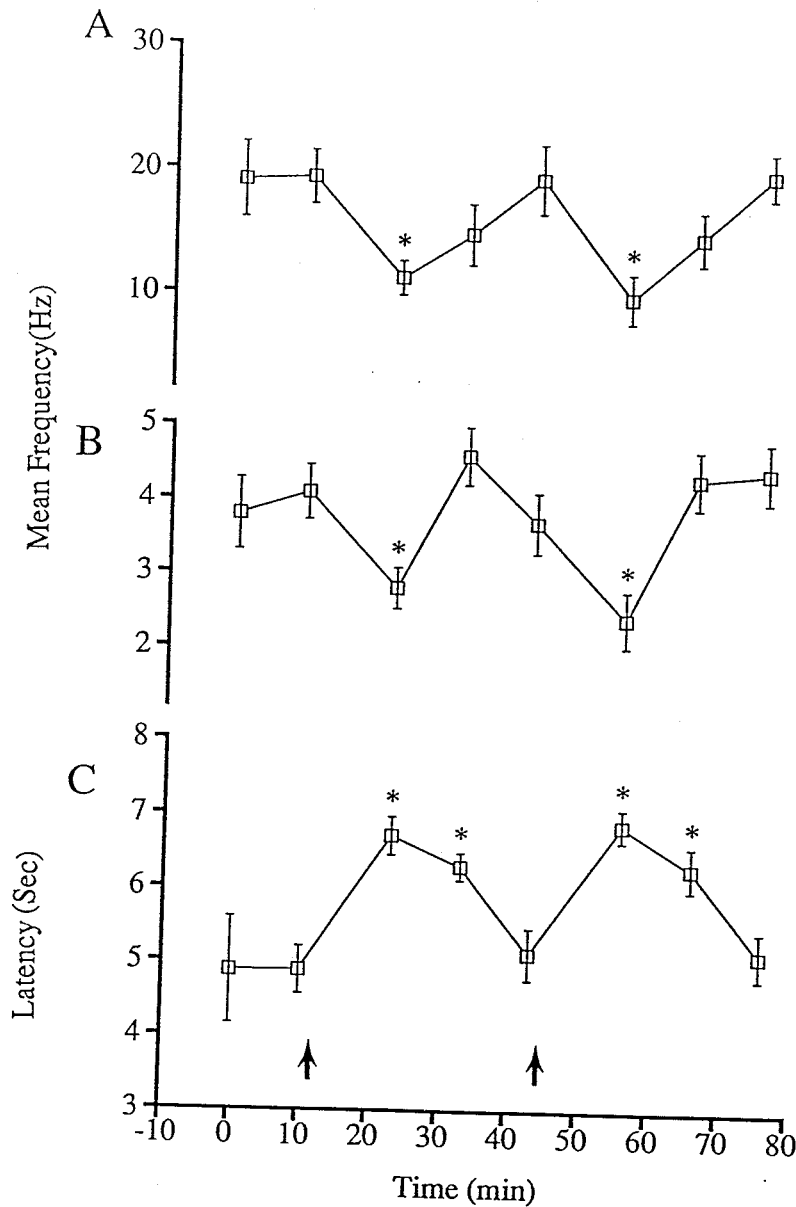


Fig. 21. The effects of the DRN stimulus on the noxious radiant heat-evoked activity in NPF neurones and the TF-EMG latency. A: Noxious heat-evoked activity of NPF neurones. B: The spontaneous activity of the same cells immediately prior to the noxious stimulus. C: The tail-flick latency. The arrows indicate electrical stimulation of the DRN (10 Hz, 0.2 ms, 0.3 mA) for 30 s. Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 8$).

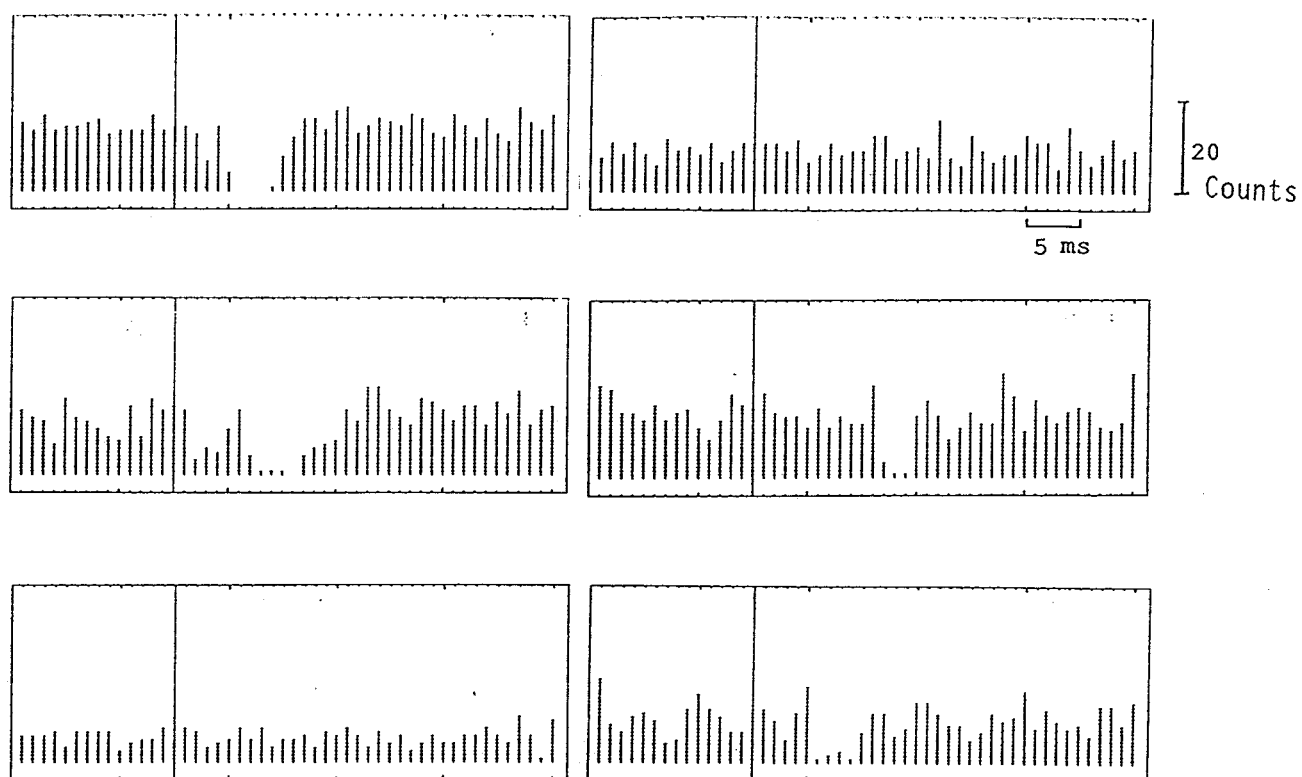


Fig. 22. Peristimulus histograms of 6 glutamate-driven NPF neurones tested by a single pulse (1.0 Hz, 0.2 ms, 0.6 mA) stimulation in the DRN. The vertical lines indicate the point of stimulation. Bin width = 1 ms. Each histogram is comprised of 400 sweeps.

from the NPF. Although two of these neurones showed no inhibition to single pulse DRN stimulation, a 1.0 s train of pulses (10 Hz, 0.2 ms, 0.3 mA) did produce an inhibition of 50 - 62 s on these neurones.

Serotonergic Involvement in the Inhibitory Projection from the DRN to the NPF (Experiment 10).

A group of 6 rats was treated with bilateral injections of 5,7-DHT, a specific serotonin neurotoxin (Bjorklund et al., 1974), into the lateral ventricles. Within 24 hours of the injection, all six animals showed behavioral signs of hyperaggressiveness and hyperactivity. Two weeks after the 5,7-DHT treatment the animals showed a mean weight loss of 6.8 gm compared to a weight gain of 72.2 gm for control animals over the same time period (Table 3). The two 5,7-DHT treated animal brains tested using the immunofluorescence technique showed a marked reduction of 5-HT immunofluorescence in the DRN compared to the controls (Fig. 23).

1) Studies on 5,7-DHT Pretreated Animals

NPF neurones were also tested in animals treated with 5,7-DHT as previously described. The effects of noxious radiant heat on NPF neurones in these rats are shown in Fig. 24 and should be compared to identical experiments in non-pretreated animals shown in Fig. 19. Note that the noxious heat-evoked response in the pretreated animals was

Table 3. Changes in Weight, Behavioural Activity
and Immunofluorescence Reaction in 5,7-DHT Treated Rats

5, 7-DHT Treated Group				Control Group			
Animal No.	Weight Gain in Two Weeks Mean gm	Hyperaggressiveness or Hyperactivity	Immunofluorescence Reaction	Animal No.	Weight Gain in Two Weeks Mean gm	Hyperaggressiveness or Hyperactivity	Immunofluorescence Reaction
1	-1.2	Yes	No	1	8.1	No	Yes
2	-1.8	Yes	NT	2	6.8	No	NT
3	-3	Yes	NT	3	6.9	No	Yes
5	2	Yes	NT	4	8.3	No	NT
5	-1.3	Yes	No	5	4.8	No	NT
6	3	Yes	NT	6	8.4	No	NT
Mean	-6.8			Mean	72.2		

NT = not tested.



Fig. 23. An illustration of serotonergic immunofluorescence in the DRN region of a control rat (top) and a 5,7-DHT treated animal (bottom).

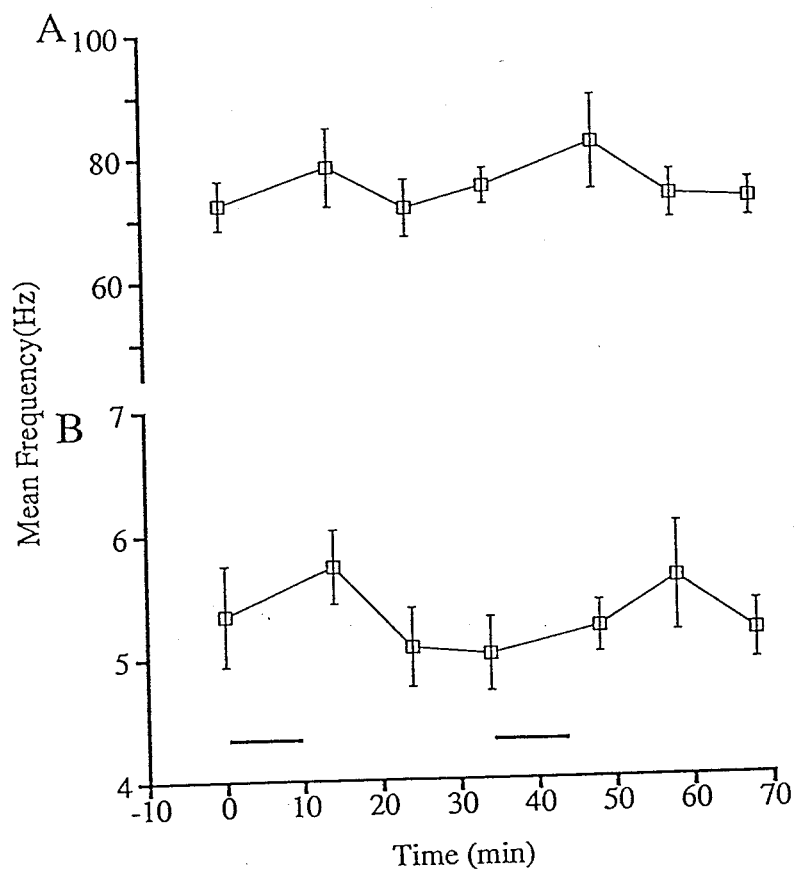


Fig. 24. A: EA (bars) fails to inhibit the evoked activity of NPF neurones in response to noxious radiant heat in 5,7-DHT treated animals. B: Spontaneous activity of the same neurones immediately prior to noxious radiant heat applications. Each point represents the mean \pm SE and was compared to its corresponding control value (n=6).

significantly higher (mean: 72.6 Hz) compared to the non-pretreated group (mean: 25.3 Hz) even though the heat pulse was the same temperature in the two groups. The spontaneous activity of NPF neurones in 5,7-DHT treated animals was also greater than that in non-pretreated animals. EA, in the 5,7-DHT pretreated animals, failed to produce an inhibition of NPF neurones previously seen in the non-pretreated animals. In fact there was a tendency (though not significant) for EA to enhance the activity of these neurones (Fig. 24).

Similarly, stimulating the DRN in 5,7-DHT pretreated animals at the same parameters previously used failed to elicit inhibition of NPF neurones (Fig. 25A) or prolong the tail-flick reflex (Fig. 25C). Compare these results to those obtained in identical experiments in non-pretreated animals (Fig. 21). Indeed, in the pretreated animals, DRN stimulation produced an increased firing rate in spontaneous and nociceptor-evoked activity of NPF neurones.

It is interesting that only NCLN neurones were found in the DRN of 5,7-DHT treated animals. Twenty-five of these neurones were found in six 5,7-DHT pretreated animals compared to 51 in 24 non-treated animals. The mean frequency of discharge was 1.6 Hz which did not differ from the controls. There were 35 CL and 32 NCL neurones in non-treated animals, but none were found in 5,7-DHT treated animals.

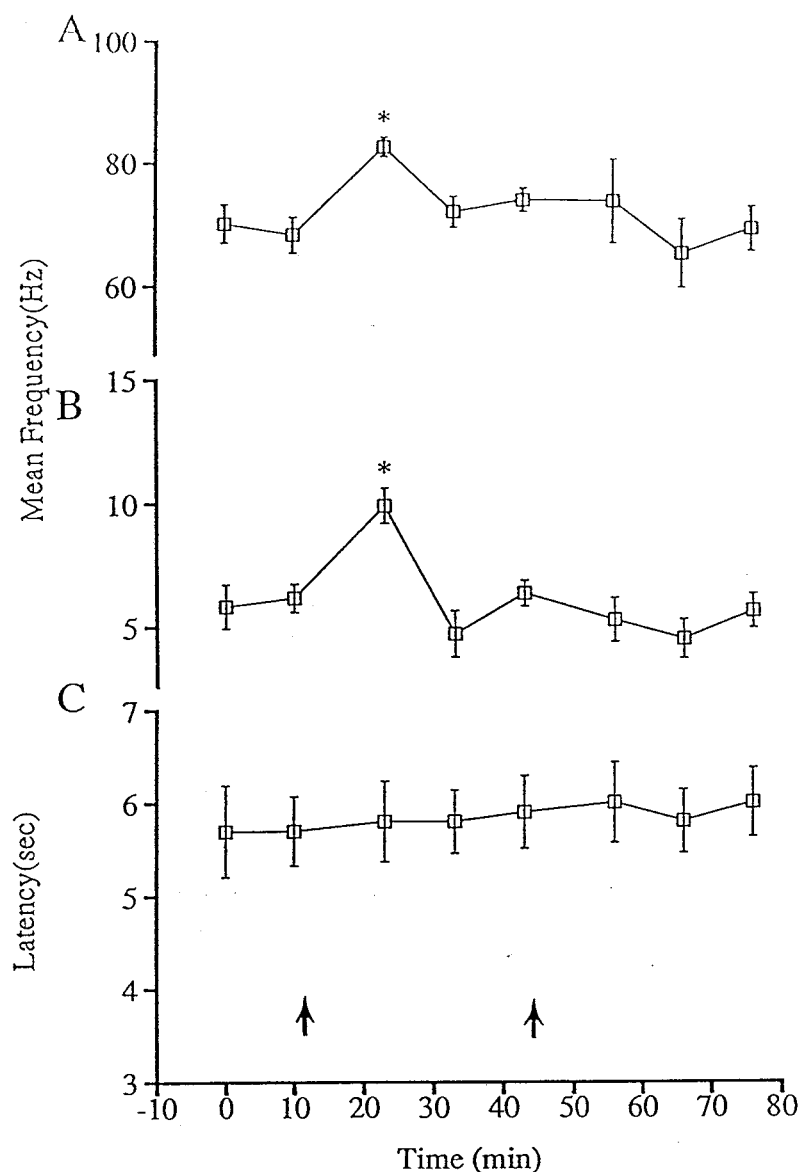


Fig. 25. Stimulation of the DRN for 30 s (arrows, 10 Hz, 0.2 ms, 0.3 mA) fails to inhibit the noxious radiant heat-evoked activity in NPF neurones in 5,7-DHT treated animals (A). Spontaneous activity of the same cells immediately prior to the noxious activity (B). The tail-flick latency (C). Each point representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n=6$).

2) Iontophoretic Studies

Iontophoretic studies were also used to test the idea that 5-HT is the mediator of the inhibition in the NPF produced by DRN stimulation. Alaproclate, a specific 5-HT uptake inhibitor, iontophoretically applied to NPF neurones which were activated by 10 s pulses of glutamate reduced the discharge rate at an ejection current of 45 nA (n=7) but not at 15 (n=5) or 30 nA (n=7; Fig. 26 and 27; Table 4). Tests for current effects by iontophoretically applied Na⁺ at 45 nA from the 0.95 % NaCl filled barrel, failed to alter the activity of these neurones (n=3, Fig.26; Table 4). 5-HT iontophoretically applied to glutamate-activated NPF neurones was inhibitory. Fig. 28 illustrates the mean frequency of 12 NPF neurones in which the protocol was identical. Preliminary tests in each experiment were done to establish currents to use for each drug. 5-HT was applied at a current (usually 30 nA) which reduced the glutamate-evoked activity to about 50 % of control. After several responses, alaproclate concomitantly applied at 30 nA, which had been previously shown in these neurones not to alter the discharge rate, was found to enhance the inhibitory response (Fig. 28A). Alaproclate was tested alone and failed to alter the glutamate-evoked responses. Also alaproclate did not alter the inhibitory effect of GABA (30 - 45 nA) on these neurones (Fig. 28B). An example of the effect of iontophoretic

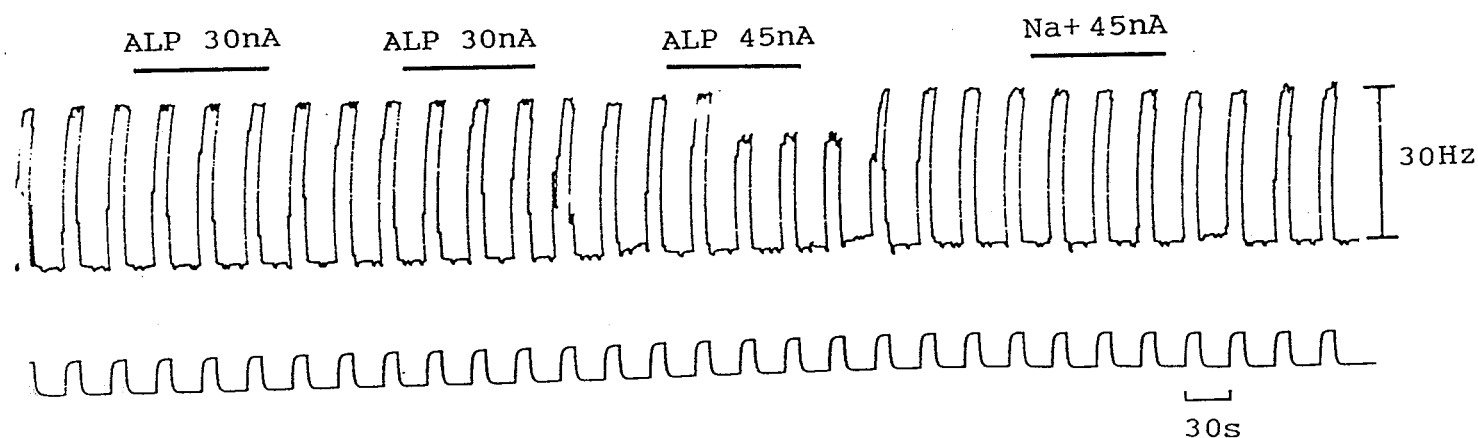


Fig. 26. An example of the effect of iontophoretic application of alaproclate at 30 and 45 nA on a NPF neurone. Na^+ ejected by a current of 45 nA applied through the NaCl (0.95%) barrel had no effect. The lower trace represents 10 s iontophoretic applications of glutamate. The bars above the top trace represent the duration of iontophoretic applications of alaproclate and Na^+ .

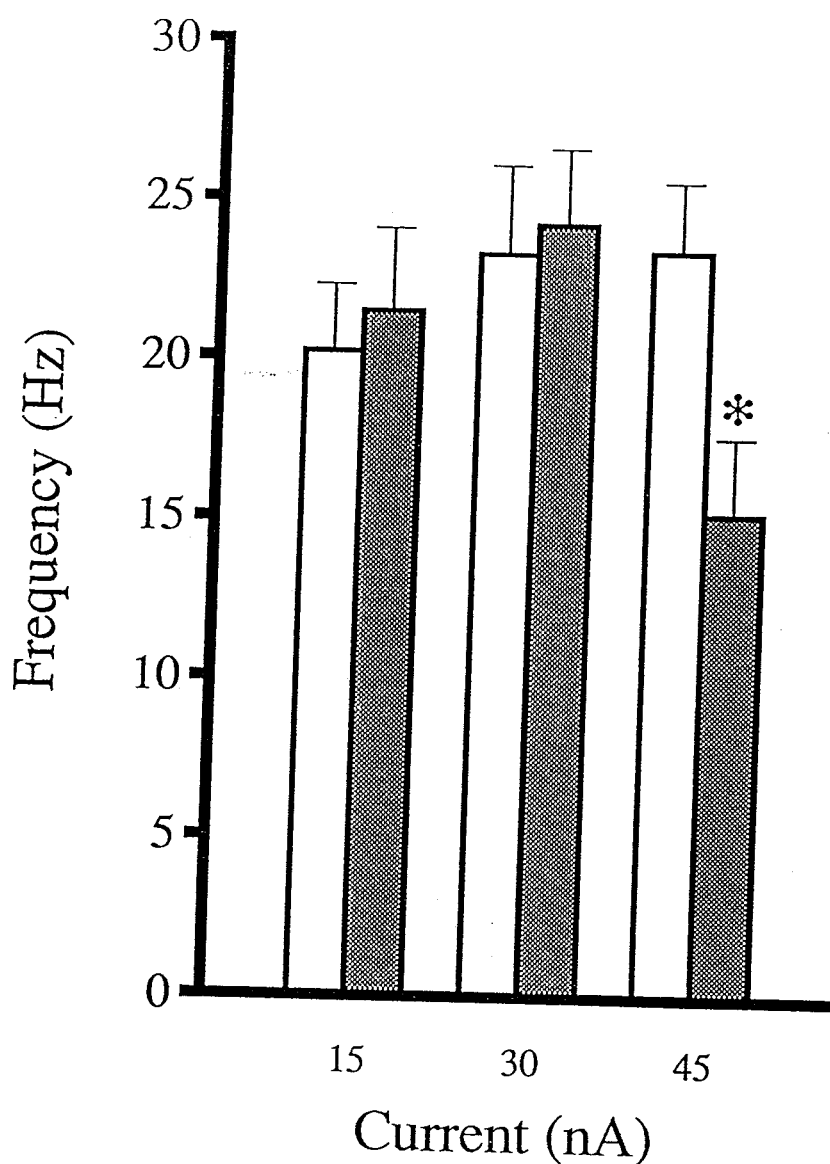


Fig. 27. The effects of iontophoresis of alaproclate on NPF neurones activated by glutamate. The open and filled bars represent the discharge frequency before and during the iontophoresis of alaproclate at the current specified below, respectively. Each bar representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 5-7$).

Table 4. Iontophoretic Application of Alaproclate and Na^+ on NPF Neurons Activated by Glutamate

Number of Neurons	Alaproclate						Na^+	
	15 nA		30 nA		45 nA		45 nA	
	No. Tests	f	No. Tests	f	No. Tests	f	No. Tests	f
1	3	16.6/16.0	3	15.99/18.91	3	16.67/8.54		
2	6	17.18/18.06	5	17.09/16.95	6	15.56/11.47		
3	1	21.15/29.13	2	24.13/23.49	4	22.51/13.64		
4	2	28.08/28.47	2	28.64/29.37	2	27.63/12.07	2	28.48/28.05
5	2	17.90/17.70	2	16.90/18.60	1	19.00/14.70		
6			3	24.16/25.07	2	24.48/18.63	2	23.43/25.77
7			3	35.95/35.65	2	38.00/27.33	2	38.30/40.70
Mean (\pm SE)		20.18/21.47 (2.1) (2.6)		23.27/24.22 (2.8) (2.4)		23.41*/15.20 (2.2) (2.4)		30.07/31.51 (4.4) (4.7)

f = Average frequency before/during iontophoresis (spike/s);

* = $p < 0.05$.

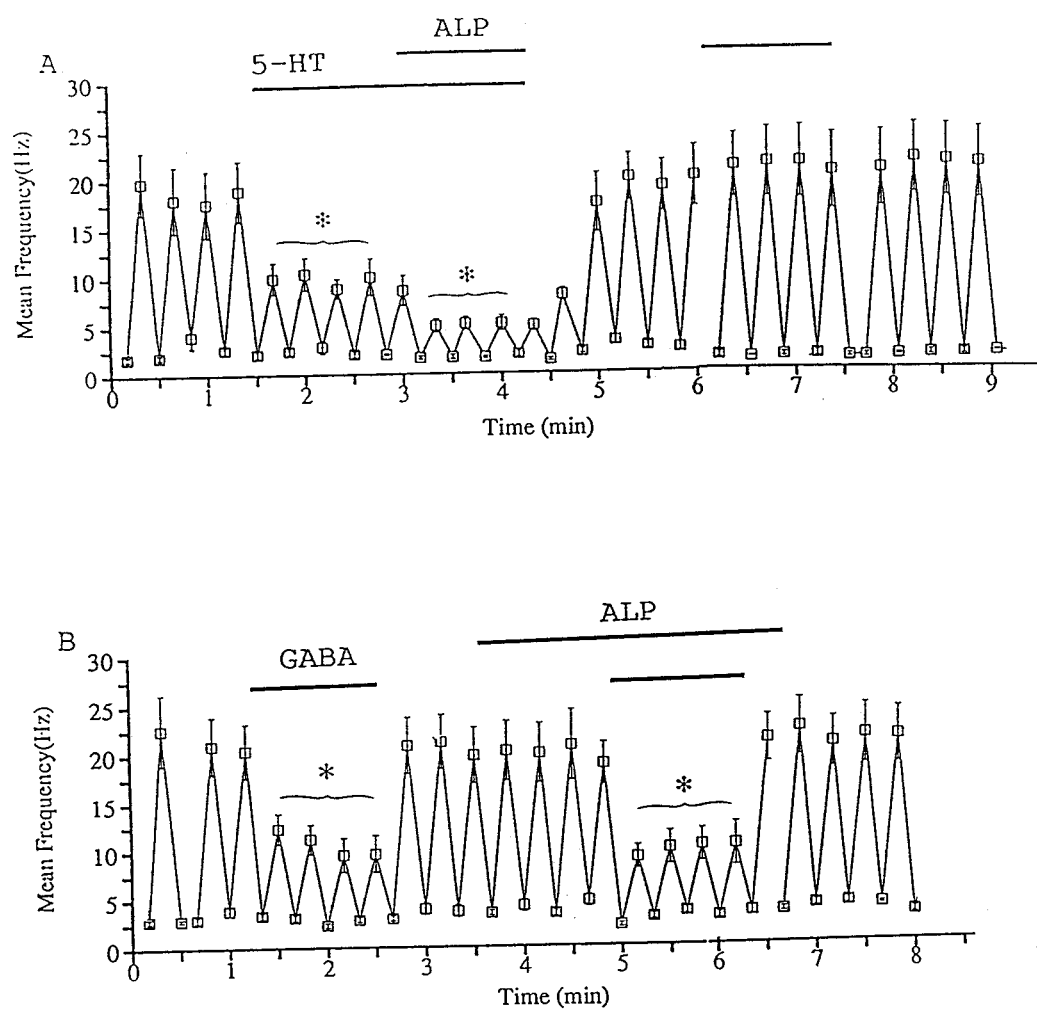


Fig. 28. Ionophoretic application of alaproclate enhances the inhibition produced by 5-HT but not GABA on NPF neurones. A: The firing rate of NPF neurones activated by glutamate (10 s pulses at 45 nA) was reduced by 5-HT application. A greater inhibition was observed when alaproclate (30 nA) was applied concurrently. B: Alaproclate did not enhance the inhibition effect of GABA (45 nA). The period of application of 5-HT, alaproclate and GABA are indicated by labelled bars. Each point represents the mean \pm SE (* $p < 0.05$, $n = 12$).

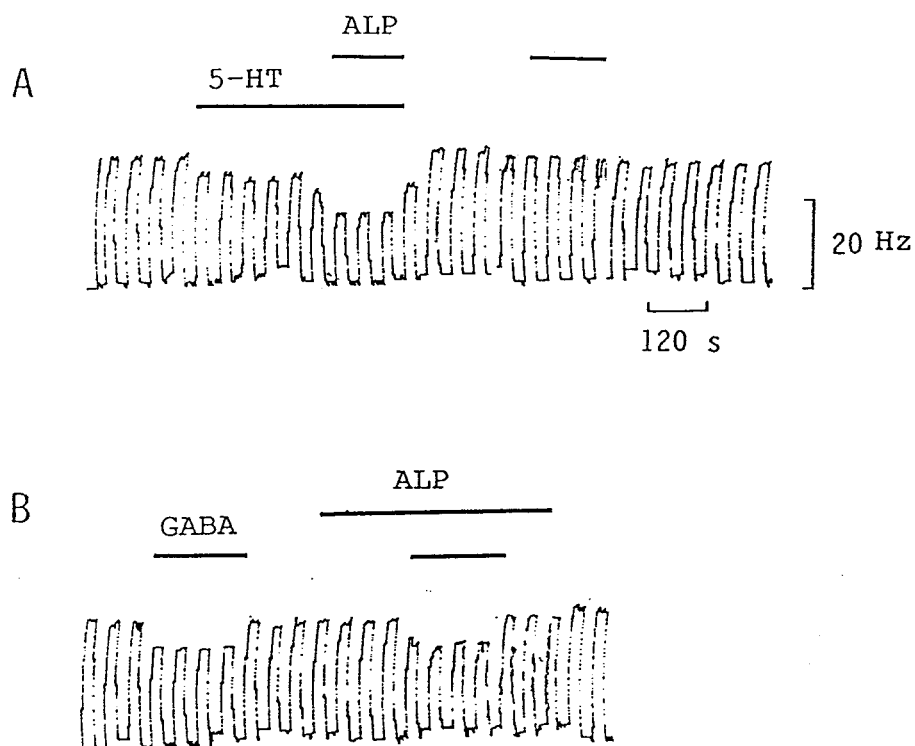


Fig. 29. An example of the effect of iontophoretic application of 5-HT, alaproclate and GABA on a NPF neurone. The firing rate of an NPF neurone driven by glutamate was reduced by 5-HT application. A greater inhibition was observed when alaproclate was applied concurrently (A). Alaproclate did not enhance the inhibitory effect of GABA (B). The bars above the trace represent the duration of iontophoretic application.

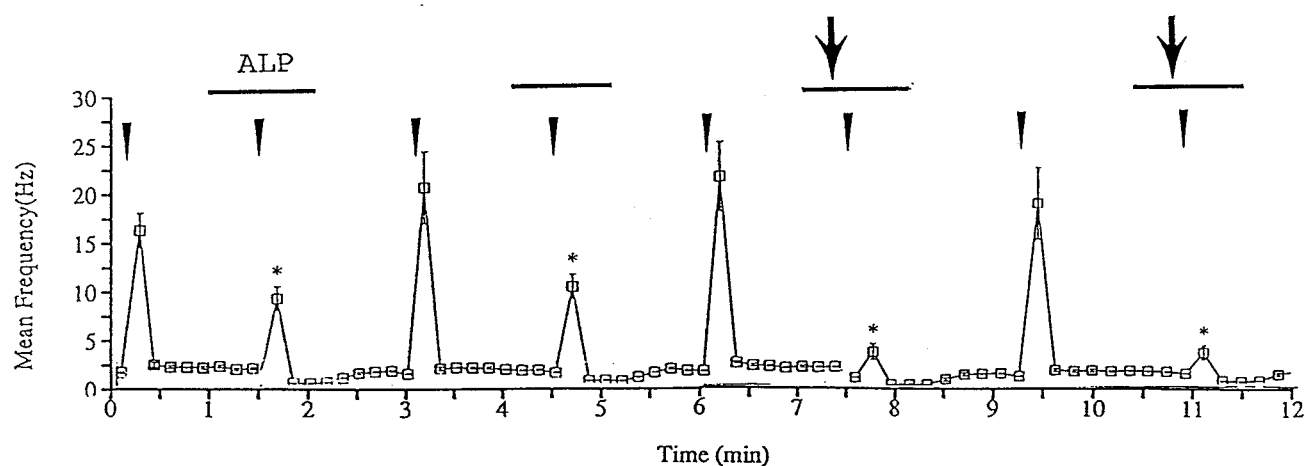


Fig. 30. The effect of iontophoretically applied alaproclate on noxious radiant heat-evoked activation of NPF neurones and inhibition by DRN stimulation. Noxious radiant heat-evoked firing of NPF neurones was decreased during the application of alaproclate (30 nA, bars above trace) and further decreased by a combination of alaproclate and DRN stimulation (arrows, 10 Hz, 0.2 ms, 0.3 mA) for 1.0 s. Each point represents the mean \pm SE and was compared to its corresponding control value (* p <0.05, n =12).

alaproclate application on 5-HT- and GABA-mediated inhibition of an NPF neurone is shown in Fig. 29.

Activation of NPF neurones, illustrated in Fig. 30, by noxious radiant heat to the tail was decreased by the application of alaproclate (30 nA). A combination of alaproclate and DRN stimulation (10 Hz, 0.2 ms, 0.3 mA) produced an even greater inhibition of these neurones. An example of the effect of iontophoretic application of alaproclate and DRN stimulation on nociceptor-evoked activity in an NPF neurone is shown in Fig. 31.

Stimulation (10 Hz, 0.2 ms, 0.3 mA) of the DRN for 1, 2, 4 s produced a prolonged inhibition in the activity of NPF neurones whose background activity was maintained by the constant iontophoretic release of glutamate. The inhibitory response had a sudden onset, which sometimes took several seconds to reach maximum effect, long duration and sudden offset. An example from one experiment is illustrated in Fig. 32 A, B. The mean (\pm SE) inhibitory durations were 51.2 (\pm 7.9), 107.7 (\pm 8.8) and 186.4 (\pm 15.3) s after 1, 2 and 4 s stimulation, respectively (Fig. 32, 33 and Table 5). Thus, the duration of the inhibition was correlated with the duration of stimulation. However, the magnitude of the inhibition was the same regardless of the period of stimulation (Fig. 32, 33, 34 and Table 5).

Alaproclate iontophoretically applied to NPF neurones at 30 nA had no effect on the firing rate of these neurones before the stimulation of the DRN. However, a greater inhibitory response in magnitude was

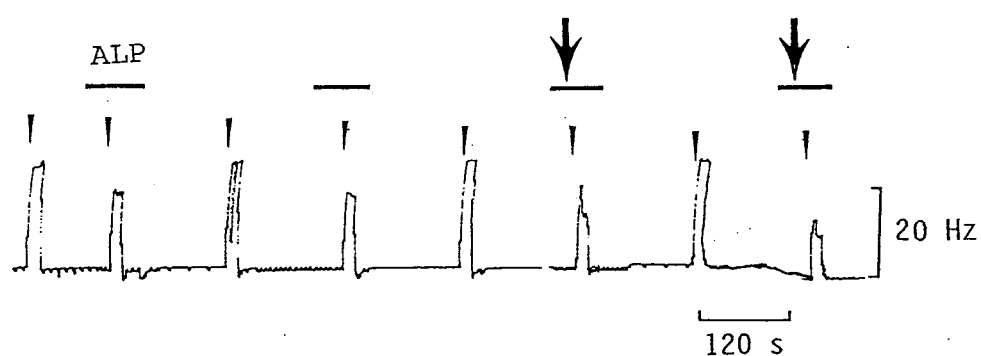


Fig. 31. An example of the effect of iontophoretically applied alaproclate and DRN stimulation on noxious radiant heat-evoked activation of an NPF neurone. Noxious radiant heat-evoked firing of the NPF neurone was decreased during the application of alaproclate (30 nA, bars above trace) and further decreased by a combination of alaproclate and DRN stimulation (arrows, 10 Hz, 0.2 ms, 0.3 mA) for 1.0 s.

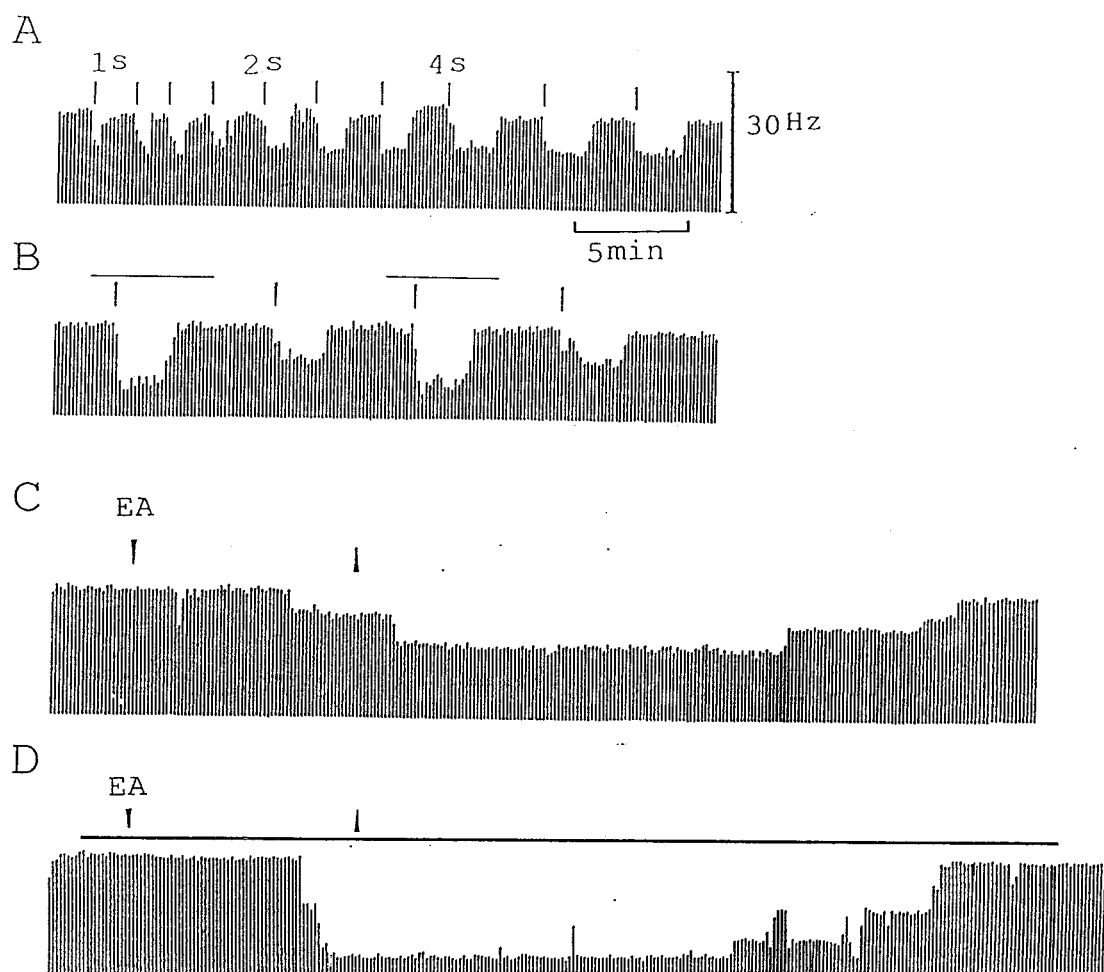


Fig. 32. The effects of alaproclate on DRN- and EA-evoked inhibition of a glutamate-driven NPF neurone. The traces are continuous and show the discharge frequency of an NPF cell (bin width = 10 s). The marks above the trace indicate the points and durations of DRN stimuli. The bars above the trace indicate the periods of iontophoretic alaproclate application at 30 nA. EA was applied for 10 min with the onset at the downward arrow and the offset at the upward arrow. A and B show the effect of different periods of DRN stimulation and the effect of alaproclate on the inhibition. C and D similarly show the inhibition produced by EA and its enhancement by alaproclate.

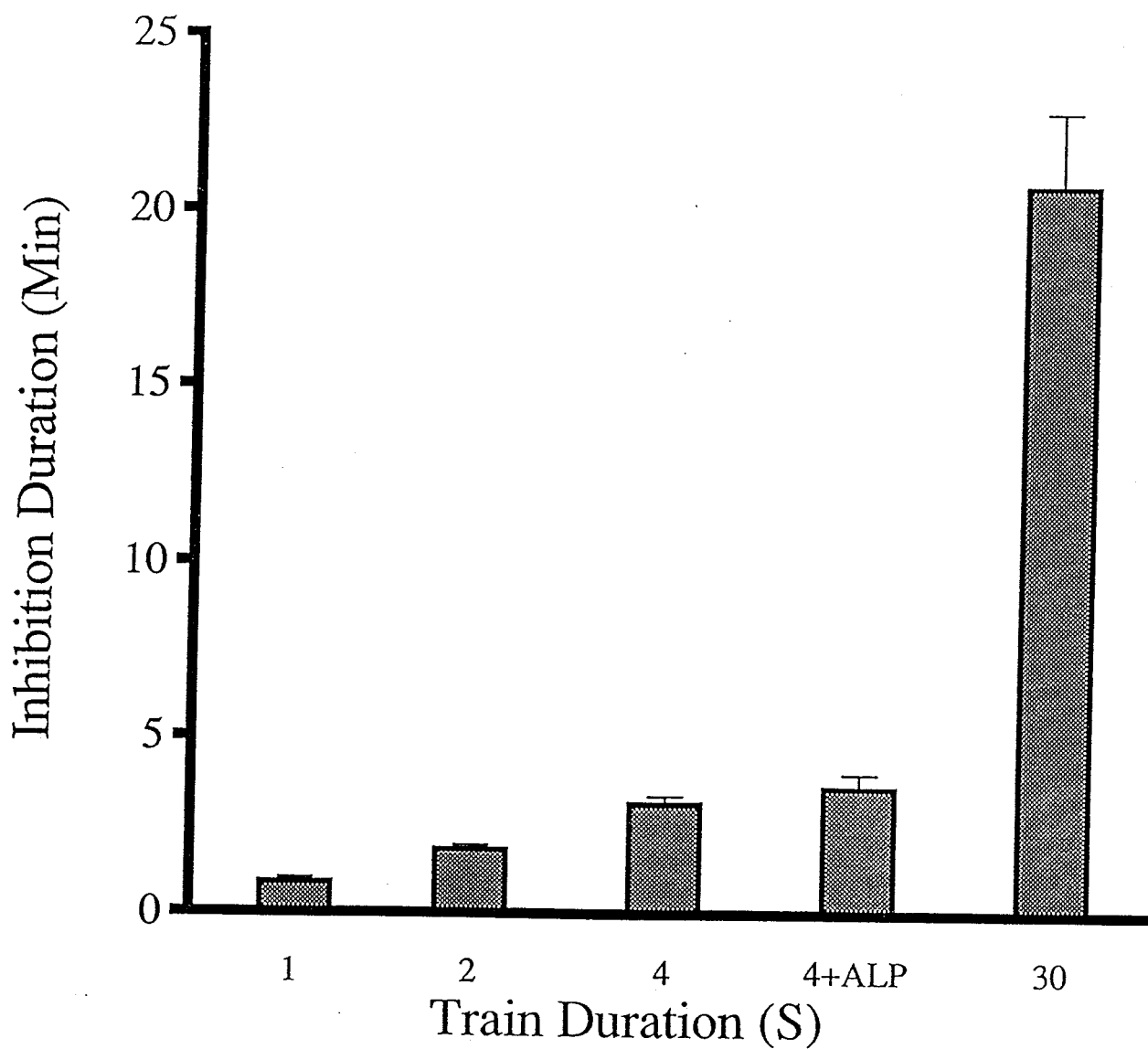


Fig. 33. The duration of the NPF inhibition produced by DRN stimulation of 1, 2, 4, and 30 s. The 4+ALP bar represents a 4 s stimulus combined with iontophoresis of alaproclate at 30 nA. Each bar represents the mean \pm SE and was compared to its corresponding control value ($n=4-11$).

Table 5. The Effect of DRN Stimulation on NPF Neurones Driven by Glutamate

Neurone Tested	1 S			2 S			4 S			4 S + ALP 30 nA			30 S		
	n	f	d	n	f	d	n	f	d	n	f	d	n	f	d
1	5	32.0/26.8	47.0	3	32.0/24.6	93.3	2	31.0/20.5	140.0						
2	1	17.3/10.7	50.0	2	24.0/13.0	100.0	1	23.2/13.4	220.0						
3	3	28.5/24.3	60.0	3	29.2/23.5	103.3	2	27.5/18.2	170.0						
4	4	36.7/26.9	84.3	3	32.2/21.3	151.3	2	35.8/19.3	285.0	5	34.7/13.4	342.0	1	33.7/30.6	21.6
5	5	30.5/25.6	47.8	5	31.6/20.5	27.5	3	29.5/20.6	136.7	2	30.5/11.6	145.0			
6	7	33.1/20.2	58.2	12	39.2/30.4	109.6	3	41.8/34.4	162.5	6	41.3/14.1	208.3			
7	9	28.6/24.5	58.9	6	30.5/22.6	135.0	3	32.5/23.5	203.3	4	32.0/13.6	188.0	1	27.9/21.3	26.0
8	2	32.5/20.8	50.0	2	32.5/26.9	110.0	2	32.5/24.5	210.0	2	32.5/12.2	215.0	1	32.2/20.2	19.3
9	4	24.7/15.3	90.0	3	25.1/16.4	136.7	5	25.4/16.7	243.3	2	24.8/11.5	285.0	2	24.0/10.2	16.7
10				2	39.0/30.4	60	2	39.2/26.5	160.0	2	39.4/19.6	170.0			
11							2	39.7/26.7	120.0	2	43.0/10.4	160.0			
Mean (\pm SE)		29.3/21.7 (1.9) (1.9)	51.2 (7.9) n=9		31.5/23.0 (1.6) (1.8)	107.6 (8.8) n=10		32.6/22.2 (1.8) (1.7)	186.4 (15.3) n=11		34.8/13.3 (2.2) (1.0)	214.2 (23.8) n=8		29.5/18.1 (2.2) (2.7)	20.7 (0.1) n=4

n = Number of tests;

f = Average frequency before/after DRN stimulation (spike/s);

d = Duration of inhibition (s);

d* = Duration of inhibition (min.).

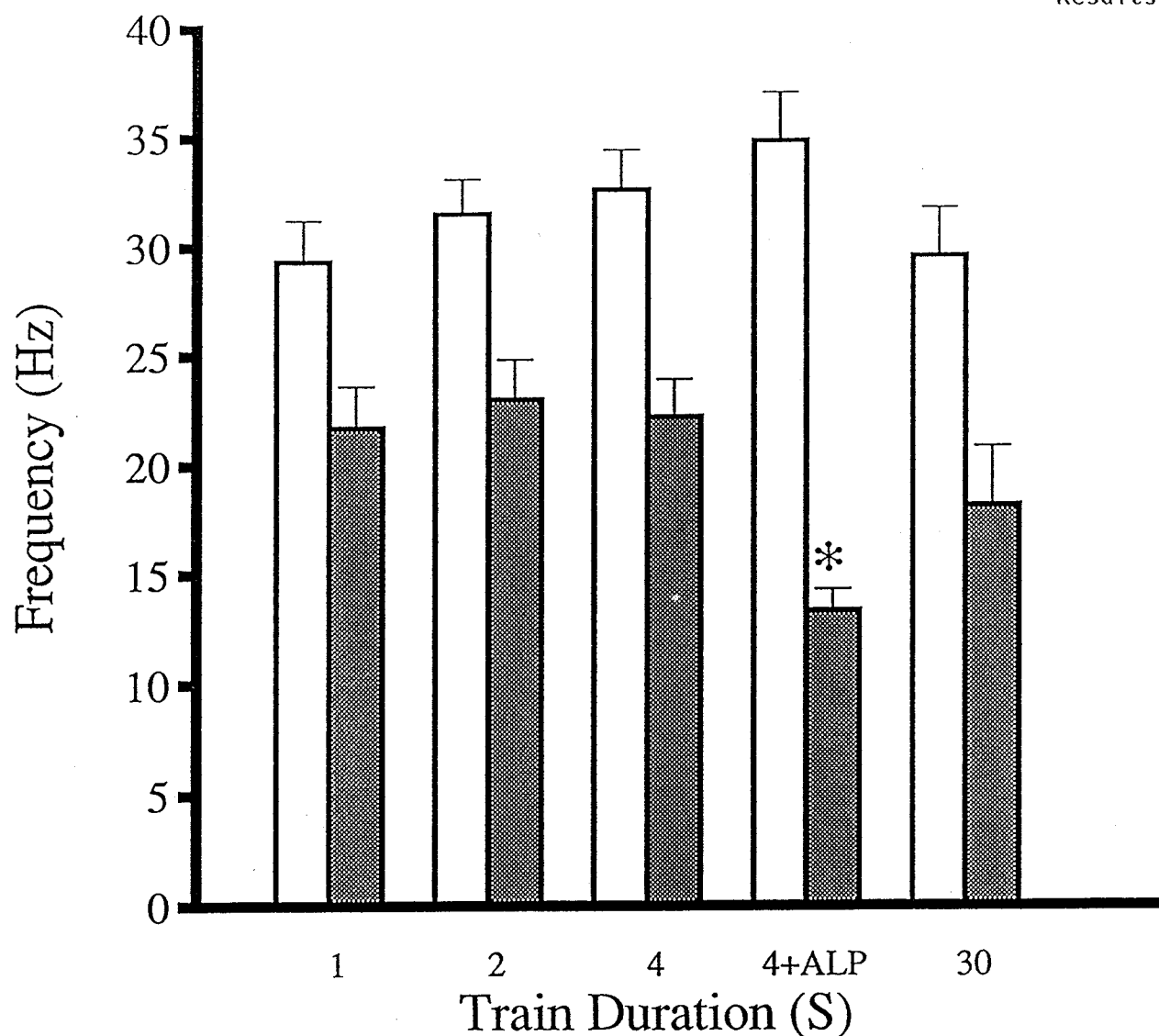


Fig. 34. The magnitude of the NPF inhibition produced by DRN stimulation. The open and filled bars represent the NFP neurones frequency of discharge before and after DRN stimulation, respectively. The 4+ALP bar represents a 4 s stimulus combined with iontophoresis of alaproclate at 30 nA. Each bar represents the mean \pm SE. In each case the responses after stimulation were significantly greater than the corresponding control values. Alaproclate enhanced the effect of a 4 s stimulus ($n=4-11$, $*p<0.05$,). The data are also expressed in Table 5.

observed when alaproclate ejected at this current was combined with a 4 s stimulation of the DRN (Fig. 32, 34 and Table 5). However, there was no difference in duration of the inhibition (Fig. 32, 33 and Table 5).

EA on the same neurone illustrated in Fig. 32C and D produced a prolonged inhibition which was clearly reached in 2 steps. Repeating the procedure in the presence of iontophoretically released alaproclate at a current of 30 nA markedly increased the magnitude of the inhibition while the duration of inhibition was almost identical. The data on the 5 neurones tested with alaproclate in this manner are illustrated in Fig. 35A and B and Table 6.

Another study was performed using cyproheptadine, a serotonin antagonist, to determine whether the ascending pathway from the DRN to the NPF is serotonergic. Fig. 36 shows that stimulation of the DRN (10 Hz, 0.2 ms, 0.3 mA) for 1, 2 and 4 s evoked inhibition of increasing duration on a glutamate-driven NPF neurone. Alaproclate, iontophoretically applied (30 nA), enhanced the inhibition evoked by a 4 s stimulation of the DRN. However alaproclate did not alter the inhibitory effect of GABA on this neurone. After the administration of cyproheptadine (5 mg/kg, i.v.), the inhibitory effect of DRN stimulation using the same parameters was blocked. Also the spontaneous activity of this neurone was increased significantly compared to the control. However, cyproheptadine did not alter the inhibition produced by GABA. The grouped data for 5 neurones tested in this manner are shown in Fig. 37 and Table 7. in which the spontaneous activity of the NPF neurones

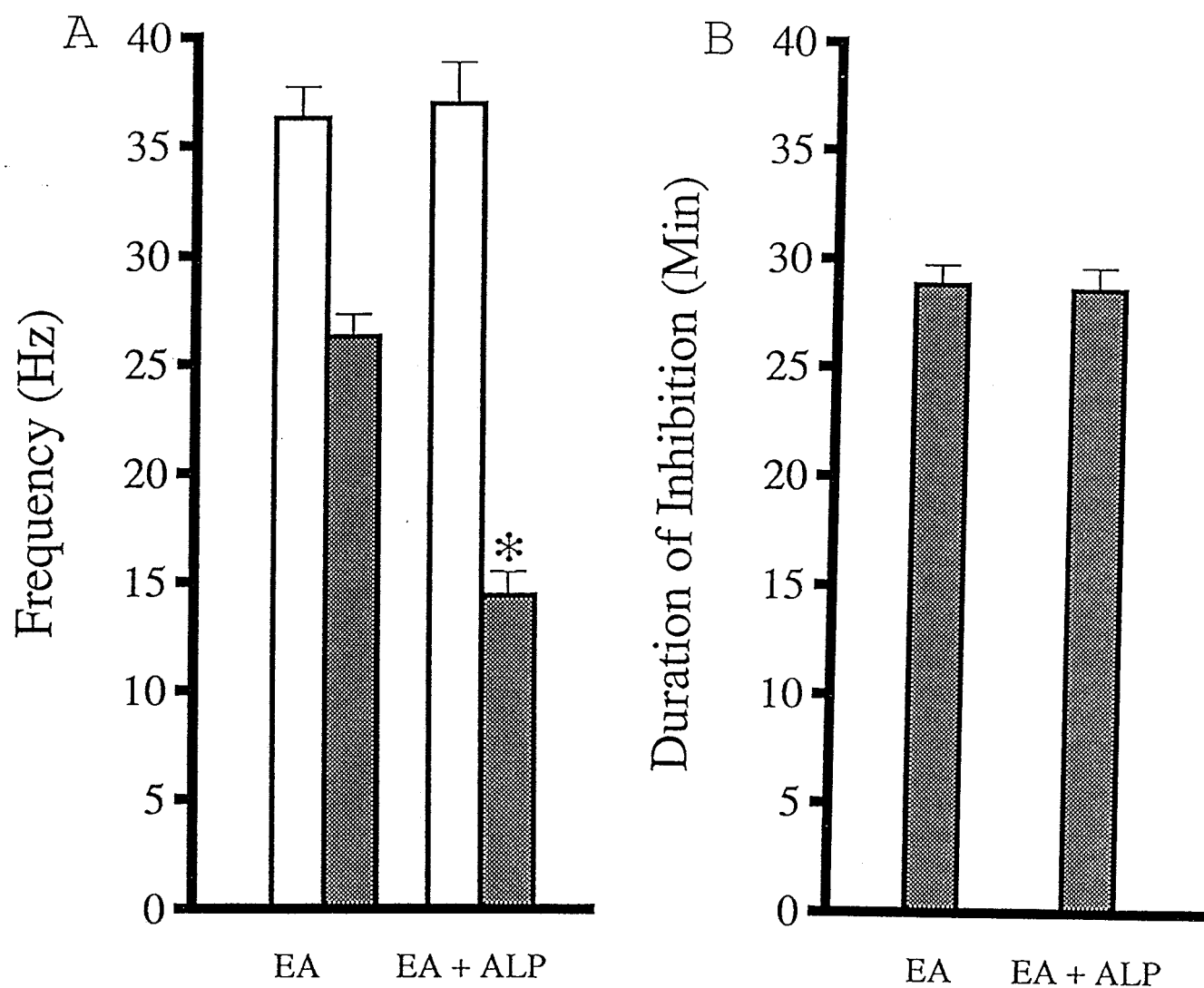


Fig. 35. Alaproclate iontophoretically applied at 30 nA enhances the magnitude (A) but not the duration of inhibition (B) of EA-evoked inhibition of NPF neurones. Each bar representing the mean \pm SE was compared to its corresponding control value (* $p < 0.05$, $n = 5$).

Table 6. The Effect of Alaproclate on EA-induced Inhibition of Glutamate-Driven NPF Neurones

Neurone	EA			EA + Alaproclate 30nA		
	f'	L	d	f''	L	d
Tested						
1	34.83/26.96	4.6	31	34.33/14.52	5.2	30
2	39.59/29.83	5.3	28	42.96/13.64	6.3	29
3	39.66/26.95	5.0	30	39.61/18.44	5.8	31
4	32.24/24.26	6.3	26	32.57/13.02	6.7	28
5	35.02/23.28	5.8	29	35.26/12.23	6.4	25
Mean (\pm SE)	36.27/26.26 (1.45) (1.05)	5.4 (0.30)	28.8 (0.9)	*36.94/14.37 (1.90) (1.09)	6.08 (0.59)	28.6 (1.0)

f' = Average frequency of EA control group,

f'' = Average frequency of EA + alaproclate,

L = latency (min),

d = duration of inhibition (min),

* = $p < 0.05$ which was compared to control value f'. n = 5.

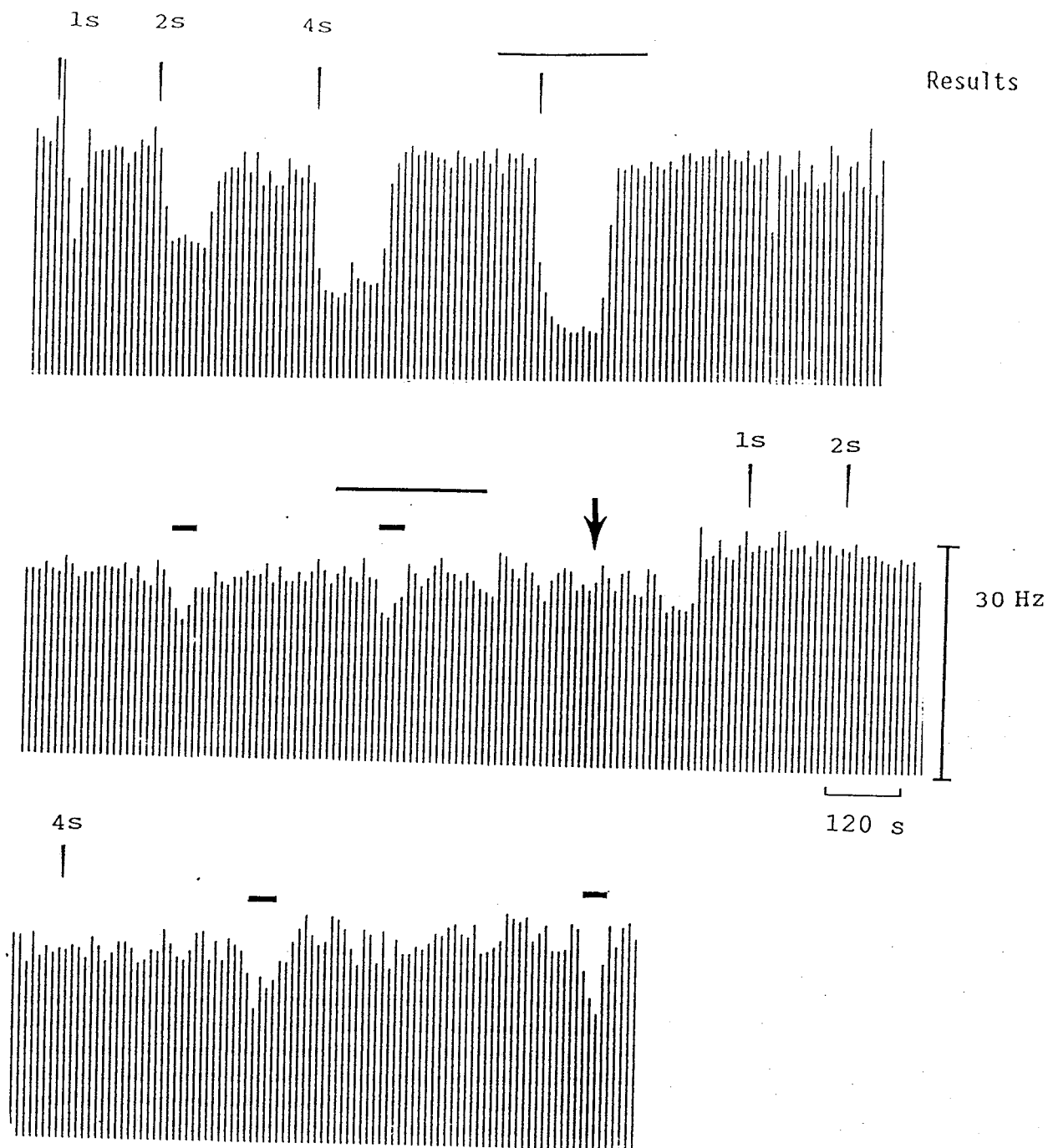


Fig. 36. Cyproheptadine (5 mg/kg. i.v.) blocks the DRN-evoked inhibition of a glutamate-driven NPF neurone. The traces are continuous and each bin equals 10 s. The marks above the trace indicate the points and durations of a DRN stimulus. The long bars above the trace indicate the periods of iontophoretic alaproclate application at 30 nA. The heavy short bars above the trace indicate the application of iontophoretic GABA at 30 nA. The arrow represents the administration of cyproheptadine.

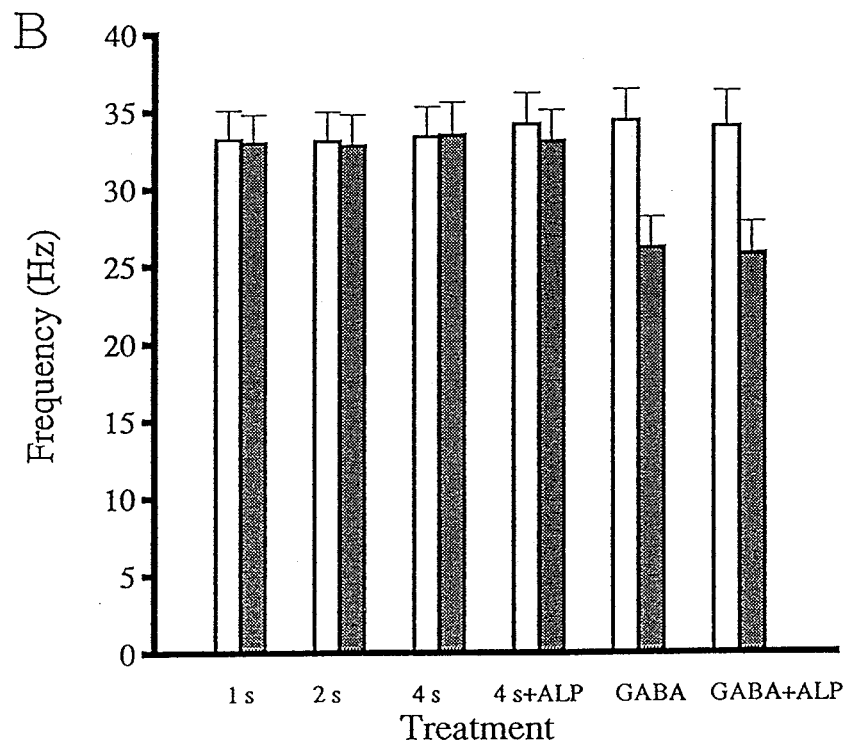
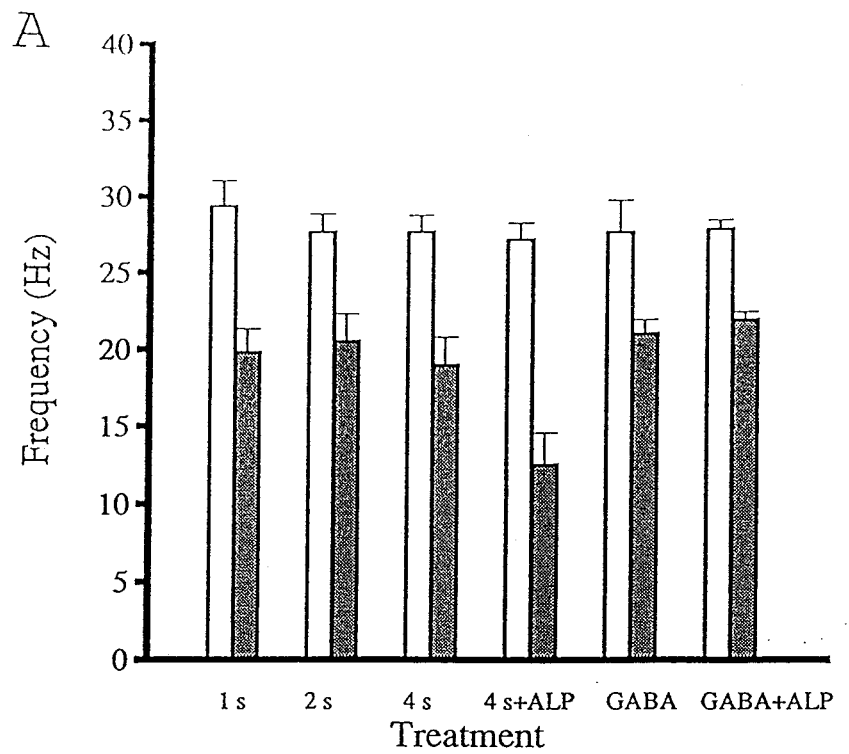


Fig. 37. Cyproheptadine blocks the DRN-evoked inhibition of glutamate-driven NFP neurones. The arrangement is the same as shown by Fig. 34. A: control, B: after administration of cyproheptadine (5 mg/kg, i.v.). Each bar represents the mean \pm SE (n = 5).

Table 7. Cyproheptadine Blocks the DRN-Evoked Inhibition of Glutamate-Driven NFP Neurons

Neurons	Control											
	1S			2S			4S			4S+ALP 30nA		
	n	f	d	n	f	d	n	f	d	n	f	d
Tested												
1	2	31.2/24.0	36.0	2	30.2/24.4	80.4	1	28.4/22.6	150.2	1	28.0/11.3	170.0
2	3	28.4/20.4	60.4	2	27.2/23.4	98.6	1	26.4/21.4	180.0	1	25.4/10.3	164.2
3	2	24.3/16.7	51.8	2	24.0/17.3	100.4	2	25.0/18.2	149.3	2	24.4/10.0	170.2
4	2	34.3/21.5	48.2	2	30.4/22.3	110.3	1	31.2/20.4	130.0	1	30.2/18.3	120.4
5	2	28.4/16.3	40.0	2	26.4/15.3	90.2	1	26.8/12.5	120.3	1	27.2/9.8	130.3
Mean (\pm SE)		29.3/19.8 (1.7) (1.5)	47.3 (4.3)		27.6/20.5 (1.2) (1.8)	95.9 (5.0)		27.0/19.0 (1.1) (1.8)	144.2 (10.2)		27.1/12.0 (1.1) (1.6)	143.0 (10.0)
Cyproheptadine												
Tested												
1	2	36.8/36.6	0	2	37.0/36.8	0	1	37.4/37.8	0	1	37.9/36.8	0
2	3	32.2/32.0	0	2	32.8/31.0	0	1	33.0/32.6	0	1	34.3/32.0	0
3	2	28.4/28.0	0	2	27.6/28.0	0	2	28.0/27.3	0	2	29.2/28.4	0
4	2	38.2/37.6	0	2	37.8/38.4	0	1	38.2/38.6	0	1	39.1/38.6	0
5	2	30.2/30.6	0	2	30.4/30.0	0	1	30.6/31.0	0	1	30.2/29.6	0
Mean (\pm SE)		33.2/33.0 (1.9) (1.8)	0		33.1/32.8 (1.9) (2.0)	0		33.4/33.5 (1.9) (2.1)	0		34.2/33.1 (2.0) (2.0)	0
GABA												
Tested												
1	2	36.8/36.6	0	2	37.0/36.8	0	1	37.4/37.8	0	1	37.9/36.8	0
2	3	32.2/32.0	0	2	32.8/31.0	0	1	33.0/32.6	0	1	34.3/32.0	0
3	2	28.4/28.0	0	2	27.6/28.0	0	2	28.0/27.3	0	2	29.2/28.4	0
4	2	38.2/37.6	0	2	37.8/38.4	0	1	38.2/38.6	0	1	39.1/38.6	0
5	2	30.2/30.6	0	2	30.4/30.0	0	1	30.6/31.0	0	1	30.2/29.6	0
Mean (\pm SE)		33.2/33.0 (1.9) (1.8)	0		33.1/32.8 (1.9) (2.0)	0		33.4/33.5 (1.9) (2.1)	0		34.2/33.1 (2.0) (2.0)	0
GABA + ALP 30nA												
Tested												
1	2	36.8/36.6	0	2	37.0/36.8	0	1	37.4/37.8	0	1	37.9/36.8	0
2	3	32.2/32.0	0	2	32.8/31.0	0	1	33.0/32.6	0	1	34.3/32.0	0
3	2	28.4/28.0	0	2	27.6/28.0	0	2	28.0/27.3	0	2	29.2/28.4	0
4	2	38.2/37.6	0	2	37.8/38.4	0	1	38.2/38.6	0	1	39.1/38.6	0
5	2	30.2/30.6	0	2	30.4/30.0	0	1	30.6/31.0	0	1	30.2/29.6	0
Mean (\pm SE)		33.2/33.0 (1.9) (1.8)	0		33.1/32.8 (1.9) (2.0)	0		33.4/33.5 (1.9) (2.1)	0		34.2/33.1 (2.0) (2.0)	0

n = Number of tests;

f = Average frequency before/duration and after DRN stimulation (spikes/S);

d = Duration of inhibition (S).

Results

was increased after administration of cyproheptadine. Also cyproheptadine blocks EA-evoked inhibition of a glutamate-driven NPF neurone (Fig. 38).

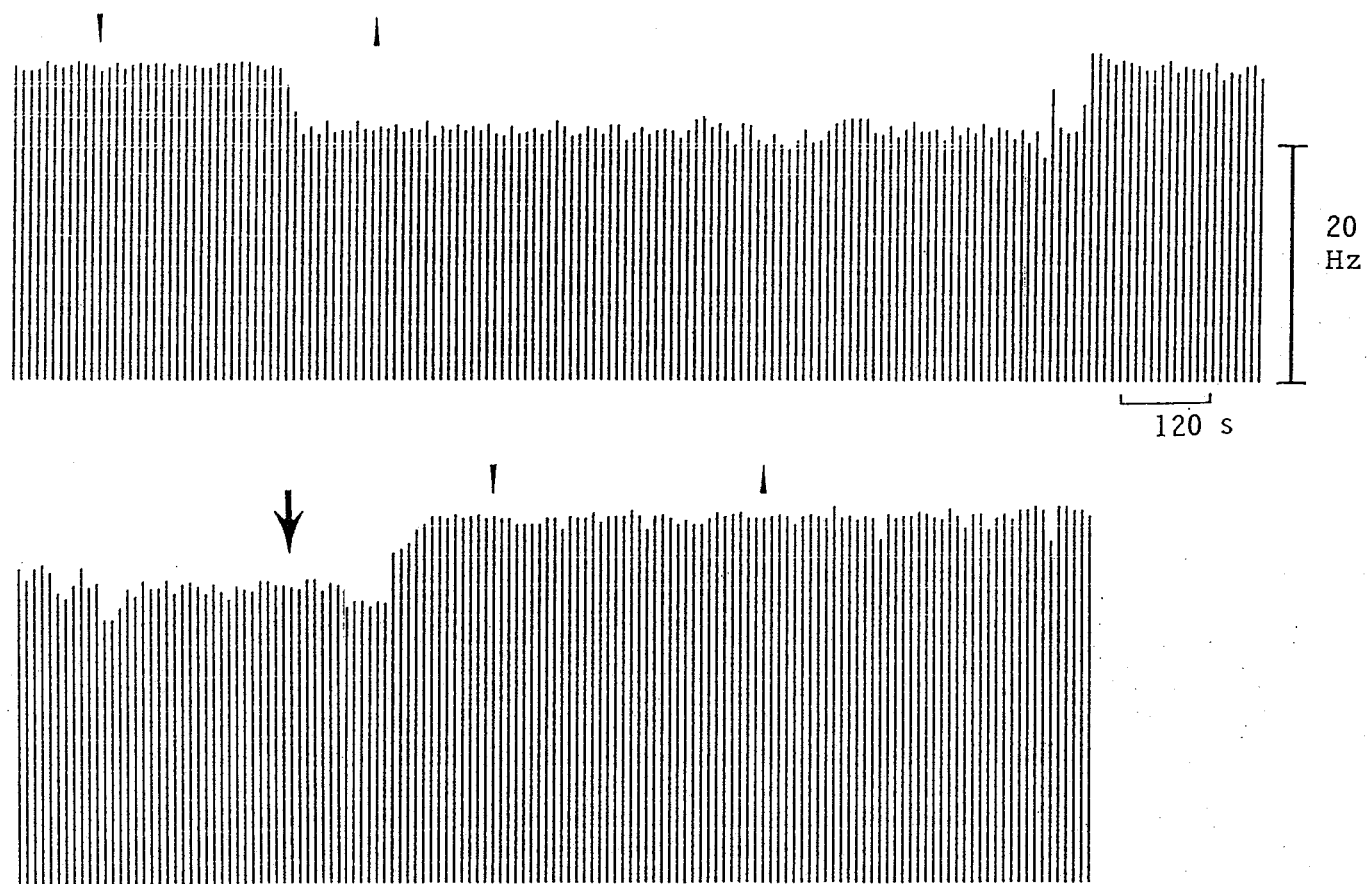


Fig. 38. Cyproheptadine blocks EA-evoked inhibition of a glutamate-driven NPF neurone.

DISCUSSION

EA would seem to activate many systems involving a number of neurotransmitters. Evidence for this is that antagonists for the following neurotransmitters have been shown to block EA: GABA and glycine (McLennan et al., 1977), substance P (Romita et al., 1992), acetylcholine and catecholamines (Han, 1986). This study focuses on a pathway involving of 5-HT.

This study shows that EA applied bilaterally for 10 min in the rat produces a reproducible long-lasting inhibition of nociceptive transmission in the spinal cord. This is reflected by an inhibition of WDR dorsal horn neurones and a prolongation of the tail-flick reflex latency which parallel each other in time. Thus the nociceptor-evoked activity in these dorsal horn neurones was inhibited following EA even though the receptive field was exposed to the noxious heat for a longer period of time.

The data from this study may be compared to a similar study reported by Pomeranz and Cheng (1979) in the anaesthetized cat. The intensity of the EA stimulus appears to be comparable in the two studies although Pomeranz and Cheng (1979) stimulated for a period of 30 min compared to 10 min in the present study. I found the magnitude of the nociceptor-evoked activity of WDR spinal cord neurones to be depressed to approximately 50% of the control value whereas Pomeranz and Cheng (1979) reported a mean depression of 16%. They speculated that this minimal EA effect was due to poor acupuncture point placement of the

needles or to excessive depth of chloralose anaesthesia. It is a common finding clinically that complete failure of EA analgesia occurs if the needles are inappropriately placed. Another possibility for the difference seen in the two studies is the difference in EA points used: Pomeranz and Cheng (1979) used Futo (Stomach 32) and Yangling (Gallbladder 34) ipsilaterally, whereas I used Zusanli (stomach 36) and Shangjuxu (stomach 38) bilaterally. Pomeranz and Cheng (1979) found that the depression of these WDR neurones took 20 min of EA stimulation to reach the peak and 20 min to wear off. The protocol used in the present study did not permit a determination of the onset of the EA-induced spinal cord inhibition although NPF neurones were inhibited after 4 - 8 min of stimulation. Similarly, the WDR neuronal activity returned to control levels within about 20 min of EA offset. Another similarity between the two studies is that EA was found to be effective at acupuncture points and ineffective at non-acupuncture points.

Wu et al. (1986), in chloralose anaesthetized cats, applied EA at 5 Hz for 15-20 min at various points. Although they demonstrated good inhibition of spinal cord WDR neurones during EA, the effect was eliminated almost immediately following the cessation of stimulation. This is surprising since they seemed to use a rather intense stimulus which they described as "generally below 3 mA". Their tests for a supraspinal involvement on only 2 neurones were inconclusive.

Thus it is clear that the results of the present study much more closely resemble those reported by Pomeranz and Cheng (1979) than those described by Wu et al. (1986). In particular, the prolonged time course of the inhibition following EA was comparable in this study to that of Pomeranz and Cheng (1979). It is also consistent with prolonged effects observed in other reported animal and human studies (McLennan et al., 1977; Yee, 1973).

Opinions differ as to whether effective EA can be elicited by activating only A β fibres or whether it is necessary to involve A δ and C-fibres as well. The present results clearly favour the former in agreement with Liu et al. (1986), Pomeranz and Paley (1979) and Toda and Ichioka (1978). All these investigators used a relatively mild EA stimulus which was just sufficient to produce muscular contraction. On the other hand, at least in experimental animals, it seems clear that high intensity stimulation, activating A δ and C-fibres, does produce a greater inhibition (Bing et al., 1990; Chung et al., 1984a,b). Bing et al. (1990) argue, and the evidence supports the notion, that the acupuncture stimulus activates an inhibitory system known as "diffuse noxious inhibitory controls" (DNIC, LeBars et al., 1975, 1976, 1979a,b, 1980, 1986; Villanueva, et al., 1986a,b). Bing et al. (1990) used a manual form of acupuncture in which a needle was inserted at the Zusanli point to a depth of 0.5 - 1.0 cm and lifted, thrust and rotated in a clockwise and anticlockwise fashion. They found a similar magnitude and

time-course of the antinociceptive effect when an adjacent non-acupuncture point was stimulated. Thus, whether effective acupuncture is specific to acupuncture points would seem to depend on the intensity of the stimulus applied. However, one could also argue that, due to the close proximity of the non-acupuncture point to the acupuncture point in the Bing et al. (1990) study, it is quite possible that the intense manual stimulus at the non-acupuncture point also stimulated the acupuncture point.

Bing et al. (1990) felt that their stimulus was noxious and activated A δ and C-fibres. It is also important to note that the onset of the inhibition in the studies by Bing et al. (1990) and Chung et al. (1984a,b) occurred almost immediately after the onset of the stimulus. With milder forms of acupuncture stimuli there tends to be a delay of several minutes to reach peak effects (Chan and Fung, 1975; Chapman et al., 1980; Cheng and Pomeranz, 1980; McLennan, et al., 1977; Pomeranz and Cheng, 1979; Takeshige, et al., 1981, 1985). Bao et al. (1989) found C-fibre activation was not important in acupuncture analgesia but was for DNIC. The stimulation parameters used for clinical acupuncture vary widely but I feel it is safe to say that in the great majority of cases, and in my personal practice, the intensity of the stimulus is not considered noxious to the patient.

I found that the spinal cord effects of EA were eliminated if conduction in the spinal cord was blocked suggesting the involvement of

a supraspinal loop in the mediation of the spinal cord inhibition. These effects are consistent with the results of Pomeranz et al. (1977 and 1979) and Shen, et al. (1975) in the cat.

Cold-blocking the spinal cord produced a decrease in the latency of the TF-EMG and enhanced the nociceptor-evoked activity of dorsal horn neurones. This is consistent with the previous finding of Necker and Hellon (1978) and Sinclair et al. (1988) and is thought to be the consequence of blocking the tonic descending inhibition in the spinal cord. However, this enhanced responsiveness in the cold-blocked state would not seem to be responsible for blocking the effects of EA since EA was also ineffective when the responses were reduced by decreasing the intensity of the noxious stimulus.

Since the EA-induced increase in NCL dorsal raphe neurones was not blocked by bilateral lesions of the DLF but was eliminated by bilateral lesions of the VLT, it would seem that the ascending limb of the supraspinal loop was mediated by the VLT. This is in agreement with acupuncture studies by Li, et al. (1983) on spinal cord lesioned patients. On the other hand, the DLF would appear to be involved in the descending inhibition since the effects of EA on dorsal horn neurones and the tail-flick reflex were blocked after bilateral DLF lesions. Bilateral lesions of the ascending or descending pathways were necessary to block the EA-induced spinal cord inhibition. This was not unexpected since EA was also applied bilaterally. My findings on the location of

the ascending and descending pathways are in agreement with the conclusion reached by Shen, et al. (1975) in studies on cats.

The above experiments were difficult in that movement induced by the onset of the current often resulted in the "loss" of the cell. This occurred in about 40% of the cells studied and the data were discarded in such cases. However, due to the rigid fixing of the animal in the stereotaxic headholder and spinal frame and perhaps due to the use of carbon-fibre recording electrodes, I was successful in many cases.

The findings obtained in my work in urethane anaesthetized rats are in agreement with most investigators in that CL DRN neurones are unresponsive to natural peripheral stimuli (Nakahama, et al., 1981; Shima, et al., 1986, 1987). Aghajanian et al. (1978) found that low intensity stimulation of the sciatic nerve produced a transient inhibition of these CL cells. They found that the response rapidly adapted with a higher frequency of stimulation which perhaps partially accounts for the failure to observe the effect upon natural stimulation. The slow rate of discharge by these cells also makes it difficult to observe an inhibition. However, although inconsistent with my work, Sanders et al. (1980) found many DRN units were inhibited by noxious stimuli in chloralose or pentobarbital anaesthetized rats.

Aghajanian and his group did not concentrate on the NCL DRN neurones although they reported that some exhibited an excitation to a

sciatic nerve stimulus which would not be in the noxious range (Aghajanian et al., 1978; Aghajanian and Haigler, 1974; Haigler, 1976).

Interestingly, the findings in the work reported here are very similar to that reported by Shima and his colleagues in the anaesthetized (Nakahama, et al., 1981) or conscious cat (Shima, et al., 1986). A common finding was that CL neurones were not responsive to natural stimuli and about half of the irregularly discharging cells were responsive to noxious stimuli. They termed the latter NCL neurones. Generally the cells responding to a noxious stimulus also responded to a non-noxious stimulus. Therefore, perhaps it is not surprising that the great majority (12/15) of NCL neurones tested in my study were facilitated by EA. The magnitude of the facilitation was not great, approximately doubling the normally slow discharge rate of the cell. The onset of this facilitation occurred only after a few min of EA stimulation.

The finding that only NCL neurones responded to EA and noxious stimuli is quite different from the report by the Beijing group (1986). They reported that approximately half of the regularly firing neurones, or in my terminology, the CL neurones, were facilitated by EA. In addition, they found that only 2 of the 14 neurones facilitated by EA were activated by noxious stimuli.

Although the present work was not concerned with investigating whether NCL DRN neurones are involved in modulating spinal cord sensory

transmission, there is ample evidence for the involvement of DRN in descending systems. One of the major relay sites in this descending projection is the nucleus raphe magnus (NRM). Interestingly, the NRM has been implicated in EA. Liu et al. (1986) reported that EA applied to the "Zusanli" point activated NRM neurones and inhibited spinal cord nociceptive responses in dorsal horn neurones. Du and Chao (1976) found that a lesion in the medulla which included the NRM significantly decreased the inhibitory effect of EA on the viscerosomatic reflex. Therefore it seems quite possible that the NCL neurones in the DRN which are activated by EA project to brainstem nuclei such as the NRM which, in turn, project to the spinal cord to inhibit nociceptive transmission. Literature reports clearly show that this projection is predominantly in the DLF. Thus, the finding that the effects of EA on the spinal cord are blocked by DLF lesions fits with the involvement of this descending system in EA.

It seems likely that neurones ascending in the SMT participate in the activation of NCL DRN neurones by noxious stimuli and EA. I have shown that this activation takes place after lesions in the DLF but is abolished by lesions in the VLT where SMT neurones project. As previously mentioned SMT neurones project directly to the PAG.

As mentioned above the DRN of the PAG has been implicated as the upper tier in a descending antinociceptive system (Basbaum and Fields, 1979). However the DRN clearly has rostral projections to many areas

that have also been implicated in nociception or antinociception. My work examined the connection between the DRN and the NPF of the medial thalamus and the influences of EA on this pathway. NPF neurones exhibiting the characteristics described by Andersen and Dafny (1983a) were relatively easy to locate and monitor for long periods of time. Cells were selected for study only if they were excited by noxious radiant heat applied to the tail. As was the case with Andersen and Dafny (1983a) and Benabid et al. (1983), I found a number of NPF neurones responded to a noxious stimulus with an inhibition. These neurones, however, were not further examined. While histology revealed that the recording locations appeared to be within the NPF, the boundaries of this structure are not clearly defined and it is possible that some of the cells were in an adjacent medial thalamic nucleus. In any case the cells under study exhibited a rather vigorous response to noxious radiant heat of the tail; much more so than the NCL neurones of the DRN. Neurones in the STT are likely the major source of excitatory input to the NPF upon a noxious peripheral stimulus. Other pathways may also indirectly activate the NPF.

EA consistently inhibited this nociceptor-evoked activity. In studies where the background activity of the cells was increased with iontophoretic glutamate release, the onset of inhibition ranged from 4 - 8 min after initiating EA. There appeared to be a reciprocal relationship between the EA-induced inhibition of these NPF neurones and

the excitation of NCL neurones in the DRN suggesting that the activation of the DRN neurones was responsible for the NPF inhibition. The long delay in the onset of these effects would seem to rule out the activation of a neuronal pathway to account for the findings. Perhaps prolonged EA stimulation results in the release of a chemical in sufficient concentration to produce these effects. In this regard a number of investigators have found EA to release endogenous opioids (Cheng et al., 1979; Han, et al., 1986; He et al., 1979; He and Dong, 1983; He, 1987; Sjolund et al., 1977; Zhang, A. et al., 1986; Zhou, et al., 1982; Zou, et al., 1986;). Indeed, Shima et al., (1987) found the opiate, morphine, activated NCL DRN neurones. Moss et al. (1981) also reported that serotonin-containing neurones in DRN include numerous leucine-enkephalin immunoreactive cells. The finding by several groups that the opioid antagonist, naloxone, antagonizes the effects of EA is also consistent with this idea (Han, et al., 1986; Mayer, et al., 1976, 1977; Pomeranz, 1977; Pomeranz and Cheng, 1979; Zhou et al., 1986).

At first glance, it is difficult to reconcile with this proposal the finding that the NPF inhibition evoked by EA is often abrupt and sometimes appears in steps (eg. Fig 32 C and D). One would expect a more gradual onset and offset if the effect was due to a build up of a chemical substance. However, stimulation of the DRN also produces a prolonged inhibition of NPF neurones that is correlated with the duration of stimulation (Figs. 32 A,B; 33). Significantly, the onset

and offset of this inhibition is also abrupt. It is not known whether stimulation of the DRN produces a prolonged discharge of the activated cells which then suddenly stop. If this does not occur, the stimulus must produce a sustained state of activation, either within the NPF or in structures which impinge upon the NPF, where the net effect is inhibition.

Dafny and colleagues previously reported that DRN stimulation influences the activity of NPF neurones. In an early study they reported that DRN stimulation activated NPF neurones with a latency of 20-25 ms (McClung and Dafny, 1980). Later, Andersen and Dafny (1983a,b) reported an inhibitory effect. However there are differences between their findings and mine. For example, I found that DRN stimulation produced only inhibition in NPF neurones, whereas, Andersen and Dafny (1983a) found some neurones to be facilitated and that the higher the stimulus intensity the greater the likelihood of producing excitation (Andersen and Dafny, 1983b). They speculated that the excitation was due to current spread to the adjacent reticular formation. They used a bipolar concentric stimulating electrode with a 500 μm tip separation whereas I used a monopolar carbon-fibre electrode (8 μm diam, < 15 μm length) as the stimulating electrode. Another difference is that they appeared to produce a rather short period of inhibition in NPF neurones upon DRN stimulation. For example, Fig.8 in Andersen and Dafny (1983b) illustrates an inhibition of less than a 4 s using stimulation

parameters of a 2.0 s train, 20 Hz, 0.2 ms pulse width and 0.5 mA intensity. I found in glutamate-driven NPF neurones that a 2.0 s stimulus (10 Hz, 1.0 ms, 0.3 mA) produced a mean inhibition of 107 s.

In any case the inhibitory effect would appear to be mediated by serotonin. The evidence in my study in support of this is that the inhibitory effect of EA or DRN stimulation on NPF neurones is eliminated in animals treated with the neurotoxin, 5,7-DHT, or the 5-HT antagonist, cyproheptadine. Also, the inhibitory effect is enhanced by the iontophoretic release of the specific 5-HT neuronal uptake blocker, alaproclate, at the recording sites. Finally, 5-HT iontophoretically applied to NPF neurones is inhibitory.

I am confident that the 5,7-DHT treatment was very effective in destroying 5-HT neurones in the DRN. The animals used all exhibited the reported behaviour of 5-HT depletion by 5,7-DHT, namely hyperactivity, hyperaggressiveness and weight loss. In addition, the histochemical examination of two animals confirmed the marked 5-HT depletion.

The conduction velocity of the DRN neurones projecting to the NPF ranged from 0.4 - 5.8 m/s with a mean of 1.85 m/s. Thus, these neurones would seem to have similar axonal diameters as the CL neurones which have a similar conduction velocity of 1 m/s and are therefore considered unmyelinated (Wang and Aghajanian, 1977a,b).

Alaproclate produced effects consistent with the blockade of 5-HT uptake. It increased the magnitude of the inhibition on NPF neurones by

iontophoretic 5-HT, DRN stimulation and EA. It is interesting that these inhibitions were enhanced using currents which produced no changes in the background activity. This is perhaps surprising since there is evidence (presented later) that the DRN serotonergic pathway to the NPF is tonically active. However, it must be remembered that the spacial distribution of alaproclate release differs from that of synaptically released 5-HT. Perhaps the presumably increased 5-HT release upon DRN stimulation or EA allowed for an interaction to occur between alaproclate and endogenous 5-HT. It would appear that the effect of alaproclate is specific for 5-HT in that the release at the same currents which enhanced the activity of 5-HT failed to alter the inhibitory effect of GABA.

Similarly, cyproheptadine, which was very effective in eliminating the effect of DRN stimulation or EA on NPF neurones, did not alter the inhibition produced by GABA.

Dafny and colleagues have also provided evidence that the projection from the DRN to the NPF involves 5-HT. Andersen and Dafny (1983b) also found that 5,7-DHT pretreatment enhanced the nociceptor-evoked activity of NPF neurones and blocked the DRN-induced inhibition. In addition, Andersen and Dafny (1982) reported that iontophoresis of 5-HT onto NPF neurones was inhibitory. More recently they showed that iontophoretic release of glutamate, morphine or 5-HT into the DRN had an inhibitory effect on nociceptor-evoked activity in NPF neurones. They

conclude that neurones which are activated by DRN stimulation and inhibit NPF neurones originate in the DRN (and are not axons *en passage*) and contain opioid and 5-HT receptors (Dafny et al., 1990).

Clearly the inhibition produced on NPF neurones by EA and DRN stimulation resemble each other very closely. They both exhibit a sudden onset, prolonged duration, sudden offset and respond in an identical manner to drugs which would be expected to alter 5-HT neuronal transmission. They differ in that DRN stimulation produces a rapid inhibition (found to be 6-12 ms in single pulse studies), whereas, EA-induced inhibition is delayed. Therefore, EA must elicit other effects which, in turn, activate DRN neurones to inhibit NPF cells.

The serotonergic-mediated inhibition of NPF neurones would appear to be tonically active. This is evident from the finding that 5,7-DHT pretreatment results in an increase in the spontaneous activity of NPF neurones and a marked increase to noxious stimuli. In addition, systemically administered cyproheptadine increases the background activity of these neurones. Finally, alaproclate, applied iontophoretically with high currents, inhibits the activity of these cells.

It would seem that a serotonin-mediated inhibitory pathway is the predominant pathway from the DRN to the NPF. Pretreating the animals with 5,7-DHT completely eliminates the inhibition and, in fact, converts the inhibition to a facilitation. Thus, there must also be an

excitatory pathway from the DRN to the NPF that is normally masked under my experimental conditions.

It is also clear that the NCL neurones of the DRN must be the neurones responsible for the inhibition of NPF neurones. All of the NCL neurones tested were shown electrophysiologically to have a direct projection to the NPF, whereas none of the other DRN neurones tested projected to this site. The probability of finding neurones which did project was likely increased by positioning the stimulating electrode in the NPF at a site where an NPF neurone could be shown to be inhibited by DRN stimulation, and secondly, using a relatively larger stimulating current (0.3 mA).

Since the evidence is good that the pathway is serotonergically-mediated and NCL neurones in the DRN are the projecting neurones, it follows that there are neurones other than CL neurones in the DRN which are a serotonergic. However, there seems to be no question that CL neurones are also serotonergic since I was unable to locate these neurones in 5,7-DHT pretreated rats. This was also the case in earlier work by Aghajanian (1972) and Andersen and Dafny (1983a) in 5,7-DHT and PCPA pretreated rats, respectively.

A recent report by Reichling and Basbaum (1991) is particularly pertinent to the present study. In a double-labeling study using two retrograde tracers they examined the projections from the rat PAG to the nucleus raphe magnus (NRM) and several forebrain structures. One of

these forebrain structures was the medial thalamus with the injection being centred in the NPF. Neurones labeled in the PAG were found in the ventrolateral, lateral, dorsal and ventral regions including the DRN. Approximately 75% of the retrogradely labeled neurones were located ipsilateral to the injection site. Interestingly, about 20 % of the PAG neurones labeled from the medial thalamus also projected to the NRM. Thus, it is possible that the same neurones modulate nociceptive transmission by both descending and ascending projections. In support of this idea, any treatment that influenced the nociceptor-driven neurones in the NPF influenced spinal cord WDR neurones in a like manner. For example, EA and DRN stimulation inhibit both. Similarly, the effects of stimulating the DRN on the NPF and spinal cord were both blocked in 5,7-DHT treated animals.

It could be argued that the inhibition in the NPF upon DRN stimulation in this study is simply due to activation of a descending inhibitory system which inhibits nociceptive transmission at the spinal cord level which, in turn, results in a reduced response at the NPF. Although I did not examine this possibility, Qiao and Dafny (1988) and Dafny et al. (1990) found the DRN to NPF inhibition still existed after sectioning the dorsal half the spinal cord.

A summary of the findings of this study are depicted schematically in Fig. 39. A noxious stimulus activates STT neurones and perhaps others which are excitatory to the NPF in the medial thalamus. The same

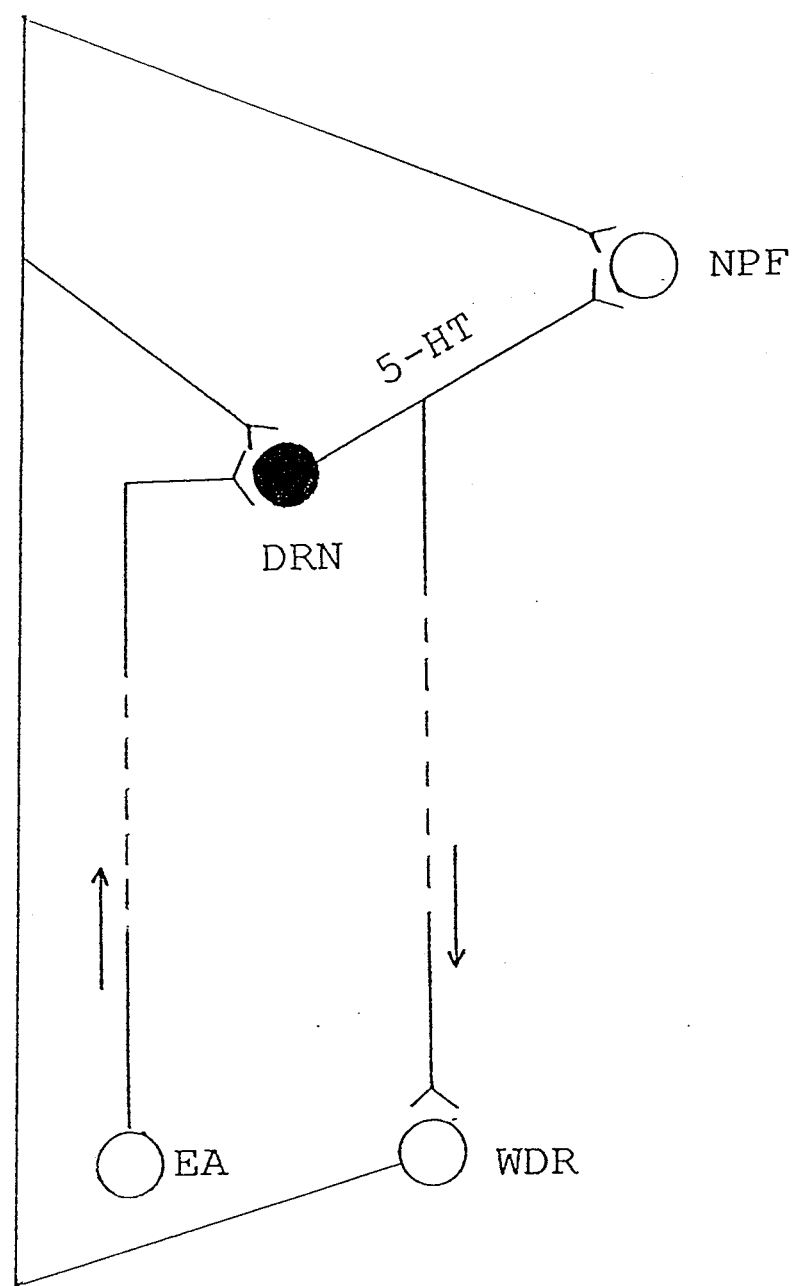


Fig. 39. Schematic arrangement of neurones activated by EA and a noxious stimulus. Filled circle indicates an inhibitory neurone.

stimulus probably activate SMT neurones which excite NCL neurones in the DRN. EA activates large diameter peripheral fibres. The activated neurones in the spinal cord project supraspinally through the VLT, and directly or indirectly activate NCL neurones in the DRN. These neurones are serotonergic in nature, project directly to the NPF and exert a tonic inhibitory effect on nociceptive NPF neurones. These DRN neurones also likely project to brainstem nuclei, such as the NRM, which are involved in a descending inhibitory projection to spinal cord nociceptive transmission via the DLF. Thus a supraspinal loop is involved in the EA suppression of nociceptive transmission in the spinal cord.

The most important discovery from this work is the identification of a group of neurones in the DRN that participate in the modulation of nociceptor-driven activity in the medial thalamus. These are neurones that exhibit irregular background activity, respond to natural peripheral stimuli and have been termed NCL neurones. The evidence is that these NCL DRN neurones are serotonergic, whereas, it was previously believed that only the CL neurones were serotonergic. There is considerable evidence in the literature that serotonergic neurones in the DRN participate in the modulation of nociception and therefore it was naturally assumed that the CL neurones were responsible. However, I found no evidence of CL neurones responding to noxious stimuli or EA. I

believe it is the NCL serotonergic neurones which are an important link in these inhibitory modulation system on nociceptive pathways.

Secondly, all the evidence from this work points to an important aspect of the antinociceptive activity of EA being the excitation of NCL DRN neurones. EA was found to be dependent on a serotonergic system and activated NCL but not CL neurones. It would also appear that the NCL neurones influence both descending and ascending system to modulate nociceptive pathways. Any treatment which altered NCL neuronal activity produced a similar effect on the EA-induced inhibition in the spinal cord and NPF.

CONCLUSIONS

1. Electroacupuncture (EA) stimulation at sites on "Zusanli" (st. 36) and "Shangjuxu" (st. 38) for 10 min (10 Hz, 1.0 ms) was found to produce a long-lasting inhibition of wide dynamic range (WDR) spinal cord dorsal horn neurones and to prolong the latency of the tail-flick reflex in the lightly anaesthetized rat.
2. Stimulation of acupuncture points "Zusanli" and "Shangjuxu" produced inhibition of WDR neurones. The same stimulation parameters applied to non-acupuncture points in the gastrocnemius muscle produced no inhibition.
3. This inhibition was effectively produced at an EA stimulation intensity which did not activate fibres smaller than AB.
4. There is a supraspinal involvement in the EA-induced inhibition of spinal cord nociceptive transmission.
5. The ascending arm of the supraspinal loop was found to be in the ventrolateral tract (VLT) and the descending arm is located in the dorsolateral funiculi (DLF).
6. Non-clock-like (NCL) neurones in the dorsal raphe nucleus (DRN) respond to both noxious and non-noxious stimuli but clock-like (CL) and non-clock-like-non-responding (NCLN) neurones do not respond to either.

Conclusions

7. EA facilitated the discharge of NCL neurones in the dorsal raphe nucleus (DRN) but not CL or NCLN neurones.
8. There is a direct projection of only NCL DRN neurones to the nucleus parafascicularis (NPF). All neurones displayed constant but widely ranging latency (0.6 ms to 9.0 ms). The conduction velocity was estimated to range from 0.40 - 5.8 m/s.
9. There are 3 types of spontaneously active NPF neurones: slow, bursting and fast firing. The slow and some bursting NPF neurones were facilitated by noxious stimulation.
10. EA inhibited the nociceptor-driven NPF neurones.
11. Simultaneously recording from a NCL neurone in the DRN and a NPF neurone revealed that EA produced an increase in the discharge rate of DRN neurones concomitantly with a decrease in the firing rate of NPF neurones.
12. The inhibition of NPF neurones produced by DRN stimulation had a sudden onset, offset and a prolonged time course that was correlated with the duration of stimulation. However, the magnitude of the inhibition was the same regardless of the period of stimulation.
13. The effect of single pulse DRN stimulation on glutamate-driven NPF neurones showed an inhibitory response in most cells tested with latencies ranging from 6.0 to 12.0 ms. These latencies were in a similar range to those found by antidromic activation of DRN neurones from the NPF.

Conclusions

14. Behavioral signs of hyperaggressiveness, hyperactivity as well as weight loss were observed in 5,7-dihydroxytryptamine (5,7-DHT) treated animals. There was also a marked reduction of 5-HT neurones in the DRN when examined using the indirect immunofluorescence technique. Only NCLN neurones were recorded in 5,7-DHT treated animals; CL and NCL neurones could not be found.
15. EA fails to inhibit NPF neurones in response to noxious radiant heat in 5,7-DHT treated animals.
16. Stimulation of the DRN fails to inhibit, but rather increases, the noxious stimulation-evoked activity in NPF neurones in 5,7-DHT treated animals.
17. 5-HT iontophoretically applied to glutamate-driven NPF neurones was inhibitory. Alaproclate, a 5-HT uptake inhibitor, when iontophoretically applied at low current (30 nA) failed to alter the activity of glutamate-driven NPF neurones but enhanced the inhibitory responses of 5-HT but not that of GABA.
18. The inhibitory response of NPF neurones to stimulation of the DRN or EA was enhanced by iontophoretically applied alaproclate at currents which do not alter the firing rate alone. Alaproclate iontophoretically applied at high current to glutamate-driven NPF neurones reduced the discharge rate.
19. Cyproheptadine, a serotonin antagonist, blocked the inhibition of NPF neurones produced by the stimulation of the DRN, but not the GABA-induced inhibition.

Conclusions

20. Cyproheptadine blocked the inhibition of glutamate-driven NPF neurones produced by EA.

SUMMARY

Electroacupuncture (EA) applied to "Zusanli" (st. 36) and "Shangjuxu" (st. 38) acupuncture points (10 min, 10 Hz, 1.0 ms) was found to produce a long-lasting inhibition of wide dynamic range (WDR) in the spinal cord dorsal horn neurones and to prolong the latency of the tail-flick reflex in the lightly anesthetized rat. This inhibition was effectively produced at a stimulation intensity which not activate fibres smaller than AB. The effects of EA were eliminated by cold-blocking the spinal cord rostral to the recording site suggesting a supraspinal involvement in the EA-induced inhibition of spinal cord nociceptive transmission. EA also facilitated the discharge of non-clock-like (NCL) dorsal raphe neurones (DRN). Bilateral lesions of the ventrolateral tract (VLT), but not the dorsolateral funiculi (DLF), blocked this effect suggesting that the ascending arm of the loop is via the VLT. The descending arm is located in the DLF since bilateral lesions of the DLF blocked the spinal cord effects of EA.

Evidence in the literature suggests that the DRN may be involved in the above supraspinal loop as well as in an ascending inhibitory pathway to the nucleus parafascicularis (NPF). Examination of the DRN revealed there are three types of neurones: clock-like (CL), NCL and non-clock-like non-responding neurones (NCLN). The NCL neurones were excited by noxious and non-noxious natural peripheral stimuli as well as EA. The other neurones were non-responsive to these stimuli. NCL

neurones of the DRN were also antidromically activated by NPF stimulation indicating that the projection from the DRN to the NPF is direct. Stimulation of the DRN produced an inhibition of NPF neurones which had a sudden onset and offset and a duration that was correlated with the length of stimulation. EA also produced long-lasting inhibition of these cells. The inhibitory pathway from the DRN to the NPF, which is activated by EA and presumably mediated by NCL neurones, would appear to be serotonergic. The evidence favoring this idea is that the inhibition evoked by DRN stimulation or EA is enhanced by alaproclate (a 5-HT uptake blocker) and blocked by 5,7-dihydroxytryptamine (a 5-HT neurotoxin), or cyproheptadine (a serotonin antagonist).

BIBLIOGRAPHY

- Abrams, G. H., and Recht, L. Neuropeptides and their role in pain and analgesia. *Acupuncture and Electro-Therapeutics, Res., Int. J.* 7:105-121, 1982.
- Aghajanian, G. K. Influence of drugs on the firing of serotonin-containing neurons in brain. *Federation Proceedings* 31:91-96, 1972a.
- Aghajanian, G. K., Haigler, H. J. and Bloom, F. E. Lysergic acid diethylamide and serotonin; Direct actions on serotonin-containing neurones in rat brain. *Life Sci.* 11:615-622, 1972b.
- Aghajanian, G. K. and Haigler, H. J. L-Tryptophan as a selective histochemical marker for serotonergic neurones in single-cell recording studies. *Brain Res.* 81:364-372, 1974.
- Aghajanian, G. K., Wang, R. Y and Baraban, J. Serotonergic and non-serotonergic neuron of the dorsal raphe: reciprocal changes in firing induced by peripheral nerve stimulation. *Brain Res.* 153; 169-175, 1978.
- Ai, M. and Wu, Z. The influence of electroacupuncture on the ultrastructure of synapses in medial region of thalamus. In: "Advances in Acupuncture and Acupuncture Anaesthesia.", Ed., T. Chang, Tiantan Xili Beijing China. The People's Medical Publishing House, pp. 449, 1979.
- Aimone, L. D. and Gebhart, G. F. Stimulation-produced spinal cord inhibition from the midbrain in the rat is mediated by an excitatory amino acid neurotransmitter in the medial medulla. *J. Neuroscience*, 6:1803-1813, 1986.
- Aimone, L. D., Jones, S. L. and Gebhart, G. F. Stimulation-produced descending inhibition from the periaqueductal gray and nucleus raphe magnus in the rat: mediation by spinal monoamines but not opioids. *Pain* 31:123-136, 1987.
- Albe-Fessard, D. and Kruger, L. Duality of unit discharges from cat centrum medianum in responses to natural and electrical stimulation. *Neurophysiol.* 25:3-20, 1962.

Bibliography

- Andersen, E and Dafny, N. Microiontophoretically applied 5-HT reduces responses to noxious stimulation in the thalamus. *Brain Res*, 241:176-178, 1982.
- Andersen, E. and Dafny, N. An ascending serotonergic pain modulation pathway from the dorsal raphe nucleus to the parafascicularis nucleus of the thalamus. *Brain Res*. 269:57-67, 1983a.
- Andersen, E. and Dafny, N. Dorsal raphe stimulation reduces responses of parafascicular neurons to noxious stimulation. *Pain* 15:323-331.1983b.
- Anderson, S. A. and Holmgren, E. On acupuncture analgesia and the mechanism of pain. *American J. chinese Medicine*. 3:311-334, 1975.
- Armstrong, J. M. and Millar, J. Carbon fiber microelectrodes. *J. Neurosci. Meth.* 1:279-287, 1979.
- Asarch, K. B., Ransom, R. W. and Shih, J. C. 5-HT-1a and 5-HT-1b selectivity of two phenylpiperazine derivatives: evidence for 5-HT-1b heterogeneity. *Life Sci*, 36:1265-1271, 1985.
- Bao, H., Zhou, Z., Yu, Y. and Han, J. C fibre is not important in acupuncture analgesia but is for DNIC. *Acupuncture Res.*, 14:139-140, 1989.
- Basbaum, A., Clanton, C. H., and Fields, H. L. Opiate and stimulus-produced analgesia: functional anatomy of a medullospinal pathway. *Proceedings of the National Academy of Sciences of the United States of America* 73:4685-4688, 1976.
- Basbaum, A. I. and Fields, H. L. Endogenous pain control mechanism: review and hypothesis. *Ann. Neurol.* 4:451-462, 1978.
- Basbaum, A. I. and Fields, H. L. The Origin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat: Further studies on the anatomy of pain modulation. *J. Comp. Neurol.* 187:513-532, 1979.
- Basbaum, A. I., Clanton, C. H. and Fields, H. L. Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographic study of pain modulating systems. *J. Comp. Neurol.* 178, 209-224, 1978.
- Behbehani, M. M. and Fields, H. L. Evidence that an excitatory connection between the periaqueductal gray and nucleus raphe magnus mediates stimulation produced analgesia. *Brain Res*. 1979.

Bibliography

- Behbehani, M. M. and Pomeroy, S. L. Effect of morphine injected in periaqueductal gray on the activity of single units in nucleus raphe magnus of the rat. *Brain Res.* 149:266-269, 1978.
- Beijing Medical College, Dept. of Biophysics and Physiology. The effect of electro-acupuncture and some exogenous monoamines on unit discharges of the dorsal nucleus raphe. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press, pp. 93-97, 1986.
- Beitz, A. J. The organization of afferent projections to the midbrain periaqueductal grey of the rat. *Neuroscience*, 7:133-159, 1982.
- Benabid, A. L., Henriksen, S. J., McGinty, J. F. and Bloom, F. E, Thalamic nucleus ventro-postero-lateralis inhibits nucleus parafascicularis response to noxious stimuli through a non-opioid pathway. *Brain Res.* 280:217-231, 1983.
- Bennett, G. J. and Mayer, D. J. Inhibition of spinal cord interneurons by narcotic microinjection and focal electrical stimulation in the periaqueductal central gray matter. *Brain Res.* 172:243-257, 1979.
- Berge, O. G. Effects of 5-HT receptor agonists and antagonists on a reflex response to radiant heat in normal and spinally transected rats. *Pain* 13:253-266, 1982.
- Berge, O. G. Response latencies in the tail-flick test depend on tail skin temperature. *Neurosci. Lett.* 86:284-288, 1988.
- Bing, Z., Villanueva, L. and LeBars, D. Acupuncture and diffuse noxious inhibitory controls: Naloxone-reversible depression of activities of trigeminal convergent neurons. *Neuroscience* 37:809-818, 1990.
- Bjorklund, A., Baumgarten, H. G., and Nobin, A. Chemical lesioning of central monoamine axons by means of 5,6-dihydroxytryptamine and 5,7-dihydroxytryptamine. *Adv. Biochem. Psychopharmacol.* 10:13-33, 1974.
- Blomqvist, A. and Craig, A. D. Organization of spinal and trigeminal input to the PAG. In "The Midbrain Periaqueductal Gray Matter", Edited by Depaulis, A. and Bandler, R., Plenum Press, pp.345-363, 1990
- Bobillier, P., Petitjean, S. D., Ligier, M. and Seguin, S. Differential projections of the nucleus raphe dorsalis and nucleus raphe central as revealed by autoradiography. *Brain Res.* 85:205-210, 1975.

Bibliography

- Boivie, J. and Perl, E. R. Neural substrates of somatic sensation. In: "MTP international review of science, physiology series one." vol.3, neurophysiology, Ed, Hunt, C.C., University Park Press, Baltimore, pp.303-411, 1975.
- Bonate, P. L. Serotonin receptor subtypes: functional, physiological, and clinical correlates, *Clinical Neuropharmacol.* 14:1-16, 1991.
- Bradley, P. B., Engel, G. and Feniuk, W. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacol.* 25:563-566, 1986.
- Bradshaw, C. M., Stoker, M. J. and Szabadi, E. Comparison of the neuronal responses to 5-hydroxytryptamine, noradrenaline and phenylephrine in the cerebral cortex: Effects of haloperidol and methysergide. *Neuropharmacol.* 22:677-685, 1983.
- Bramwell, G. J. and Gonye, T. Responses of midbrain neurones to microiontophoretically applied 5-hydroxytryptamine: comparison with the response to intravenously administered lysergic acid diethylamine. *Neuropharmacol.* 15:457-461, 1976.
- Brockhaus, A. and Elger, C. E. Hypoanalgesic efficacy of acupuncture on experimental pain in man. Comparison of laser acupuncture and needle acupuncture. *Pain* 43:181-185, 1990.
- Brown, A. G. Cutaneous afferent fibre collaterals in the dorsal columns of the cat. *Exp. Brain Res.* 5:293-305, 1968.
- Brown, A. G. Effects of descending impulses on transmission through the spinocervical tract. *J. Physiol.* 219:103-125, 1971.
- Brown, A. G. Organization in the spinal cord: the anatomy and physiology of identified neurones. Springer-Verlag, Berlin, 1981.
- Brown, A. G. and Iggo, A. A quantitative study of cutaneous receptors and afferent fibres in the cat and rabbit. *J. Physiol.* 193:707-733, 1967.
- Brown, A. G. and Franz, D. N. Responses of spinocervical tract neurones to natural stimulation of identified cutaneous receptors. *Exp. Brain Res.* 7:231-249, 1969.
- Brown, A. G., Hamann, W. C. and Martin, H. F. Effects of activity in non-myelinated afferent fibres on the spinocervical tract. *Brain Res.* 98:243-259, 1975.

Bibliography

- Brown, A. G., Fyffe, R. E. W., Noble, R., Rose, P. K. and Snow, P. J. The density, distribution and topographical organization of spinocervical tract neurones in the cat. *J. Physiol.* 300:409-428, 1980.
- Burgess, P. R., Petit, D., and Warren, R. M. Receptor types in cat hairy skin supplied by myelinated fibers. *J. Neurophysiol.* 31:833-848, 1968.
- Burstein, R. Cliffer, K. D. and Giesler, G. J. Direct somatosensory projections from the spinal cord to the hypothalamus and telencephalon. *J. Neurosci.* 7:4159-4164, 1987.
- Burstein, R. Cliffer, K. D., and Giesler, G. J. Cells of origin of the spinohypothalamic tract in the rat. *J. Comp. Neurol.* 291:329-344, 1990.
- Cadden, S. W. Villanueva, L., Chitour, D. and LeBars, D., Depression of activities of dorsal horn convergent neurones by propriospinal mechanisms triggered by noxious inputs; Comparison with diffuse noxious inhibitory controls (DNIC). *Brain Res.* 275:1-11, 1983.
- Carstens, E. Inhibition of rat spinothalamic tract neuronal responses to noxious skin heating by stimulation in midbrain periaqueducal gray or lateral reticular formation. *Pain* 33:215-224, 1988.
- Carstens, E. and Campbell, I. G. Parametric and pharmacological studies of midbrain suppression of the hind limb flexion withdrawal reflex in the rat. *Pain* 33:201-213, 1988.
- Cervero, F., Iggo, A. and Molony, V. Responses of spinocervical tract neurones to noxious stimulation of the skin. *J. Physiol.* 267:537-558, 1977.
- Cervero, F. Somatic and visceral input to the thoracic spinal cord of the cat: Effects of noxious stimulation of the biliary system. *J. Physiol.* 337:51-67, 1983.
- Cervero, F. and Wolstencroft, J. A positive feedback loop between spinal cord nociceptive pathways and antinociceptive areas of the cat's brain stem. *Pain* 20:125-138, 1984.
- Chan, S. H. H. and Fung, S. J. Suppression of polysynaptic reflex by electro-acupuncture and a possible underlying presynaptic mechanism in the spinal cord of the cat. *Exp. Neurol.* 48:336-342, 1975.

Bibliography

- Chang, H. T. Interactions in thalamus of afferent impulses from acupuncture point and site of pain. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press, pp.21-30, 1986.
- Chang, K. L., Wei, C. P., Lin, Y. M., Chou, L. P., Chou, L. H., Lo, C. L. and Chang, T. C. Effect of stimulation of midbrain raphe nuclei on the discharge on pain sensitive cells in nucleus parafascicularis of the thalamus and its significance in acupuncture analgesia. *Acta Physiologica Sinica*. 31:209-218, 1979.
- Chaouch, A., Menetrey, D., Binder, D. and Besson, J. M. Neurons at the origin of the medial component of the bulbopontine spinoreticular tract in the rat: an anatomical study using horseradich peroxidase retrograde transport. *J. Comp. Neurol.* 214:309-320, 1983.
- Chapman, C. R., Chen, A. C. and Bonica, J. J., Effects of intrasegmental electrical acupuncture on dental pain: evaluation by threshold estimation and sensory decision theory. *Pain* 3:213-227, 1977.
- Chapman, C. R., Colpitts, Y. M., Benedetti, C., Kitaeff, R. and Gehrig, J. D. Evoked potential assessment of acupuncture analgesia: Attempted reversal with naloxone. *Pain* 9:183-197, 1980.
- Cheng, J., Ye, Z. and Ou, S. The effects of naloxone on the inhibitory action of electro-acupuncture upon cortical potentials evoked by tooth pulp stimulation in rabbits. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House, pp. 497, 1979.
- Cheng, S. S. R. and Pomeranz, B. Electroacupuncture analgesia could be mediated by at least two pain-relieving mechanisms: Endorphin and non-endorphin systems. *Life Sciences* 25:1957-1962, 1979.
- Cheng, S. S. R. and Pomeranz, B. Electroacupuncture analgesia is mediated by stereospecific opiate receptors and is reversed by antagonists of type 1 receptors. *Life Sciences* 26:631-638, 1980.
- Choi, J. J., Srikantha, K. and Wu, W. H. A comparison of electroacupuncture transcutaneous electrical nerve stimulation and laserphotobiostimulation on pain relief and glucocorticoid excretion. *Acupuncture and Electro-Therapeutics Res., Int. J.* 11:45-51, 1986.
- Christensen, B. N. and Perl, E. R. Spinal neurons specifically excited by noxious or thermal stimuli: marginal zone of the dorsal horn. *J. Neurophysiol.* 33:293-307, 1970.

Bibliography

- Chu, L. S. W., Yeh, S. D. J. and Wood, D. D. "Acupuncture manual , a western approach", Marcel Dekker, Inc, 1979.
- Chung, J. M., Fang, Z. R., Hori, Y., Lee, K. H. and Willis, W. D. Prolonged inhibition of primate spinothalamic tract cells by peripheral nerve stimulation. *Pain* 19:259-275, 1984a.
- Chung, J. M., Lee, K. H., Hori, Y., Endo, K. and Willis, W. D. Factors influencing peripheral nerve stimulation produced inhibition of primate spinothalamic tract cell. *Pain* 19:277-293, 1984b.
- Chung, K., Kevetter, G. A., Willis, W. D. and Coggeshall, R. E. An estimate of the ratio of propriospinal to long tract neurons in the sacral spinal cord of the rat. *Neurosci. Lett.* 44:173-177, 1984.
- Conrad, L., Christiana, C. A., Leonard, M and Plaff, D. W. Connections of the Median and dorsal raphe nuclei in the rat: An autoradiographic and degeneration study. *J. Comp. Neurol.* 156:179-206, 1974.
- Craig, A. D. Spinocervical tract cells in cat and dog, labeled by the retrograde transport of horseradish peroxidase. *Neurosci. Lett.* 3:173-177, 1976.
- Dafny, N. and Gildenberg, P. Morphine effects on spontaneous, nociceptive, antinociceptive and sensory evoked responses of parafasciculus thalami units in morphine naive and morphine dependent rats. *Brain Res.* 323:11-20, 1984.
- Dafny, N., Reyes-Vazquez-C and Qiao, J. T. Modification of nociceptively identified neurons in thalamic parafascicularis by chemical stimulation of dorsal raphe with glutamate, morphine, serotonin and focal dorsal raphe electrical stimulation. *Brain Res. Bull.* 24:717-23, 1990.
- Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta Physiol. Scand.* 62, suppl. 232:1-55, 1964.
- Davis, H. S. Possible dorsal horn output correlation with acupuncture anesthesia. *Proceedings of the NIH Acupuncture Conference.* Bethesda, MD., Feb. 28- Mar. 1, 1973.

Bibliography

- Dept. of Physiology, Hunan Medical College. Effect of peripheral stimulation and acupuncture on unit discharges in the nucleus raphe magnus of the cat. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House, pp. 352, 1979.
- Descarries, L., Watkins, K. C., Garcia, S. and Beaudet, A. The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study, J. Comp. Neuro. 207:239-254, 1982.
- Devore, J. L. Probability and statistics for engineering and the sciences. Brooks/Cole Publishing Company, pp 343-421, 1982.
- Dong, W. K., Ryu, H. and Wagman, I. H. Nociceptive responses of neurons in medial thalamus and their relationship to spinothalamic pathways. J. Neurophysiol. 41:1592-1613, 1978.
- Du, H. J. and Chao, Y. F. Localization of central structures involved in descending inhibitory effect of acupuncture on viscerosomatic reflex discharges. Scientia Sinica 19:137-148, 1976.
- Du, H. J., Chao, Y. F. and Zheng, R. K. Inhibitory effect of raphe stimulation on viscerosomatic reflexes in cat and its relationship with acupuncture analgesia. Acta Physiologica Sinica 30:1-9, 1978.
- Du, H. J. and Zhao, Y. F. Relationship between nucleus raphe magnus and higher brain regions in inhibition of viscerosomatic reflex. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed. T. Chang, Science Press, pp. 70-81, 1986.
- Duggan, R. M. An overview of completed research on acupuncture. J. Traditional. Acup. 2:43-50, 1978.
- Eide, P. K. and Hole, K. Acute and chronic treatment with selective serotonin uptake inhibitors in mice: effects on nociceptive sensitivity and response to 5-methoxy-N, N-dimethyltryptamine. Pain 32:333-340, 1988.
- El-Etr, A. A. and Pesch, R. N. Preliminary report on somatosensory cerebral evoked potentials during the stimulation of the Ho Ku point. Proceedings of the NIH Research Conference. Feb. 28- Mar. 1, 1973.

Bibliography

- Enevoldson, T. P. and Gordon, G. Spinocervical neurons and dorsal horn neurons projecting to the dorsal column nuclei through the dorsolateral fascicle: a retrograde HRP study in the cat. *Exp. Brain Res.* 75:621-630, 1989.
- Fang, F. G., Moreau, J. L. and Fields, H. L. Dose-dependent antinociceptive action of neurotensin microinjected into the rostroventromedial medulla of the rat. *Brain Res.* 420:171-174, 1987.
- Fields, H. L. and Anderson, S.D. Evidence that raphe-spinal neurons mediate opiate and midbrain stimulation-produced analgesia. *Pain* 5:333-349, 1978.
- Fields, H. L., Basbaum, A. I., Clanton, C. H. and Anderson, S. D. Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons. *Brain Res.* 126:441-453, 1977a.
- Fields, H. L., Clanton, C. H., and Anderson, S. D. Somatosensory properties of spinothalamic neurons in the cat. *Brain Res.* 120:49-66, 1977b.
- Fields, H. L. and Basbaum, A. I. Brainstem control of spinal pain-transmission neurons. *Ann. Rev. Physiol.* 40:217-48, 1978.
- Freeman, T. B., Campbell, J. N. and Long, D. M. Naloxone does not affect pain relief induced by electrical stimulation in man. *Pain* 17:189-195, 1983.
- Gaary, A. E. Acupuncture analgesia. *Acupuncture and Electro-Therapeutics Res., Int. J.* 1:219, 1975.
- Gallager, D. W. and Pert, A. Afferents to brain stem nuclei (brain stem raphe, nucleus reticularis pontis caudalis and nucleus gigantocellularis) in the rat as demonstrated by microtophoretically applied horseradish peroxidase. *Brain Res.* 144:257-275, 1978.
- Gasser, H. S. Unmyelinated fibers originating in dorsal root ganglia. *J. Gen. Physiol.* 33:651-690, 1950.
- Gaw, A. C., Chang, L. W. and Shaw, L. C. Efficacy of acupuncture on osteoarthritic pain. *New Eng. J. Med.* 293:375-378, 1975.
- Gebhart, G. F., Sandkuhler, J., Thalhammer, J. G. and Zimmermann, M. Inhibition in spinal cord of nociceptive information by electrical stimulation and morphine microinjection at identical sites in midbrain of the cat. *J. Neurophysiol.* 51:75-89, 1984.

Bibliography

- Ghia, J. N., Mao, W., Toomey, T. C. and Gregg, J. N. Acupuncture and chronic pain mechanisms. *Pain* 2:285-299, 1976.
- Giesler, G. J., Menetrey, D., Guilband, G. and Besson, J. Lumbar cord neurons at the origin of the spinothalamic tract in the rat. *Brain Res.* 118:320-324, 1976.
- Giesler, G. J., Cannon, J. T., Urca, G. and Liebeskind, J. C. Long ascending projections from substantia gelatinosa Rolandi and the subjacent dorsal horn in the rat. *Science* 202:984-986, 1978.
- Giesler, G. J., Menetrey, D. and Basbaum, A. Differential origins of spinothalamic tract projections to medial and lateral thalamus in the rat. *J. Comp. Neurol.* 184:107-126, 1979.
- Giesler, G. J., Spiel, H. R. and Willis, W. D. Organization of spinothalamic tract axons within the rat spinal cord. *J. Comp. Neurol.* 195:243-252, 1981.
- Giesler, G. J., Nahin, R. L. and Madsen, A. M. Postsynaptic dorsal column pathway of the rat. I. Anatomical studies. *J. Neurophysiol.* 51:260-275, 1984.
- Giesler, G. J. and Cliffer, K. D. Postsynaptic dorsal column pathway of the rat. II. evidence against an important role in nociception. *Brain Res.* 326:347-356, 1985.
- Glennon, R. Central serotonin receptors as targets for drug research. *J. Med. Chem.* 30:1-12, 1987.
- Greguss, P. A model for making acupuncture consistent with eastern concepts of biological information processing. *Proceedings from the NIH Acupuncture Conference, Feb. 28-Mar. 1, 1973.*
- Grossmann, W. and Jurna, I. Depression by morphine of activity in the ventrolateral tract evoked from cutaneous A-fibers. *Eur. J. Pharmacol.* 29:171-174, 1974.
- Grossman, M. L., Basbaum, A. I. and Fields, H. L. Afferent and efferent connections of the rat tail flick reflex (a model used to analyze pain control mechanisms). *J. Comp. Neurol.* 206:6-16, 1982.
- Guilbaud, G., Besson, J. M., Oliveras, J. L. and Liebeskind, J. C. Suppression by LSD of the inhibitory effect exerted by dorsal raphe stimulation on certain spinal cord interneurons in the cat. *Brain Res.* 61:417-422, 1973.

Bibliography

- Haigler, H. J. Morphine, ability to block neuronal activity evoked by a nociceptive stimulus. *Life Sciences*, 19:841-858, 1976.
- Hall, J. G., Duggan, A. W., Johnson, S. M. and Morton, C. R. Medullary raphe lesions do not reduce descending inhibition of dorsal horn neurones of the cat. *Neurosci. Lett.* 25:25-29, 1981.
- Hall, J. G., Duggan, A. W. Morton, C. R. and Johnson, S. M. The location of brainstem neurones tonically inhibiting dorsal horn neurones of the cat. *Brain Res.* 244:215-222, 1982.
- Han, J. Role of central neuro-transmitters in acupuncture analgesia: In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp. 241-255, 1986.
- He, L. Effect of iontophoretic etorphine and electroacupuncture on nociceptive response from nucleus lateralis anterior of thalamus in rabbits. *Acupuncture and Electro-Therapeutics Res., Int. J.* 6:151-157, 1981.
- He, L. Involvement of endogenous opioid peptides in acupuncture analgesia. *Pain* 31:99-121, 1987.
- He, L. and Dong, W. Activity of opioid peptidergic system in acupuncture analgesia. *Acupuncture Electro-Therapeutics Res., Int. J.* 8:257-266, 1983.
- He, L., Du, L., Jiang, J., Shi, Z. and Wang, M. Effects of naloxone on analgesia from caudate nucleus stimulation in rabbits. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House. pp. 480, 1979.
- He, L., Dong, W. Q. and Wang, M. Effects of iontophoretic etorphine and naloxone, and electroacupuncture on nociceptive responses from thalamic neurones in rabbits. *Pain* 44:89-95, 1991.
- He, S. Wang, P and Shen, K. Inhibitory effect of cord dorsum stimulation on evoked potential of bulbar reticular formation. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House. pp. 361, 1979.
- Henning, C. W., Steinhoff, W. C. and Braughler, S. A. Tonic immobility and the serotonergic system in chickens: differential blockade of receptors by cyproheptadine and cinanserin. *Physiology and Behavior*, 44:15-20, 1988.

Bibliography

- Holstege, G. Descending pathways from the periaqueductal gray and adjacent areas. In "The Midbrain Periaqueductal Gray Matter", Edited by Depaulis, A. and Bandler, R., Plenum Press. pp.239-265, 1991.
- Hosobuchi, Y., Rassier, J., Bloom, F. E. and Guillemin, R. Stimulation of human periaqueductal gray for pain relief increases immunoreactive β -endorphin in ventricular fluid. *Science*, 203, 279-281, 1977.
- Hung, C., Li, X., Zhang, C., Liu, S., Pan, X. and Zhou, Z. Effect electrical stimulation of midbrain periaqueductal gray on acupuncture analgesia in rats. *Acta Physiologica Sinica* 34:343-357, 1982.
- Hunskar, S., Berge, O. G., Broch, O. J. and Hole, K. Lesions of the ascending serotonergic pathways and antinociceptive effects after systemic administration of p-chloroamphetamine in mice. *Pharmacol. Biochem. Behav.* 24:709-714, 1986.
- Hynynen, K. H., Ketovouri, H., Mattila, M. A. K., Mattila, S., Nokso-Koivisto, V. M. and Pontinen, P. J. Heart-rate variation as a pain indicator in conscious subjects undergoing acupuncture analgesia. *Acupuncture and Electro-Therapeutics Res., Int. J.* 6:47-55, 1981.
- Jacob, W. L. A case of severe chronic depression treated with acupuncture. *The AAMA Rev.* 2:33-34, 1990.
- Jurna, I. Aminophylline differentiates between the depressant effects of morphine on the spinal nociceptive reflex and on the spinal ascending activity evoked from afferent C fibers. *Europ. J. Pharmacol.* 71:393-400, 1981.
- Kevetter, G. A. and Willis, W. D. Spinothalamic cells in the rat lumbar cord with collaterals to the medullary reticular formation. *Brain Res.* 238:181-185, 1982.
- Kevetter, G. A. and Willis, W. D. Collaterals of spinothalamic cells in the rat. *J. Comp. Neurol.* 215:453-464, 1983.
- Kneisley, L. W., Biber, M. P. and LaVail, J. H. Brain stem projections to the spinal cord using retrograde transport of horseradish peroxidase in Rhesus monkeys. *Neurology (Minneap.)* 26:350, 1976.
- Kuypers, H. G. J. M. and Maisky, V. A. Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. *Neurosci. Lett.* 1:9-14, 1975.

Bibliography

- LeBars, D., Menetrey, D., Conseiller, C. and Besson, J. M. Depressive effects of morphine upon lamina V cells activities in the dorsal horn of the spinal cat. *Brain Res.* 98:261-277, 1975.
- LeBars, D., Menetrey, D. and Besson, J. M. effects of morphine upon the lamina V type cells activities in the dorsal horn of the decerebrate rat. *Brain Res.* 113:293-310, 1976.
- LeBars, D., Dickenson, A. H. and Besson, J. M. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain* 6:283-304, 1979a.
- LeBars, D., Dickenson, A. H. and Besson, J. M. Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. *Pain* 6:305-327, 1979b.
- LeBars, D., Dickenson, A. H. and Besson, J. M. Microinjection of morphine within nucleus raphe magnus and dorsal horn neurones activities related to nociception in the rat. *Brain Res.* 189:467-481, 1980.
- LeBars, D., Dickenson, A. H., Besson, J. M. and Villanueva, L. Aspects of sensory processing through convergent neurons. In: "Spinal afferent processing", Ed., Yaksh. T.L., Plenum Press, New York pp. 467-504, 1986.
- Ledergerber, C. P. Electroacupuncture in obstetrics. *Acupuncture and Electro-Therapeutics Res., Int. J.* 2:105-118, 1976.
- Lee, P. K., Andersen, T. W. Modell, J. H. and Saga, S. A. Treatment of chronic pain with acupuncture. *JAMA.* 232:1133-1135, 1975.
- Lewis, V. A. and Gebhart, G. F. Evaluation of the periaqueductal central gray (PAG) as a morphine-specific locus of action and examination of morphine-induced and stimulation-produced analgesia at coincident PAG loci. *Brain Res.* 124:283-303, 1977.
- Li, S., Jiang C and Chen, G. The relationship between needling sensation and acupuncture effects, with special reference to their ascending pathway in the spinal cord. *Acupuncture and Electro-Therapeutics Res., Int. J.* 8:105-110, 1983.
- Liebeskind, J. C., Guilbaud, G., Besson, J. M. and Oliveras, J. L. Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. *Brain Res.* 50:441-446, 1973.

Bibliography

- Liebeskind, J. C., Guilbaud, G., Besson, J. M. and Oliveras, J. L. Analgesia from electrical stimulation of the periaqueductal gray matter in the cat; behavioral observations and inhibitory effects on spinal cord interneurons. *Brain Res.* 269:57-67, 1983.
- Light, A. R., and Perl, E. R. Central termination of identified cutaneous afferent units with fine myelinated fibers. *Soc. Neurosci, Abstr.* 3, 486, 1977.
- Light, A. R., and Perl, E. R. Reexamination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibers. *J Comp. Neurol.* 186:117-132, 1979.
- Lindberg, U. H., Thorberg, S. O., and Bengtsson, S. Inhibitors of neuronal monoamine uptake. 2. selective inhibition of 5-hydroxytryptamine uptake by α -amino acid esters of phenethyl alcohols. *J. Med. Chem.* 21:448-456, 1978.
- Lin, B., Lui, Z., Wu, C., Tang, J., Liu, L. and Zhu, H. The effects of cauterization of supraoptic nucleus area and the mammillary complex of the rats on analgesic effect of electric needling of "Zusanli". In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House, pp.335, 1979.
- Linzer, M. and Atta, L. V. The Electrophysiological assessment of acupuncture's effect on single thalamic neuron in the cat: neural coding for pain at a thalamic level. *Proceedings of the NIH Acupuncture Research Conference*, Feb. 28-Mar. 1, 1973.
- Liu, R. P. Laminar origins of spinal projection to the periaqueductal gray of the rat. *Brain Res.* 264:118-122, 1983.
- Liu, X., Zhu, B. and Zhang S. Relationship between electroacupuncture analgesia and descending pain inhibitory mechanism of nucleus raphe magnus. *Pain* 24:383-396, 1986.
- Lloyd, D. P. C. and Chang, H. T. Afferent fibers in muscle nerves. *J. Neurophysiol.* 11:199-208, 1948.
- Looney, G. L. Acupuncture for sensorineural hearing impairment. *Proceedings of the NIH Acupuncture Research Conference*, Feb. 28-Mar. 1, 1973a.
- Looney, G. L. Effect of acupuncture anesthesia upon peripheral vascular hemodynamics. *Proceedings of the NIH Acupuncture Research Conference*, Feb. 28-Mar. 1, 1973b.

Bibliography

- Looney, G. L. A neurologic basis for acupuncture (autonomic theory)(No. 3). Acupuncture and Electro-Therapeutics Res., Int. J. 1:210, 1975.
- Lu, H. C. "The Yellow Emperor", a translated acupuncture book, Academy of Oriental Heritage, 1973.
- Lund, R. D. and Webster, K. E. Thalamic afferents from the dorsal column nuclei. an experimental anatomical study in the rat. J. Comp. Neurol. 130:301-311, 1967.
- Luo, F., Yuan, J., Yang, S., and Zhang, X. Inhibitory effect on nociceptive discharges of parafascicular nucleus of thalamus by electrical stimulation. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House. pp. 333, 1979.
- Matsumoto, T., Ambruso, V., Martin, F. and Hayes, Jr. Effect of acupuncture: I. Gastrointestinal atony following vagotomy. Proceedings from the NIH Acupuncture Research Conference, Feb. 28-Mar. 1, 1973.
- Maunz, R. A., Pitts, N. G. and Peterson, B. W. Cat spinoreticular neurons: locations, responses and changes in responses during repetitive stimulation. Brain Res. 148:365-379, 1978.
- Mayer, D. J. and Liebeskind, J. C. Pain reduction by focal electrical stimulation of the brain: an anatomical and behavioral analysis. Brain Res. 68:73-93, 1974.
- Mayer, D. J., Price, D. D., Barber, J. and Rafii, A. Acupuncture analgesia: Evidence for activation of a pain inhibitory system as a mechanism of action. In:"Advances in Pain Research and Therapy" Ed., Bonica, J. J. and Albe-Fessard, D., Raven Press, New York, Vol.I. pp. 751-754, 1976.
- Mayer, D. J., Price, D. D. and Rafii, A. Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. Brain Res. 121:368-372, 1977.
- McClung, R. E. and Dafny, N. The parafascicular nucleus of the thalamus exhibits convergence input from the dorsal raphe and the spinal tract of the trigeminal nerve. Brain Res. 525-531, 1980.
- McLennan, H., Gilfillan, K. and Heap, Y. Some pharmacological observations on the analgesia induced by acupuncture in rabbits. Pain 3:229-238, 1977.

Bibliography

- Menetrey, D., Chaouch, A. and Besson, J. M. Location and properties of dorsal horn neurons at origin of spinoreticular tract in lumbar enlargement of the rat. *J. Neurophysiol.* 44:862-877, 1980.
- Mosko, S. S. and Jacobs, B. L. Midbrain raphe neurons: spontaneous activity and response to light. *Physiol. and Behav.* 13:589-593, 1974.
- Nakahama, H., Shima, K., Yamamoto, M. and Aya, K. Regularity of the spontaneous discharge of neurons in the nucleus raphe dorsalis of the cat. *Neurosci. Lett.* 23: 161-165, 1981.
- Necker, R. and Hellon, R. F. Noxious thermal input from the rat tail: modulation by descending inhibitory influences. *Pain* 4:231-242, 1978.
- Numoto, M. N. and Donaghy, R. M. P. Physiological responses to acupuncture. *Proceedings of the NIH Acupuncture Research Conference*, Feb. 28-Mar. 1, 1973.
- Nyquist, J. K. and Greenhoot, J. H. Unit analysis of nonspecific thalamic responses to high intensity cutaneous input in the cat. *Exp. Neurol.* 42:609-622, 1974.
- Ogren, S. O. and Holm, A. C. Test-specific effects of the 5-HT reuptake inhibitors alaproclate and zimelidine on pain sensitivity and morphine analgesia. *J. Neural. Trans.* 47:253-271, 1980.
- Ogren, S. O., Holm, A. C., Hall, H. and Lindberg, U. H. Alaproclate, a new selective 5-HT uptake inhibitor with therapeutic potential in depression and senile dementia. *J. Neural. Trans.* 59:265-288, 1984.
- Ogren, S. O. and Berge, O. G. Evidence for selective serotonergic receptor involvement in p-chloroamphetamine-induced antinociception. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 329:135-140, 1985.
- Ogren, S. O., Nordstrom, O., Danielsson, E., Peterson, L. L. and Bartfai, T. In vivo and in vitro studies on the potentiation of muscarinic receptor stimulation by alaproclate, a selective 5-HT uptake blocker. *J. Neural. Transm.* 61:1-20, 1985.
- Oleson, T. D. and Kroening, R. J. A comparison of Chinese and Nogier auricular acupuncture points. *Amer. J. Acup.* 11:3, 1983.

Bibliography

- Oleson, T. D., and Liebeskind, J. C. Modification of midbrain and thalamic evoked responses by analgesic brain stimulation in the rat. In "Advances in Pain Research and Therapy" ed. Bonica and Albe-Fessard. Raven Press, New York, 1:487-494, 1976.
- Oleson, T. D., Twombly, D and Liebeskind, J. D. Effects of pain-attenuating brain stimulation and morphine on electrical activity in the raphe nuclei of the awake rat. *Pain* 4:211-230, 1978.
- Oliveras, J. L., Besson, J. M., Guilbaud, G. and Liebeskind, J. C. Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. *Exp. Brain Res.* 20:32-44, 1974.
- Oliveras, J. L., Redjemi, F., Guilbaud, G and Besson, J. M. Analgesia induced by electrical stimulation of the inferior centralis nucleus of the raphe in the cat. *Pain* 1:139-145, 1975.
- Oliveras, J. L., Guilbaud, G. and Besson, J. M. A map of serotonergic structures involved in stimulation producing analgesia in unrestrained freely moving cats. *Brain Res.* 164: 317-322, 1979.
- Olpe, H. R. The cortical projection of the dorsal raphe nucleus: some electrophysiological and pharmacological properties. *Brain Res.* 216:61-71, 1981.
- Ornstein, D. B. "The Complete Book of Acupuncture", Celestial Arts, 1976.
- Paxinos, G and Watson, C. The rat brain in stereotaxic coordinates, Academic Press, 1982.
- Pearl, G. S. and Anderson, K. V. Responses patterns of cells in the feline caudal nucleus reticularis gigantocellularis after noxious trigeminal and spinal stimulation. *Exp. Neurol.* 58:231-241, 1978.
- Pechura, C. and Liu, R. Spinal neurons which project to the periaqueductal gray and the medullary reticular formation via axon collaterals: a double-label fluorescence study in the rat. *Brain Res.* 374:357-361, 1986.
- Peets, J. M. and Pomeranz, B. Studies in suppression of nociceptive reflexes measured with tail flick electromyograms and using intrathecal drugs in barbiturate anesthetized rat. *Brain Res.* 416:301-307, 1987.

Bibliography

- Peroutka, S. J. Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} binding sites in rat frontal cortex. *J. Neurochem.* 47:529-540, 1986.
- Peschanski, M., Guilbaud, G. and Gautron, M. Posterior intralaminar region in rat: neuronal responses to noxious and non-noxious cutaneous stimuli. *Exp. Neurol.* 22:226-238, 1981.
- Peschanski, M., Mantyh, P. W. and Besson, J. M. Spinal afferents to the ventrobasal thalamic complex in the rat: an anatomical study using wheat-germ agglutinin conjugated to horseradish peroxidase. *Brain Res.* 278:240-244, 1983.
- Peschanski, M. and Besson, J. M. A spino-reticulo-thalamic pathway in the rat: an anatomical study with reference to pain transmission. *Neuroscience* 12:165-178, 1984.
- Philips, C. M. and Rusy, B. F. Electrical characteristics of two Chinese stimulators. *Proceedings of the NIH Acupuncture Research Conference*, Feb. 28- Mar. 1, 1973.
- Pierce, E. T., Foote, W. E. and Hobson, A. E. The efferent connection of the nucleus raphe dorsalis. *Brain Res.* 107:137-144, 1976.
- Pomeranz, B. Specific nociceptive fibers projecting from spinal cord neurons to the brain: a possible pathway for pain. *Brain Res.* 50:447-451, 1973.
- Pomeranz, B. Brain's opiates at work in acupuncture? *New Sci.* Jan. 6, 1977.
- Pomeranz, B. and Cheng, R. Suppression of noxious responses in single neurons of cat spinal cord by electroacupuncture and its reversal by the opiate antagonist naloxone. *Exp. Neurol.* 64:327-341, 1979.
- Pomeranz, B., Cheng, R. and Law, P. Acupuncture reduces electrophysiological and behavioral responses to noxious stimuli: pituitary is implicated. *Exp. Neurol.* 54:172-178, 1977.
- Pomeranz, B. and Paley, D. Electroacupuncture hypoalgesia is mediated by afferent nerve impulses: An electrophysiological study in mice. *Exp. Neurol.* 66:398-402, 1979.
- Pomeroy, S. L. and Behbehani, M. M. Physiologic evidence for a projection from periaqueductal gray to nucleus raphe magnus in the rat. *Brain Res.* 176:143-147, 1979.

- Proudfit, H. K. and Anderson, E. G. Morphine analgesia: blockade by raphe magnus lesions. *Brain Res.* 98:612-618, 1975.
- Pycock, C. J., Burns, S. and Morris, R. In vitro release of 5-hydroxytryptamine and α -aminobutyric acid from rat periaqueductal grey and raphe dorsalis region produced by morphine or an enkephalin analogue. *Neurosci. Lett.* 22:313-317, 1981.
- Qiao, J. T. and Dafny, N. Dorsal raphe stimulation modulates nociceptive responses in thalamic parafascicular neurones via an ascending pathway: further studies on ascending pain modulation pathways. *Pain* 34:65-74, 1988.
- Qiao, J. T., Xie, Y. F. and Chang, H. Y. Supraspinal mechanism involved in the inhibition of parafascicular neurons by periaqueductal gray stimulation in rabbits. *Acta Physiol. Sin.* 35: 226-230, 1983.
- Ren, M. and Han, J. Rat tail flick acupuncture analgesia model. *Chinese Medical J.* 92:576-582, 1979.
- Reichling, D. B. and Basbaum, A. I., Collateralization of periaqueductal gray neurons to forebrain or diencephalon and to the medullary nucleus raphe magnus in the rat. *Neuroscience* 42:183-200, 1991.
- Rexed, B. The cytoarchitectonic organization of the spinal cord in the cat. *J. Com. Neurol.* 96:415-466, 1952.
- Reynolds, D. V. Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science* 164:444-445, 1969.
- Richter, J. A., Baum, M., Kunkel, R., Bauerle, A., Heimisch, W., Amereller, H., Schumacher, K., Erdmann, K. and Bojzewicz, U. V. Clinical experience with electrical acupuncture analgesia in 125 patients undergoing open heart surgery. *Acupuncture Electro-Therapeutics. Res., Int. J.* 1:143-156, 1975.
- Romita, V. V., Yashpal, K., Hui-Chan, C. and Henry, J. L. Electroacupuncture analgesia in the lightly anaesthetized rat is blocked by naloxone and by the novel substance P antagonist, CP-96,345. *Canadian Pain Soc. Annual Meeting*, May 14-16, 1992.
- Ross, S. B. and Renyi, A. L. Inhibition of the neuronal uptake of 5-hydroxytryptamine and noradrenaline in rat brain by (Z)- and (E)-3-(4-bromophenyl)-N, N-dimethyl-3-(3-pyridyl) allylamines and their secondary analogues. *Neuropharmacol.* 16:57-63, 1977.

Bibliography

- Ruda, M. A. An autoradiographic study of the efferent connections of the mid-brain central gray in the cat. Ph.D. Dissertation, University of Pennsylvania, 1975.
- Samanin, R., Gumulka, W. and Valzelli, L. Reduced effect of morphine in midbrain raphe lesioned rats. *Eur. J. Pharmacol.* 10: 339-343, 1970.
- Samanin, R., Ghezzi, D., Maunon, C. and Valzelli, L. Effect of midbrain raphe lesion on the antinociceptive action of morphine and other analgesics in rats. *Psychopharmacol. (Berl.)* 33:365-368, 1973.
- Samanin, R. and Valzelli, L. Increase of morphine-induced analgesia by stimulation of the nucleus raphe dorsalis. *Eur. J. Pharmacol.* 16:298-302, 1971.
- Sanders, K. H., Klein, C. E., Mayer, T. E., Heym, C. H. and Handwerker, H. O. Differential effects of noxious and non-noxious input on neurones according to location in ventral periaqueductal grey or dorsal raphe nucleus. *Brain Res.* 186:83-97, 1980.
- Segal, M. Physiological and pharmacological evidence for a serotonergic projection to the hippocampus. *Brain Res.* 94:115-131, 1975.
- Segal, M. 5-HT antagonists in rat hippocampus, *Brain Res.* 103:161-166, 1976.
- Shen, E. Participation of Descending Inhibition in acupuncture analgesia. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp.31-38, 1986.
- Shen, E., Tsai, T. T. and Lan, C. Supraspinal participation in the inhibitory effect of acupuncture on viscerosomatic reflex discharges. *Chinese Medical J.* 1:431-440, 1975.
- Shen, E., Ma, W. H. and Lan, C. Involvement of descending inhibition in the effect of acupuncture on the splanchnically evoked potential in the orbital cortex of cat. *Sci. Sinica.* 21:677-685, 1978.
- Shen, K., He, S. and Wang, P. Effect of cord dorsum stimulation on EEG and behavior in unrestrained rabbits. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp. 185-194, 1986.

Bibliography

- Shima, K., Nakahama, H. and Yamamoto, M. Firing properties of two types of nucleus raphe dorsalis neurons during the sleep-waking cycle and their responses to sensory stimuli. *Brain Res.* 399:317-326, 1986.
- Shima, K., Nakahama, H., Yamamoto, M., Aya, K. and Inase, M. Effects of morphine on two types of nucleus raphe dorsalis neurones in awake cats. *Pain* 29:376-386, 1987.
- Sinclair, J. G., Fox, R. E. Mokha, S. S. and Iggo, A. The Effect of naloxone on the inhibition of nociceptor driven neurons in the cat spinal cord. *Quart. J. Exper. Physiol.* 65:181-188, 1980.
- Sjolund, B., Terenius, L. and Eriksson, M. Increased cerebrospinal fluid levels of endorphins after electro-acupuncture. *Acta Physiol. Scand.* 100:382-384, 1977.
- Smith, M. O. Creating a substance abuse treatment program incorporating acupuncture. *The AAMA rev.* 2:29-32, 1990.
- Soulairac, A., Gottesmann, C. and Charpentier, J. Effects of pain and of several central analgesics on cortex, hippocampus and reticular formation of brain stem. *Int. J. Neuropharmacol.* 6:71-81, 1967.
- Steinbusch, H. W. M. Distribution of serotonin immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6:557-618, 1981.
- Stone, S. A., Wenger, H. C., Ludden, C. T., Stavorski, J. M., and Ross, C. A. Antiserotonin-antihistamine properties of cyproheptadine. *J. Pharmacol. Exp. Ther.* 131:73-84, 1961.
- Stux, G. and Pomeranz, B. *Acupuncture, textbook and atlas*, Springer-Verlag, 1987.
- Sun, F., Xu, M. and Xu. S. The effects of microinjections of naloxone and p-chloroamphetamine into rabbit central gray on acupuncture. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House, pp. 481, 1979.
- Swett, J. E., McMahon, S. B. and Wall, P. D. Long ascending projections to the midbrain from cells of lamina I and nucleus of the dorsolateral funiculus of the rat spinal cord. *J. Comp. Neurol.* 231:66-77, 1985.
- Takeshige, C. Neuronal responses to acupuncture. *Acupuncture and Electro-Therapeutics Res., Int. J.* 6:57-741, 1981

Bibliography

- Takeshige, C. Differentiation between acupuncture and non-acupuncture points by association with analgesia inhibitory system. *Acupuncture and Electro-Therapeutic Res, Int. J.* 10:195-203, 1985.
- Takeshige, C., Luo, C. P. and Kamada, Y. Modulation of EEG and unit discharges of deep structures of brain during acupunctural stimulation and hypnosis of rabbits. In: "Advance in Pain Research and Therapy" Ed., Bonica, J.J. and Albe-Fessard, D., Raven Press, New York, Vol. I. pp. 781-785, 1976.
- Takeshige, C., Sato, T. and Komugi, H. Roll of periaqueductal central gray in acupuncture analgesia. *Acupuncture and Electro-Therapeutics Res, Int. J.* 5:323-338, 1980.
- Takeshige, C., Kamada, Y and Hisamitsu, T. Commonly responsive neurons in the periaqueductal gray matter and midbrain reticular formation of rabbits to acupuncture stimulation, inversion, pressure on body parts and morphine. *Acupuncture and Electro-Therapeutics Res., Int. J.* 6:57-74, 1981.
- Tjolsen, A., Berge, O. G., Eide, P. K., Brock, O. J. and Hole, K. Apparent hyperanalgesia after lesions of the descending serotonergic pathways in due to increased tail skin temperature. *Pain* 33:225-231, 1988.
- Toda, K. and Ichioka, M. Electroacupuncture: relations between forelimb afferent impulses and suppression of jaw-opening reflex in the rat. *Exp. Neurol.* 61:465-470, 1978.
- Trevino, D. L., Coulter, J. D. and Willis, W. D. Location of cells of origin of spinothalamic tract in lumbar enlargement of the monkey. *J. Neurophysiol.* 36:750-761, 1973.
- Vanegas, H, Barbaro, N. M., and Fields, H. L. Midbrain stimulation inhibits tail-flick only at currents sufficient to excite rostral medullary neurons. *Brain Res.* 321:127-133, 1984.
- Villanueva, L., Peschanski, M., Calvino, B. and LeBar, D. Ascending pathways in the spinal cord involved in triggering of diffuse noxious inhibitory controls in the rat. *J. Neurophysiol.* 55:34-55, 1986a.
- Villanueva, L., Chitour, D. and LeBars, D. Involvement of the descending spinal projections responsible for diffuse noxious inhibitory controls in the rat. *J. Neurophysiol.* 56:1185-1195, 1986b.

Bibliography

- Wagman, I. H. Propriospinal influences, especially inhibitory on dorsal horn output. Proceedings from the NIH Acupuncture Research Conference, Feb. 28-Mar. 1, 1973.
- Wall, P. D. The laminar organization of dorsal horn and effects of descending impulses. *J. Physiol.* 188:403-423, 1967.
- Wang, R. Y. and Aghajanian, G. K. Inhibition of neurons in the amygdala by dorsal raphe stimulation: mediation through a direct serotonergic pathway. *Brain Res.* 120:85-102, 1977a.
- Wang, R. Y. and Aghajanian, G. K. Antidromically identified serotonergic neurones in the rat midbrain raphe: evidence for collateral inhibition. *Brain Res.* 132:186-193, 1977b.
- Willis, W. D. and Coggeshall, R. E. "Sensory Mechanisms of the Spinal cord", second edition, Plenum Press, 1991.
- Woolf, C. J., Mitchell, D. and Barrett, G. Antinociceptive effect of peripheral segmental electrical stimulation in the rat. *Pain* 8:237-252, 1980.
- Wu, J., Xing, J., Xing, B., Lu, J. and Chen, Y. Inhibitory action of electro-acupuncture on dorsal horn neurons of the spinal cord. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press, pp. 39-49, 1986.
- Xia, L. and He, G. Controlling effect of mesencephalon periaqueductal gray matter (PAG) on nociceptive afferent impulse. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House, pp. 358, 1979.
- Xiang, L., Zhu, B. and Zhang, S. X. Relationship between electroacupuncture analgesia and descending pain inhibitory mechanism of nucleus raphe magnus. *Pain* 24:383-396, 1986.
- Yaksh, T., Robert, L., Plant, L and Rudy, T. A. Studies on the antagonism by raphe lesions of the antinociceptive action of systemic morphine. *Eur. J. Pharmacol.* 41:399-408, 1977.
- Yee, K. C. Effects of acupuncture on uterine contraction during difficult labor. *Acta Sci. and Tec. Chekiang*, 1:6-13, 1973.
- Yeziarski, R. P. Somatosensory input to the periaqueductal gray: a spinal relay to a descending control center. In "The Midbrain Periaqueductal Gray Matter", Ed by Depaulis, A and Bandler, R., Plenum Press. pp.365-386, 1990.

Bibliography

- Yezierski, R. P., Mendez, C. M. and Black, D. J. Cells of origin of the rat spinomesencephalic tract: spinal distribution and collateral projections. *Neuroscience*, in press. 1991.
- Zemlan, F. P., Leonard, C. M., Kow, L. M. and Pfaff, D. W. Ascending tracts of the lateral columns of the rat spinal cord: a study using the silver impregnatin and horseradish peroxidase techniques. *Exp. Neurol.* 62:298-334, 1978.
- Zhang, A., Pan, X., Xu, S., Cheng, J. and Mo, H. Endorphin and acupuncture analgesia. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp. 256-266, 1986.
- Zhang, G., Wei, J., Lin, Y., Zhou, L., Zhou, L., Lo, Z. and Zhang, Z. Effect of stimulation of midbrain raphe nuclei on discharges of nociceptive neurons in nucleus parafascicularis of thalamus and its significance in acupuncture analgesia. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp. 82-92, 1986.
- Zhang, X. T. Interaction in the thalamus of afferent impulses from acupuncture point and site of pain. In: "Advances in Acupuncture and Acupuncture Anaesthesia." Tiantan Xili Beijing China. The People's Medical Publishing House. pp. 26, 1979.
- Zhao, Z., Shao, D. and Yang, Z. Inhibition of evoked discharges of posterior nuclear group of thalamus induced by stimulation of central gray matter, caudate nucleus and electroacupuncture in the awake rabbits. In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp. 138-145, 1986.
- Zhou, G. Z., Chen, M. P. and Xu, S. F. The relationship between the ontogenetic development of opiate receptors and acupuncture analgesia in rabbits. *Acupuncture and Electro-Therapeutic Res., Int. J.* 7:7-16, 1982.
- Zhu, L. X., Li, C. Y., Shi, Q. Y. and Ji, C. F. Effect of iontophoretic 5-HT on raphe-spinal neurons. *Acta Pharmacol. Sinica.* 7:394-398, 1986.
- Zou, G., Yi, Q., Wu, S., Lu, Y., Wang, F., Yu, Y., Ji, X., Zhang, Z and Zhao, D. Enkephalin involvement in acupuncture analgesia-radioimmunoassay, In: "Research on Acupuncture, Moxibustion, and Acupuncture Anesthesia", Ed., T. Chang, Science Press. pp. 267-278, 1986.

APPENDIX 1

These programs were written or modified by Mr. Roland Burton (Faculty of Pharmaceutical Sciences, UBC) since 1985. They are written in Turbo Pascal 5.5 and were run on a PC clone, but typically were used on an AT. They used a Hercules card to display monochrome graphics and a PCL-720 counter and timer card for counting.

The program modules are as follows:

S4

This has a boxcar integrator in which the bin was 0.5 s. There are three channels; one stimulation channel which controls onset or offset of the radiant heat lamp. The other two channels are used for counting neurone spikes. It has four cycles per data set and 180 bins per cycle.

S41

This also has a boxcar integrator, however, the bin width is adjustable in 18ths of a second. There are two channels for stimulation and one channel for counting the neuronal signal. It has one cycle per data set and 180 bins per cycle.

S46

This program uses a boxcar integrator in which the bin width is adjustable to 1 ms or larger. An external trigger signal starts the first cycle. The #15 bin emits a trigger which delivers a stimulus to the DRN. There is only one channel for counting. It has 400 cycles per data set and 50 bins per cycle.

L.

This program uses a boxcar integrator in which the bin width is adjustable from 0.001 ms to 1.0 ms. An external trigger signal starts the first cycle. It has 40 cycles per data set.

Dumps

This program is used for a screen dump of graphics to a HP Laserjet printer. It is used for all the above programs.

GR

This program is used for text and graphics on Hercules and other screen and used for all above programs.

APPENDIX 2

THE METHODS OF SEROTONIN-IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM

Tissue preparation

The animals were anaesthetized with sodium pentobarbital (Nembutal, 60 mg/kg bodyweight, i.p.) and perfused through the left ventricle. The blood was washed out with cold, oxygen enriched, Ca^{2+} - free Tyrode's buffer (50 ml at 4°C) followed by 500 ml ice-cold 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.3 for 30 min at a pressure of 70 mm Hg. The brains were quickly removed and soaked for 90 min in fresh fixative at 4°C. After fixation, they were rinsed in 5 % sucrose dissolved in 0.1 M sodium phosphate buffer (pH 7.3) at 4°C for at least 1 day. Tissue pieces were then frozen on a cryostat with powdered dry ice. Sections, 10 μm thick, were cut on a cryostat (Dittes, Heidelberg, FRG) at -25°C and subsequently mounted on glass slides coated with chrome alum gelatine to prevent detachment of the sections during the incubation procedure. The sections were immediately processed for immunohistochemistry.

Immunofluorescence procedure

The indirect immunohistochemical procedure was used. The sections were first incubated in 0.1 M phosphate buffered saline (PBS) (pH 7.3) at room temperature for 10-30 min. They were then incubated at 4°C for 18 h with 5-HT antiserum (76 mg/ml) or pre-immune serum that had been

diluted 1:500 or 1:1000 with PBS containing 0.1 % Triton X-100. After rinsing in PBS for 30 min at room temperature, the sections were treated with fluorescein isothiocyanate conjugated sheep anti-rabbit immuno-globulin diluted 1:16 with PBS containing 0.1 % Triton-X-100. After this final incubation of 30 min at room temperature, the sections were rinsed once again in PBS for 30 min and mounted under a coverslip in a mixture of glycerine-PBS (3:1, v/v). The sections were examined in a Zeiss Universal microscope equipped with incident illumination for fluorescence. Kodak Tri-X film was used for photomicrography having exposure times between 5 s and 20 s. Alternate sections were mounted on separate slides and stained with cresyl violet.